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Method for the Determination of the Extent of Heat Treatment of Meat

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It is superfluous to dwell upon the importance of finding methods suitable to demonstrate meat-products being satisfactorily heated. There are two such methods published in the literature at our disposal, i.e. the Hypr (1) and the Coretti (2) procedures, as well as the method of the so-called precipitation test (3) adapted in Hungary.

The comparative testing of those methods are in course at our Institute, however, we cannot form a final opinion about them yet. In consequence, the method described below is not destined to be a substitution the methods known so far, its purpose is solely to make a contribution to the solution of the problem mentioned above.

In order to decide, whether the given meat, or meat-product was satisfactorily treated or not during the technological procedure, one has to follow a biochemical, or colloid-chemical feature that would change under the effect of heating (4,8). Unfortunately there is little possibility of finding in connection with meat such a single "variable" by which one could unanimously determine the degree of heat treatment.

So e.g. the decrease of enzyme-activity is influenced, besides the circumstances of heat-treatment, by other characteristics of the raw meat (pH, salt content, hydration, fat content, etc.). The difficulties arising from such circumstances cannot always be eliminated, not even with the help of methodical and computative procedures. The results therefore, can only be treated by statistical methods (5,6,7).

Materials and methods

The acid phosphatase enzyme, present in raw meat, is inactivated by the thermic effect, the degree of which is proportionate to the temperature and the duration of cooking. Consequently, the residual enzim activity will give

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information concerning the degree of heat treatment.

Among others, Simskaya (9) and Avakyan (10) were also engaged with the phosphatase activity in meat. Recently Sanders and al. gave results (11, 12) on the determination of the degree of pasteurization of milk on the basis of the above principle.

Our method has been worked out for cooked ham and sausage products.

Sampling : samples to be tested shall always be taken from the surroundings of the thermic centre, this being the spot of the slowest warming-up, i.e. most unfavourable from the point of view of heat-treatment.

The sampling recommended for canned ham, respectively, is hown by fig. 1.

The ham shall be cut off, vertically of Plane a-b and Axis a, at distance a/2, then, at the point of intersection of Axes b and c a piece shall be cut out from the larger slice, going down to a depth of about 3 cm (parallel with Axis a), with the help of a hollow metal cylinder of 2 cm inner diameter. In the case of sausages the sample also shall be taken from the geometric centre (i.e. from the point of intersection of the symmetry xes of the cylinders).

The sample thus obtained shall be cleaned with scissors from rougher fatty and connective tissue elements, put into a mortar and after chopping (whithout using sand) it shall be blended. 2,5 g shall be accurately weighted and into a 100 ml glass vessel of the Tourmix homogeniser (either a M.S.E. top-drive macerator or a Nelco top-drive homogeniser) and 50.0 ml of citrate-buffer of 0,1 M (=pH=6,5) shall be added to it. Homogenisation will be carried out 1,5 minute to 2 minutes (10 000 - 14 000 r.p.m.).

Subsequently the suspension shall be shaked up, and immediately poured into a test tube accurately up to 10 ml mark and 5,0 ml freshly prepared solution of disodium phenyl phosphate (0,218 g/100 ml) shall be added to it. Next, the test tube (which has 10 and 20 ml marks and is fitted with a glass stopper) shall be plugged, well shaken and placed in a water bath of 37,5 $\pm$ 1 C° (most suitably in a ultrathermostat) for 60 minutes. Hereupon 5,0 ml solution of trichloroaceticacid (20 g/100 ml) shall be added to it, the whole shaken up and filtered through quantitative filter paper (e.g. Macherey 640 W) into a test tube of a larger diameter. 5,0 ml of the clean filtrate be transferred with pipet, into antoher test tube, 5,0 ml solution of Na_2CO_3 of 0,5 M shall be added to it and the whole shaken up again. The p$_H$-value of the solution thus obtained must be between 9,6 and 9,7. Following, 5 drops exactly (0,09 ml), shall be added to it from the freshly prepared solution of 2,6 dibromchinon-chlorimide (4 mg/ml in abs ethanol) and, after a thorough shaking-up, it shall be left in dark at room-temperature for 30 minutes. The absorption of the blue indophenol solution shall be measured with photometer.

Preparation of the control-test : to the 10 ml homogenized sample poured into the tube add first 5,0 ml of 20 % trichloroacetic-acid, then 5,0 ml solution of disodium phenyl phosphate ; finally, shake it up and filtrate it. The filtrate

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shall be treated in a similar way to that of the test samples ; the value of the control extinction shall be substracted from the extinction of the sample tested.

The calibration curve shall be made in the usual manner (11). The μ g phenol/10 ml values, obtained in the above described manner, have been taken by us as measure of the phosphatase activity (1 μ g phenol/10 ml = 1)

By kinetic considerations it can be demonstrated that the results may be considered really as apparent activity only.
Solutions necessary for the determinations :

Citrate buffer : (pH = 6,5). Dissolve 41, 64 g Na-citrate + 1,765 g citric acid with distilled water to obtain a solution of 3000 ml. The solution, shaken up with 1 ml toluene per 1 lit. and stored in a refrigerator, may be kept and used for a long time.

Disodium-phenyl-phosphate must be freshly prepared at every occasion in the required quantity for the tests. The solution cannot be kept in the refrigerator, not even with toluene for 24 hours without decomposition (hydrolisis?) 0,218 g shall be dissolved in distilled water up to 100 ml. As there are not sufficiently pure preparations on the market (their aqueous solution containing detectable phosphatic ions), it is advisable to use exclusively the product of The British Drug Houses LTD.B.D.H. Laboratory Chemical Group (Poul - England).

The drug must be stored in a dark bottle in an exsiccator (11).

At the test special care should be taken from the following viewpoints :

the homogenization with "Turmix" cannot be substituted by the grinding of the sample, since the phosphatase is present in the meat mostly as desmoenzyme, combined with non soluble parts in water and thus its activity greatly depend on the specific surface of the homogenized substance. Reproduceability of results may be ensured by energetic homogenization of at least 1' (10 000 - 14 000 r.p.m.). This explains why the sediment itself must be shaken up with the subtrate and incubated. For this reason special attention should be taken to the correct sampling at the pouring from the vessel of the homogeniser into the test tube.

The temperature of the water-bath must be kept within $\pm$ 1 C° ; for this purpose the ultrathermostat is the most suitable. It is also clear, that the shortening of the warming-up period of the solution increases the accuracy of the determination, consequently, only thin-walled test tubes of small diameter must be used for the incubation. The temperature of the incubation greatly influences the results (e.g. at 30 C° 8,0 ; at 37 C° 11,8 ; at 46 C° 17,9 - if the incubation period is one hour).

The trichloroacetic-acid does not hydrolise the disodium-phenyl-phosphate within the limits of the measurement time (12).

In the control test the sample s all always be freed from coarse fatty and connective tissue parts. It is easy to understand that the test results are

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influenced also by the initial activity of the original raw meat. The activity of the muscular tissue-phosphatase (at the above described method) ranges from 200 U to 300 U while that of the fatty tissue ranges only from 20 U to 30 U. If, in the interest of an approximative computation, the thermal destruction may be considered as a first rate reaction, then e.g. in the case of a 90 % decrease of activity, the residual-activity of the muscular tissue will be about 20-30 U, while that of the fatty tissue around 2-3 U. Thus, a greater proportion of fatty tissues in the sample affects considerably the results.

The incubation is carried out at pH 6,5. The pH optimum of the phosphatase, to be found in the muscular tissue of the ham ranges from about 5,3 to 5,5 ; however, we do not test at the optimum but at the "actual" obtained from the average of 300 ham-samples /pH value. This has been needed to better adapt limit values to the presently used cooking technology in Hungary. Of course, there is a pH optimum in basic medium too ; very likely, we have to do with the phenomenon of isodynamics (13).

According to our observations, the basic phosphatase is more heat-sensitive (it loses its activity at lower temperatures), furthermore, the determination here encounters methodical difficulties not detailed in the present treatise.

Discussion

Factors affecting the residual-phosphatase-activity besides the circumstances of heat-treatment have been investigated.

The sample have been previously ground and placed in a thin layer into cellothen-foil. Next, the samples - enclosed in vacuum, the thickness not exceeding 1 mm - have been placed into the water bath of the ultrathermostat for 4 minutes at a temperature of 70°. With the help of approximative calculation (14,15), it can be proved that in this way the time of the heating of the entire cross-section does not exceed 20 secs, which may be omitted as compare with the total period of the heat-treatment. The duration and the temperature of the heat-treatment being identical (4 minutes at 70° C) the results of the statistical analysis, were as follows :

There has been a strongly significant connection between salt content and residual phosphatase-activity ($R = 0,62^{++}$). The regression line shows a growing tendency, which proves that the increase of the salt content has an impeding effect upon the heat destruction of the enzyme.

The connection between activity and pH ($R = -0,195$) strongly significant only, if the NaCl-effect is eliminated ($R = -0,593^{++}$) with the help of partial correlative computation. As the regression line has a decreasing tendency, it is seen that the increase of the pH (the withdrawing from the pH-optimum of the enzyme) reduces the thermal resistance of the phosphatase. We cannot, however, consider this latter conclusion, on account of the relatively small number ($N = 30$) of the measurements, as being satisfactorily verified, in spite of the significancy. However, the thermal resistance and the pH-optimum of enzyme activity often coincide (16).

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Besides of salt content and pH values the results are affected also by other factors, out of which we have lately succeeded in proving the effects of condensed phosphates. On the other hand, we have not observed a reactivation after the heat-treatment.

It can be, therefore, concluded that the results are affected, besides the condition of the heat treatment, also by the factors resulting from the fluctuation of the quality and chemical composition of meat. Nevertheless, these fluctuations are not as great as to impede the obtaining of an approximate information, concerning the heat treatment of the product.

Fig. 2 shows the heat-destruction diagrams of the enzyme in ordinary as well as in log-log scale in a ham sampl .

The aim of our present treatise can not be the discussion of the intricate problems of kinetics of heat-destruction or of the activity-determination, however, calculating with simple reaction kinetical models, we were unable to reach a final conclusion.

Dealing with the statistical evaluation of the limit values belong to our tasks scheduled for the present. Otherwise, the most effective procedure is not the indication of rigid limit values but the introduction of a control system based on mathematic-statistical considerations.

Accidental modifications of cooking technology will, of course, necessitate further collecting of data. For the evaluation of technological modifications, microbiological and organoleptic view-points are the guiding principles.

A more detailed account of our experimental data has been impossible whi hin the frame of the present paper. In this respect we intend to publish more detailed results in the near future.

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Fig. 1 - Method of sampling in the case of canned ham.

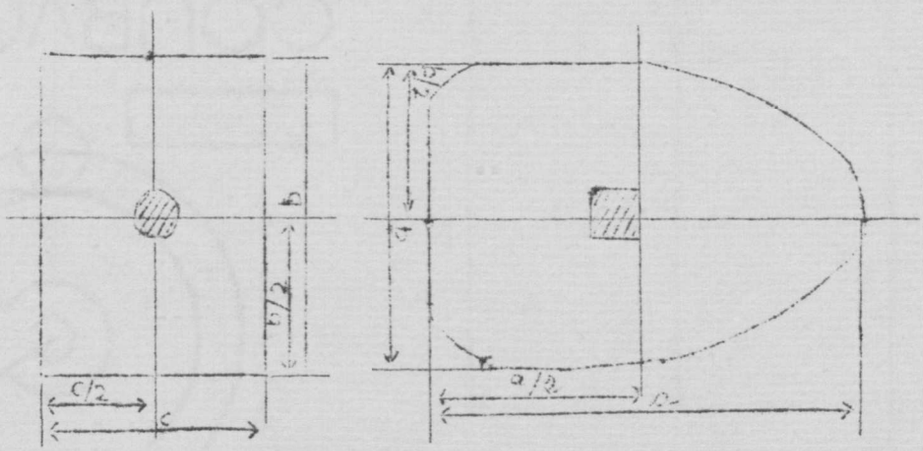
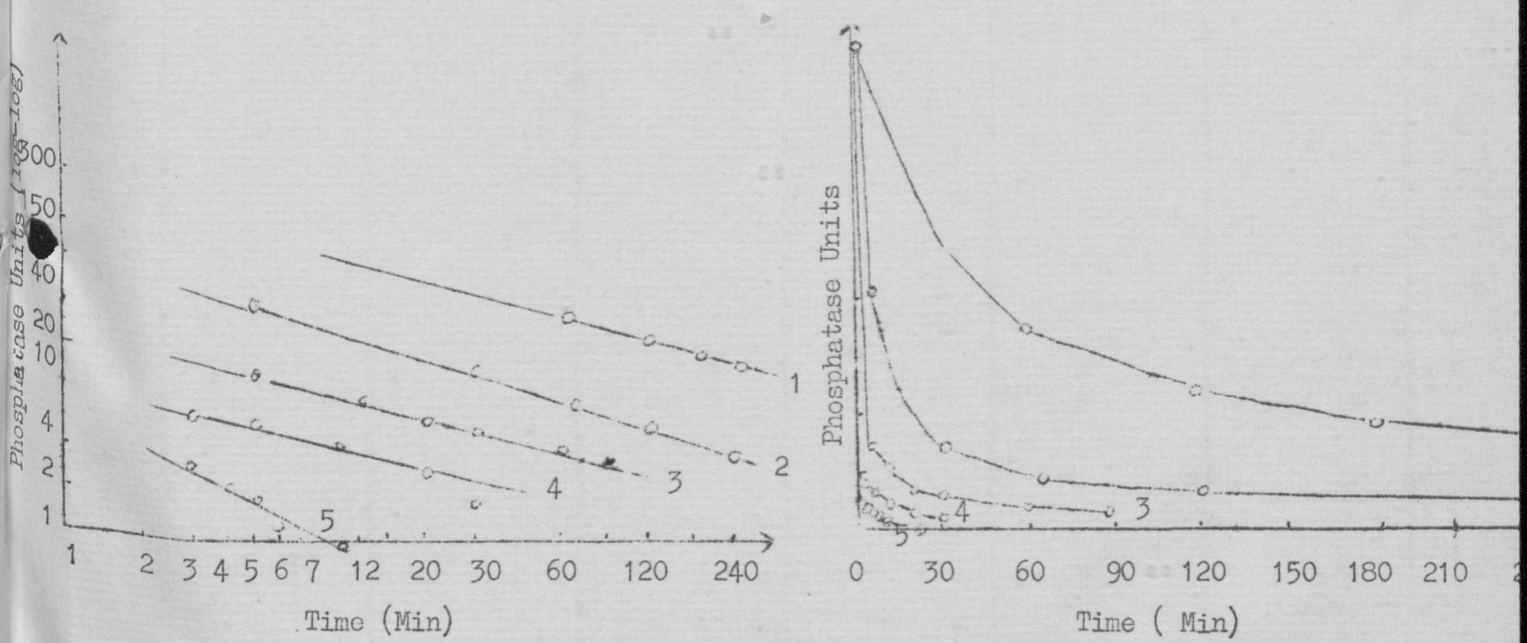


Fig. 2 - Heat-destruction curves of phosphatase enzyme in original form and on log-log scale. Ham pH = 6,25 ; NaCl = 3,5 % ; water content = 73,5 % ; initial activity = 302 ± 6 U.



1. Curve cooked at 60° C
2. " " 65° C
3. " " 70° C
4. " " 75° C
5. " " 80° C