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About the acid phosphatase test as a criterion for the heat treatment of hams and shoulders

1. Introduction

For checking correct heat treatment of cooked hams and shoulders, coagulation tests are currently used (1, 2). However, apart from difficulties in interpretation, these methods often give false results. Basing himself on earlier work of Körmendy and Gantner (4), Lind successfully used the activity of the meat acid phosphatase as an indicator for the heating rate (6a, b). A sample from the centre of the product is mixed with a buffer solution (pH 6,5) and incubated for 1 hr at 37°C with phenylphosphate as a substrate. The phenol, set free, is determined colorimetrically and is a measure for the enzyme's activity. In the relevant temperature range there is a linear relationship between the centre-temperature reached and the $^{10}\log$ of the activity.

Yet, only little systematic work is done on the capabilities of this test. The experiences reported by Suvakov c.s. (7) last year, were quite disappointing. In this report we mention some results of our work, consisting partly of model experiments and partly of experiments with industrial hams and shoulders.

2. Material and methods

a) Model experiments

Two hams from one pig were artery-pumped with 10 % of a brine (15 % salt; 0,15 % $NaNO_2$; 0,3 KNO_3 and 3 % dextrose). The brine used for the right ham contained, in addition, 5 % of a polyphosphate preparation. After draining and deboning, the hams were divided into 5 parts:

part 1 M. quadriceps femoris;
part 2 Mm. biceps femoris, semitendineus and gluteus superficialis;
part 3 Mm. glutei;
part 4 Mm. semimembranosus, gracilis and adductores
part 5 residual muscles.

Each part was separately passed twice through a meat mincer with a 2 mm holed plate. With all thus treated portions 50 g cans (58 x 32 mm) were filled which were heated at 7 temperatures, varying between 64,1 and 67,1 °C with 0,5 °C intervals. Cans filled with the same material were evenly distributed over these temperatures. Residual cans, if any, were spread over the temperatures in the middle of the range. The heating was carried out in a waterbath, the temperature rise of which was programmed in a way similar to that of the centre part of a canned ham being pasteurized. The final temperature to be reached was maintained for 30 minutes. The cans were stored under refrigeration and analysed between 2 and 9 weeks after cooking. The U.S.D.A.-flocculation test (1) which was done on a 10 x reduced scale and the Coretti test (2) were carried out. The phosphatase activity was measured with Lind's method (6a), provided that about 7,5 to 8 grammes of the sample were homogenized during 30 seconds with 4 times the weighed quantity in milliliters buffer solution, by means of an Ultraturrax-homogenizer (Janke & Kunkle; 24 000 rpm), after which

three 10 ml portions (for a duplicate and a blank determination) were pipetted.

b) Experiments with hams and shoulders

From 4 meat factories and the technological section of the Netherlands Centre for Meat Technology 39 cooked hams and 31 ditto shoulders (flat and A.M. types of different sizes) were obtained, together with their thermograms. Around the thermocouple location a sample of about 100 - 150 g was taken. From this sample a portion of about 30 g, immediately surrounding the thermocouple, was taken and freed from fat and connective tissue, and gelatine. After the pH was measured the Coretti and the phosphatase test were carried out in this portion while in the other part of the sample the U.S.D.A. test was peformed. The phosphatase test was carried out as described under 2.1. (method B) as well as with the original method of Lind (method A).

3. Results

Table I summarizes the results of an analysis of variance of the logarithm of the phosphatase activity.

Temperature, muscle group and polyphosphate all have a significant effect on phosphatase activity ($P < 0,001$). Surprisingly, there is also a highly significant interaction effect between muscle and polyphosphate. This implicates that in one muscle group polyphosphate has a larger influence on enzymic activity than in the other. Despite of the known inhibitory action of phosphate on substrate hydrolysis, ham part 1 (M. quadriceps) heated in the presence of polyphosphate was found to display a greater enzymic activity than in its absence. It must be concluded that polyphosphate noticeably inhibits the heat denaturation of the muscle proteins, a phenomenon which is also mentioned by Körmendy and Gantner (5). For ham part 4 (M. semimembranosus) things were reversed, i.e.

the inhibitory effect of phosphate outweighs the protection against denaturation in this case.

Table I.

Analysis of variance of log (phosphatase activity)

variance due to	degrees of freedom	sum of squares	variance estimate	
temperature	6	1,4905	0,2484	+++
muscle	4	0,5692	0,1423	+++
polyphosphate	1	1,2480	0,1248	+++
interaction temperature x muscle	24	0,2956	0,01232	++
interaction temperature x polyphosphate	6	0,06372	0,01062	
interaction muscle x polyphosphate	4	0,2511	0,0628	+++
interaction muscle x temp. x polyphosphate	24	0,1883	0,00785	
total	139	3,764	0,02708	+++
within samples	70	0,7808	0,00558	

$^{++}$ = significant, $P < 0,01$; $^{+++}$ = significant, $P < 0,001$

The significant interaction effect of temperature and muscle on phosphatase activity means, that heat denaturation progresses more readily in one muscle group than in the other (in the range between 64,1 and 67,1°C).

The regression equations, obtained in the model experiments, do not differ very much from those for hams and shoulders (see Table II).

There is a considerable discrepancy in the slopes of the regression lines between hams and shoulders. This necessitates the use of different equations for calculating the heating temperature from the enzymic activity, a need also appearing from the standard deviation of regression values. Lind found a regression equation for hams $y = -5,71 \log x, + 77,4$ with which our results

are in reasonable agreement. However, his standard deviation value 0,63 was considerably lower. The reason for this is likely to be the fact that in the Danish study only Pullman hams were involved, whereas in our experiments hams of several shapes and sizes were taken. The low standard deviation found in the model experiments can also be accounted for by the small variability in the samples.

In Table I the within-sample variance was given. The relative standard deviation, calculated from it, is 17 % (for an overall phosphatase activity of 107 µmol per kg of meat). Gantner and Körmendy (3) obtained a value of 11,5 % for a series of 10 determinations in the same sample on one day. Considering the effect on phosphatase activity of various factors, it could be questioned whether it is worthwhile to improve the reproducibility by modifying the determination procedure. Anyhow, from trial experiments it seems that replacing phenylphosphate by paranitrophenylphosphate as a substrate, apart from simplifying the procedure, can lead to a better reproducibility.

The meat pH may not influence the pH of the reaction mixture. The buffer solution should have adequate buffer capacity. In the Danish method this requirement is not fulfilled. A larger amount of buffer solution is therefore to be preferred (also see Gantner and Körmendy (3). A higher sensitivity, achieved by using a buffer of pH = 5,5 - the optimum for substrate hydrolysis might also benefit the reproducibility in our opinion.

The results of the coagulation tests (Tables III and IV) reveal that the U.S.D.A. - test has proved to be less satisfactory.

Table III.

Results of coagulation tests for the model experiments

heating temperature in °C	number of samples	heated below 65,6 °C (150°F) acc. to U.S.D.A.-test		acc. to Coretti-test	
		number	%	number	%
64,1	25	20	75	14	56
64,6	26	19	73	11	42
65,1	29	23	78	4	14
65,6	31	22	71	5	16
66,1	31	19	61	1	3
66,6	27	14	52	0	0
67,1	25	11	44	0	0

Table II.

Regression equations, standard deviations of regression and correlation coefficients

	regression equations		stand. dev. of regression in °C		correlation coefficient	
	method A	method B	A	B	A	B
model experiment samples		$y = -3,83 \log x + 73,1$		0,74		$-0,63^{++}$
without polyphosphate		$y = -5,33 \log x + 76,3$		0,71		$-0,70^{++}$
with polyphosphate		$y = -4,20 \log x + 73,9$		0,74		$-0,64^{++}$
all						
hams and shoulders						
hams	$y = -5,04 \log x + 74,4$	$y = -5,69 \log x + 76,6$	1,07	1,27	$-0,88^{++}$	$-0,83^{++}$
shoulders	$y = -1,46 \log x + 69,8$	$y = -1,91 \log x + 71,1$	1,31	1,40	$-0,46^{+}$	$-0,33^{+}$
all	$y = -2,25 \log x + 70,6$	$y = -1,83 \log x + 70,5$	1,66	1,73	$-0,49^{++}$	$-0,41^{+}$

+ = significant, P 0,05;
++ = significant, P 0,01

Table IV.

Results of coagulation tests for 39 hams and 31 shoulders

centre temperature (in °C) between	number	heated below 65,6 °C acc. to U.S.D.A.-test number	%	(150°F) acc. to Coretti-test number	%
61,0	1	1		1	
64,5 - 65,5	7	4	57	0	
65,6 - 66,5	28	15	54	1´	
66,6 - 67,5	8	2	25	0	
67,6 - 68,5	6	2	33	0	
68,6 - 69,5	13	3	23	0	
69,6 - 72,0	7	1	14	0	

´excentric location of the tehrmocouple

This test, according to our experience, is completely unsuitable for checking an adequate heat treatment. The Coretti-test is more satisfactory. It often occurs, however, that products heated below 65,6 °C (150°F) are not as such recognized. Contrary to the results of this study, in current work we do not unfrequently meet with hams and shoulders which display a positive Coretti-test in spite of more than adequate heat treatment (68 - 70 °C).

4. Conclusion

The phosphatase test is a valuable tool for controlling the pasteurization of hams and shoulders. Its accuracy is, however, impaired by several factors that remain unknown in quality control work. There is a 5 % chance for a ham to be condemned as being heated to an internal temperature below 65,6°C (150°F), when actually this temperature was 67,4°C. The same can be said for shoulders, actually heated to 67,8°C.