DETERMINATION OF CATALASE AND SOLUBLE PIGMENTS IN HEAT-TREATED PORK AS SANITY INDICATORS FACE TO THE AFRICAN SWINE FEVER VIRUS

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In order to find efficient indicators to give quick evaluations for African swine fever virus contamination in heat-treated pork, studies have been made on dynamic inactivation of catalase and on denaturation of meat pigment by heat.

Pork was minced and treated at different temperatures and times. Catalase and soluble pigments were evaluated by absorption spectrophotometry, before and after the heat treatment.

Results showed that the catalase inactivation and pigment insolubilisation follows an exponential curve. From these curves, thermoresistance parameters (D and Z) were calculated for each of them. Comparing the resulting catalase inactivation curves and the pigment insolubility curves to the inactivation of African Swine Fever Virus, we can determine the validity of those indicators for the evaluation of heat-treated meat as far as the virus is concerned.

INTRODUCTION

Fresh and processed pork, from countries where the African Swine Fever (ASF) appears, are considered to be important factors of the spreading of that disease. As sanity defence rule against the spreading of ASF, circulation of those raw materials and products from countries where that disease shows out to countries free from this problem and, as far as EEC is concerned, raw canned meat which reached 70°C at the critical point, during a period of 30 minutes.

In view of the small heat resistance of the ASF virus, at temperatures between 55-60°C (1, 2, 3, 4, 5, 6, 7, 8 and 9), the trade of other heat processed meat products could be allowed. The kinetics of heat inactivation of ASF virus (7 and 9) allows its characterisation in terms of D and Z values. This fact gives us the opportunity of adopting as safe indicator of thermically treated products the conception of lethal evaluation of thermic treatment (10).

The main purpose of this work is to find out indicators of easy determination for indirect evaluation of heated meat products sanity, in what ASF virus is concerned. In order to reach this objective, the kinetics of Catalase inactivation and heme-pigments denaturation, by heat, in raw pork have been studies.

MATERIALS AND METHODS

For the assays of catalase inactivation and meat pigments denaturation, finely minced shoulder and pork leg have been used. Before mincing (Moulinex cutter) the meat pieces were cleaned of visible fat and connective tissue.

1 - HEAT TREATMENTS

The finely minced raw meat was filled into glass tubes (Ø4 mm, length 25 cm; thickness 1 mm) using a plastic syringe. The tubes with rubber stoppers were plunged into a water-bath (Haake W-26, thermostat Haake E3) at constant temperature for different times. After the heat treatment the tubes were quickly chilled in icy water.

For the study of catalase inactivation and heme-pigments denaturation the temperatures used were respectively 53, 55, 56, 57, 58 and 60°C and 68.5, 70, 72, 73. 74, 75 and 76°C.

2 - EVALUATION OF WATER SOLUBLE HEME PIGMENTS

The water extraction and next evaluation of heme pigments (Hornesey 11) have been made in raw as well as in heat treated meat.

Concerning water extraction, a previous water homogenisation was made in a "Hamilton Beach Scovill, mod. 612-3, USA" followed by a double filtration (Whatman 41).

In order to make easy this last operation and the next evaluation of the meat soluble pigments as well, we used in the homogenisation the following proportions of sample and water: raw meat - 30 g, water - 99 ml; cooked meat - 30 g, water 39 ml.

For the meat pigments evaluation we mixed an aliquot of filtrate with acetone and chloride acid in a ration of 18%, 80% and 2%, respectively. 30 minutes after, this mixture was filtered by Whatman 42 followed by the reading of the absorbance value (Spectrophotometer PYE

	53°C		55°C		56°C		57°C		58°C		59°C		60°C	
min	U/g	%	U/g	%	U/g	%	U/g	%	U/g	%	U/g	%	U/g	%
0	540	100	532	100	475	100	540	100	450	100	300	100	450	100
3	-	-	-	-	-	-	-	-	-	-	205	68	170	38
4	-	-	-	-	-	-	-	-	240	53	-	-	-	-
5	-	-	-	-	-	-	355	66	-	-	130	43	78	17
7	-	-	-	-	-	-	-	-	-	-	75	25	36	8
8	-	-	-	-	-	-	-	-	153	34	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	30	10	-	-
10	-	-	-	-	-	-	245	45	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	86	19	-	-	-	-
15	-	-	296	56	-	-	185	34	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	50	11	-	-	-	-
18	-	-	-	-	-	-	-	-	36	8	-	-	-	-
20	307	57	-	-	182	38	125	23	-	-	-	-	-	-
25	-	-	-	-	-	-	80	15	-	-	-	-	-	-
30	-	-	223	42	119	25	-	-	-	-	-	-	-	-
40	273	50	209	39	91	19	-	-	-	-	-	-	-	-
50	-	-	182	34	64	13	-	-	-	-	-	-	-	-
60	260	48	162	30	-	-	-	-	-	-	-	-	-	-
70	-	-	-	-	33	7	-	-	-	-	-	-	-	-
80	245	45	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	232	43	-	-	-	-	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1 : Remaining catalase expressed in units of enzyme per gram of raw meat

	68.5°C		70.0°C		71.0°C		73.0°C		74.0°C		75.0°C		76.0°C	
min	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%
0	103.4	100.0	103.4	100.0	103.4	100.0	122.4	100.0	103.4	100.0	122.4	100.0	103.4	100.0
5	-	-	-	-	-	-	-	-	-	-	65.3	53.3	59.8	57.9
10	-	-	-	-	76.1	73.7	65.3	53.3	59.8	57.9	-	-	40.8	39.5
15	-	-	80.2	77.6	-	-	-	-	-	-	39.4	32.2	28.6	27.6
20	85.7	82.9	-	-	63.9	61.2	53	43.3	44.9	43.4	-	-	24.5	23.7
25	-	-	-	-	-	-	-	-	-	-	26.5	21.7	19	18.4
30	-	-	73.4	71.1	54.4	52.6	42.2	34.4	35.4	34.2	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-	20.4	16.7	-	-
40	-	-	-	-	-	-	35.4	28.9	28.6	27.6	-	-	-	-
45	-	-	63.9	61.8	42.2	40.8	-	-	-	-	15	12.3	-	-
50	78.9	76.3	-	-	-	-	31.3	25.6	23.1	22.4	-	-	-	-
60	-	-	61.2	59.2	36.7	35.5	27.2	22.2	-	-	-	-	-	-
75	-	-	54.4	52.6	-	-	-	-	-	-	-	-	-	-
80	70.7	68.4	-	-	-	-	-	-	-	-	-	-	-	-
110	57.1	55.3	-	-	-	-	-	-	-	-	-	-	-	-
140	50.3	48.7	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 Remaining heme expressed in µg of hemat in per gram of raw meat

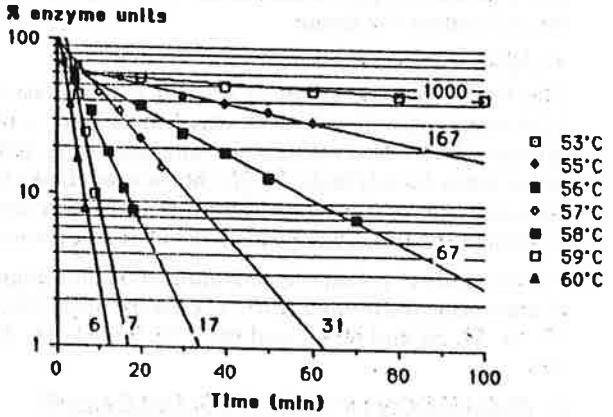

Fig. 1 Regression curves for heat inactivation of catalase. Concentration at zero has been excluded. The numbers in each curve are the D values

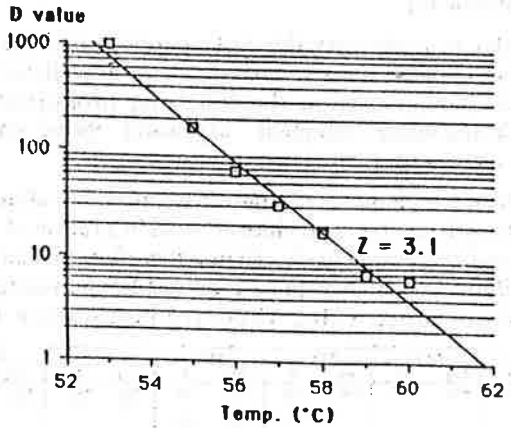

Fig. 2 Thermal inactivation time curve for the catalase. Z= 3,1 ± 0,5 (95% conf. Int.)

UNICAM SP8-100) at 640 nm (11), with a cuvette of 1 cm.

The determination of heamatina concentration in meat was calculated using the 680 factor (11), with the necessary corrections introduced by the extraction dilution (1/4 in raw meat and 1/2 in cooked meat (W/V)).

For the calculation of the water volume to be added, the moisture of meat has been subtracted (in raw as well as in cooked meat 70% was attributed for the moisture).

3 - EVALUATION OF CATALASE (12)

A previous homogenisation of approximately 10 g of each sample has been made, using a Polytron (Kinematica Basic Unit PT 10/35; Aggregate PTA 10 TSM; Control Unit PCU-6; Max. speed 27000 rpm) in the 4.5 position (max. position 11). To make it possible we made a dilution of 1/5 (P/V), with a potassium Phosphate Buffer, 50 mM, pH 7. The homogenisation obtained was centrifuged at 25000 g x 30 min running at 4°C (Sorvall RC-(C Rotor SS-34)) followed by a supernatant collection after a filtration by Whatman 41.

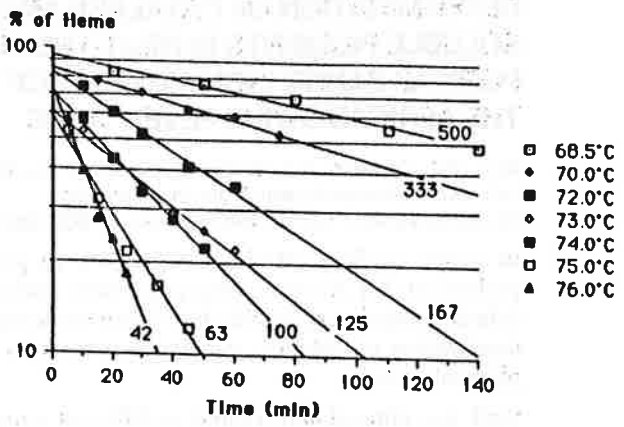

Fig.3 Regression curves for heat insolubilization at zero minutes has been excluded. The numbers in each curve are the D values

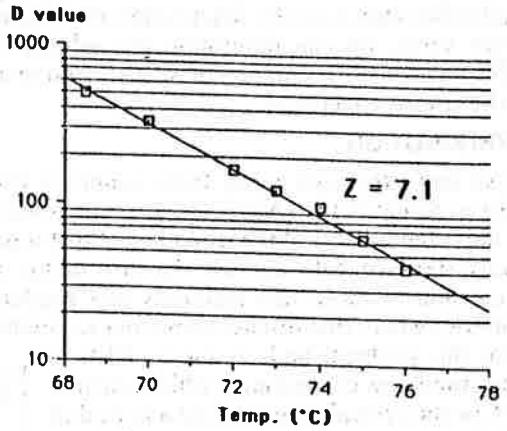

Fig. 4 Thermal denaturation curve (TDT) for heme pigment. Z=7,1 ± 0,7 (95% conf. Int.)

Subtract solution - 100 ml 30% H_2O_2 was added to 50 ml 50 mN phosphate buffer, pH 7.0. The concentration was adjusted so that the absorbance at 240 nm (A240) lies between 0.550 and 0.520.

Measurement - 100 ml of sample solution was added to 2.9 ml of subtract solution in a silica cuvette (10 mm light path). The measurements were made at room temperature. The time (seconds for the A240 decrease from 0.450 to 0.400 was applied to the following formula:

$$\frac{3.45 \times 60 \times \text{dilution factor}}{\text{seconds}} = \text{Units/g of meat}$$

4 - STATISTICAL CALCULATIONS

All calculations (D and Z) were made with an Apple Macintosh Plus personal computer. For the statistics, we used the StatView 512$^+$ (13) and for the graphical presentation, the program Cricket Graph (14).

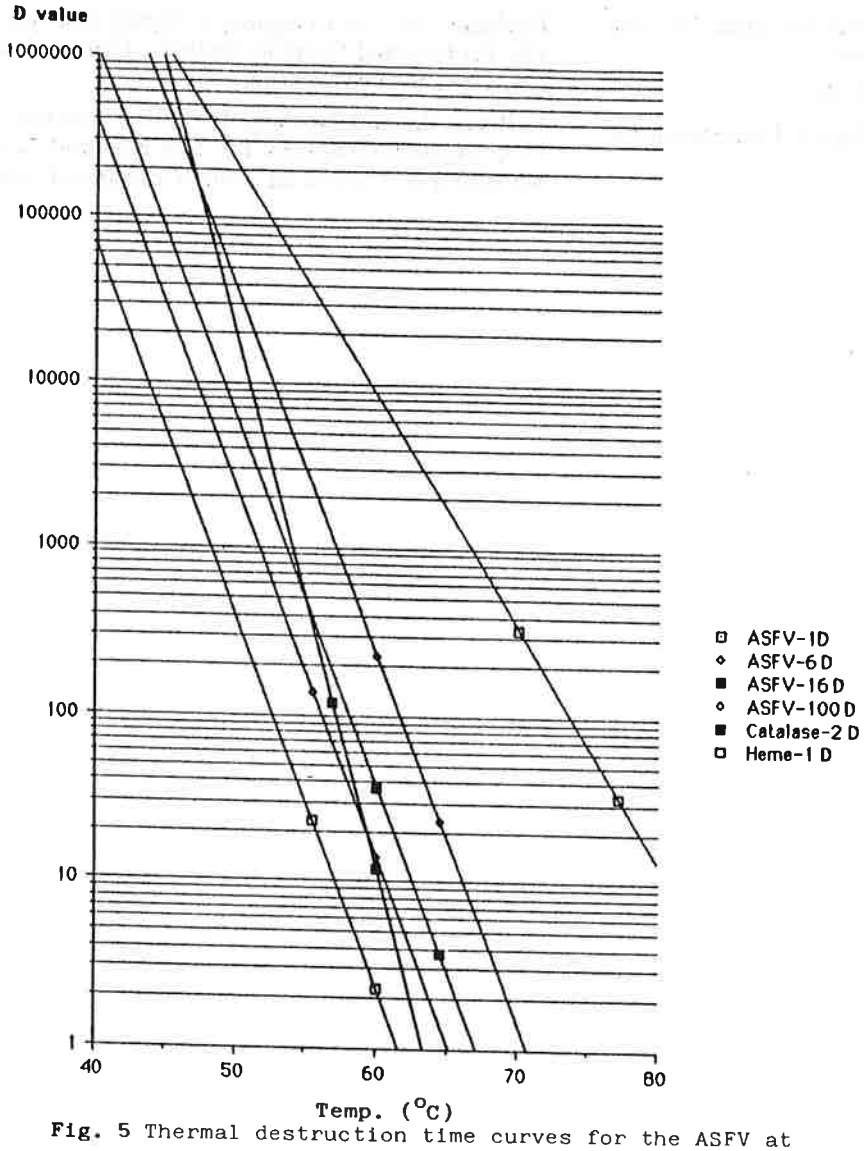

Fig. 5 Thermal destruction time curves for the ASFV at different levels of decimal reduction, compared with catalase inactivation and pigment desnaturation curves.

RESULTS AND DISCUSSION

The results concerning catalase and soluble heme pigments of raw and heat treated pork are shown in Table 1 and 2.

Fig.1 and 3 represent, respectively, the regression curves of catalase and soluble meat pigments losses at different heat treatments, in function of time. Watching both of them we can say that they follow a logarithmic curve, allowing us to find out the D value for each studied temperature (values written on each line).

Fig.2 and 4 (TDT curves) show that D values evolution in function of time, is also logarithmic, allowing us to find out the Z value for each of them. Therefore, the Z values, calculated, for the catalase inactivation and heme pigments insolubilisation in the assay conditions related before, were respectively 3, 1 and 7.1°C. In view of those results, the thermic inactivation curve of catalase as well as the thermic denaturation curve of heme pigments can be defined respectively by a D60°C = 6 min/Z = 3.1°C and 70°C = 333 min/Z = 7.1°C. ASFV known thermic inactivation curve, L60 strain, is characterised by z = 5.5°C (9). Decimal decrease timing of the same strain, being determined in fresh meat for 60°C is (D) = 2.33 mm (10).

According to these values the Fig.5 shows us ASFV thermic inactivation curves for 1D, 6D and 100D, considering D60°C = 2.33 mm and Z = 4.5°C, against the catalase and heme pigments thermic inactivation curves, respectively for 2D and 1D. Comparing them, we may conclude that the catalase determination is a good sanity indicator concerning the ASFV in heat-treated pork at temperatures below 60°C, where as the soluble heme-pigments evaluation will be safe, for the same purpose, at temperatures higher than 50°C.

These conclusions will be acceptable only for heat-treated pork and in the conditions of the assay (temperatures reaching all the meat mass in short times).

As far as processed meat products are concerned, more data will be needed, including the interference caused by seasoning and additives in thermoresistance of both indicators, as well as the influence of the different thermic treatments usually used, in terms of lethal values.

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