Modified cured hemoglobin as colouring agent for gelated pig blood plasma in livex form.

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SUMMARY:Influence of thermo-acidic denaturation and enzymic digestion of bovine hemoglobin /BHb/ on its reactivity with $NaNO_2$ was evaluated.Both procedures of the preliminary modifications of BHb increase the degree of haeme nitrosation.The colour of thermally gelated pig blood plasma /livex/,obtained after the adding 300mg% of the experimental nitrosated BHb forms was similar to that obtained with dinitrosyl ferrohemochrome-pigments preparations. Production of nitroso pigment by nitrosation of the preliminary enzymatically digested hemoglobin for processed meat products colouring seems possible,but requires further investigation on the improvement of pigment solubility.

INTRODUCTION:The search for nitrite substitute used for meat curing has been continued for many years.For a long time technologists also attempt to use the cured blood or it hemolisate for colouring purposes./PIETRZYK and ORŁOWSKA,1971,MÖHLER at al.1971/.Attempts were also made to synthesize cured meat pigment from myoglobin /KAMAREI and KAREL,1982/,hemin/PALMIN at al.1973,SHAHIDI at al.1984/ and bovine red blood cells /SHAHIDI and PEGG 1988/.The objective of this study was to investigate the possibility of the colouring agent preparation from lyophilized native bovine hemoglobin enzymatically digested and/or thermo-acidically denaturated prior to nitrosation with $NaNO_2$.

MATERIALS and METHODS:Lyophilized native bovine hemoglobin /BHb/ was the experimental material.Prior to nitrosation BHb was dissolved and modified according to the procedures given in Tab.1.

TABLE 1. Procedures of lyophilized native bovine hemoglobin modification

No.	Treatment	Modification time /h/	Methods of modification
1	N_{24}	24	Native lyophilized BHb+citric acid-phosphate buffer pH 3.2
2	N_{48}	48	/CPB/
3	E_{24}	24	Native lyophilized BHb+CBP+aspartyl proteinase from Penici-
4	E_{48}	48	llium cammemberti /AP/*
5	AT_{24}	24	Native lyophilized BHb+2M HCl+thermal treatment /45°C/60min/+
6	AT_{48}	48	+ CPB
7	ATE_{24}	24	Native lyophilized BHb+2M HCl+thermal treatment /45°C/60min/+
8	ATE_{48}	48	+CPB+AP

*Proteolytic activity of aspartyl proteinase was determined against hemoglobin at pH 3.2 and expressed in units = 800 U/Ml by CHRZANOWSKA at al./in press/.

The concentration of BHb in any of 1-8 treatments was 4.0%.Dissolved BHb modified as described above /Tab.1./was nitrosated by $NaNO_2$ for 24h.The molar ratio of nitrite to hemin and

naturiun ascorbate was 2:1 and 1:2,respectively.Additionally,NaCl and $CaCl_2$ were added to curing brine in concentration of 1.8 M/dcm^3 and 0.3 M/dcm^3,respectively.The nitrosation process was discontinued by freezing BHb samples.The degree of haeme pigment conversion to the nitrosyl pigment was determined according to HORNSEY,1956.The resulted nitrosyl pigments were used for white livex colouring i.e.thermally gelated pig blood plasma processed from stabilized blood according to the patented technology /POLISH PAT.1990,DUDA and JARMOLUK 1985/.Additionally,as a reference,the white livex was coloured using:a/.dinitrosyl ferrohemochrome /SHAHIDI at al.1985/-donated by Dr.L.J.RUBIN and b/.dinitrosyl ferrohemochrome synthesized according to SHAHIDI and PEGG,1988.The pigments used as a reference was coded:DNFH-1,DNFH-2 and DNFH-2'. The experimental and reference pigments were used at 300mg% level /calculated as hemin/apart from DNFH-2'which was also used at 1000mg% level.The coloured white livex physical colour parameters i.e.the dominant wavelength /λ d/,the excitation purity /Pe/ and the luminance /Y/ and the colour stability after 0,1,3,6 and 12 hours of continuous illumination of the samples by fluorescent white light /250Lx/ were determined by reflectance spectrophotometry.

RESULTS and DISCUSSION:The determined degree of the haeme pigment conversion to dinitrosyl pigment,irrespectivelly of the nitrosation time applied was 25.7%,31.4%,37.3% and 32.5% for the variants coded N,E,AT and ATE,respectively.Only AT modification resulted in significantly greater conversion to dinitrosyl pigment in comparison to the modification N.Tab.1.It was also observed that after 48h of preliminary modification the degree of conversion to dinitrosyl pigment for each modification applied /1-8/ averaged 28.2% and was by 7.0% smaller in comparison to that modified for 24h.Our observations suggest that reactivity of the haeme with NO depends on the strength of bonds and/or bonds destruction of globine with haeme,resulting from thermal denaturation and/or enzymic digestion of the globin moiety in the hemoglobin molecule.The solubility of the experimantal variants of nitrosated BHb in blood plasma,apart from that preliminary enzymatically digested, was very unsatisfactory and resulted in sedimentation of the pigments preparations and uneven colouring of the gel formed after plasma pasteurization.Similar phenomena were noticed for DNFH-2 and DNFH-2' and to a lesser extent for DNFH-1,The results of colour physical parameters and its stability in livex coloured with experimental pigments /1-8/ were similar to those determined for both dinitrosyl ferrohemochrome preparations.However,the colour stability of the livex coloured with DNFH-1 and DNFH-2 and 2' was much more stabile than that obtained with experimental pigments.Fig.1-3.

CONCLUSSIONS:

1.The preliminary thermo-acidic denaturation of the BHb results in increased reactivity with NO during nitrosation and concomittant reduction in subsequent pigment solubility.

2.The enzymatically digested BHb subjected to nitrosation could be used for livex and/or processed meat products colouring.

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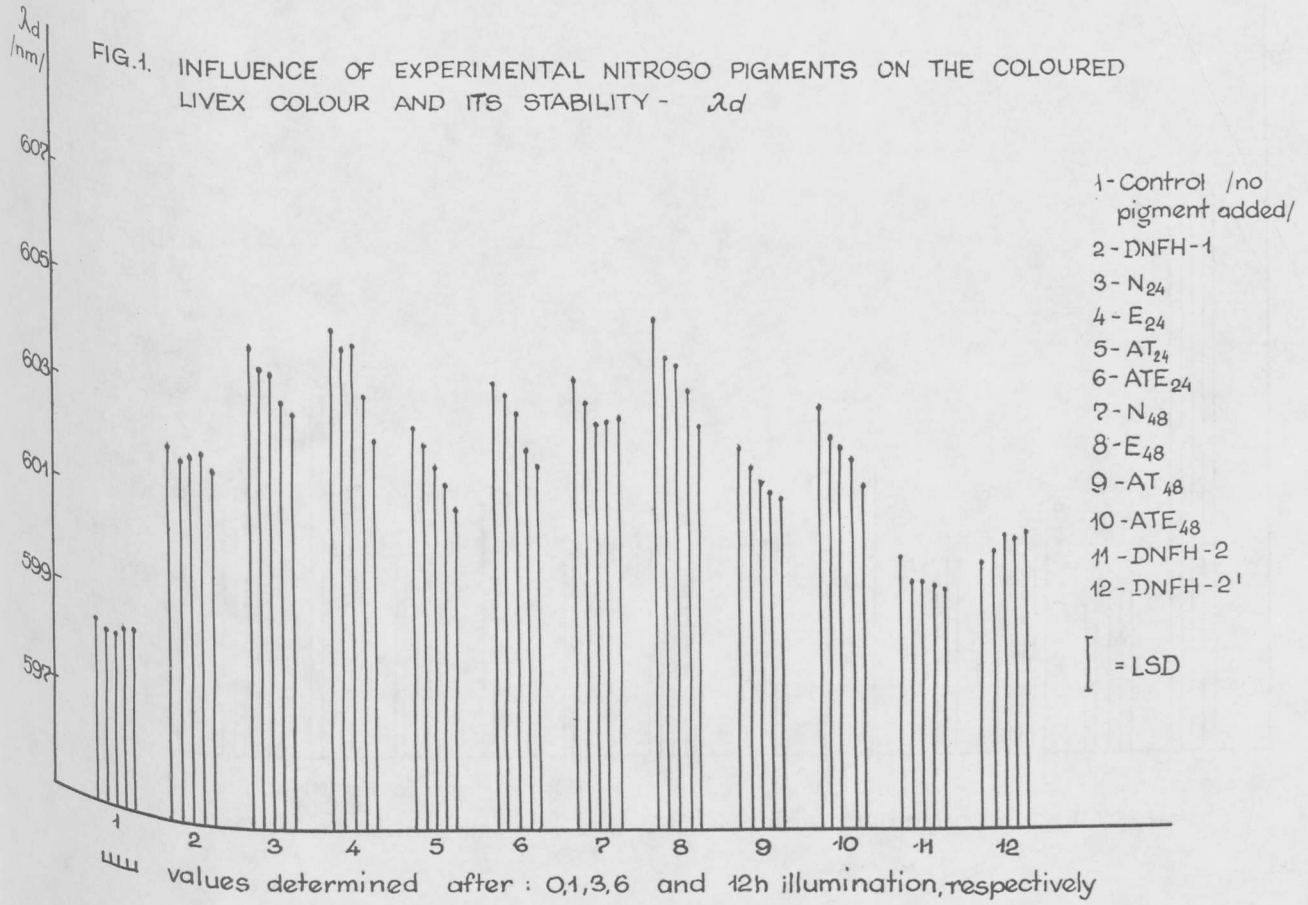
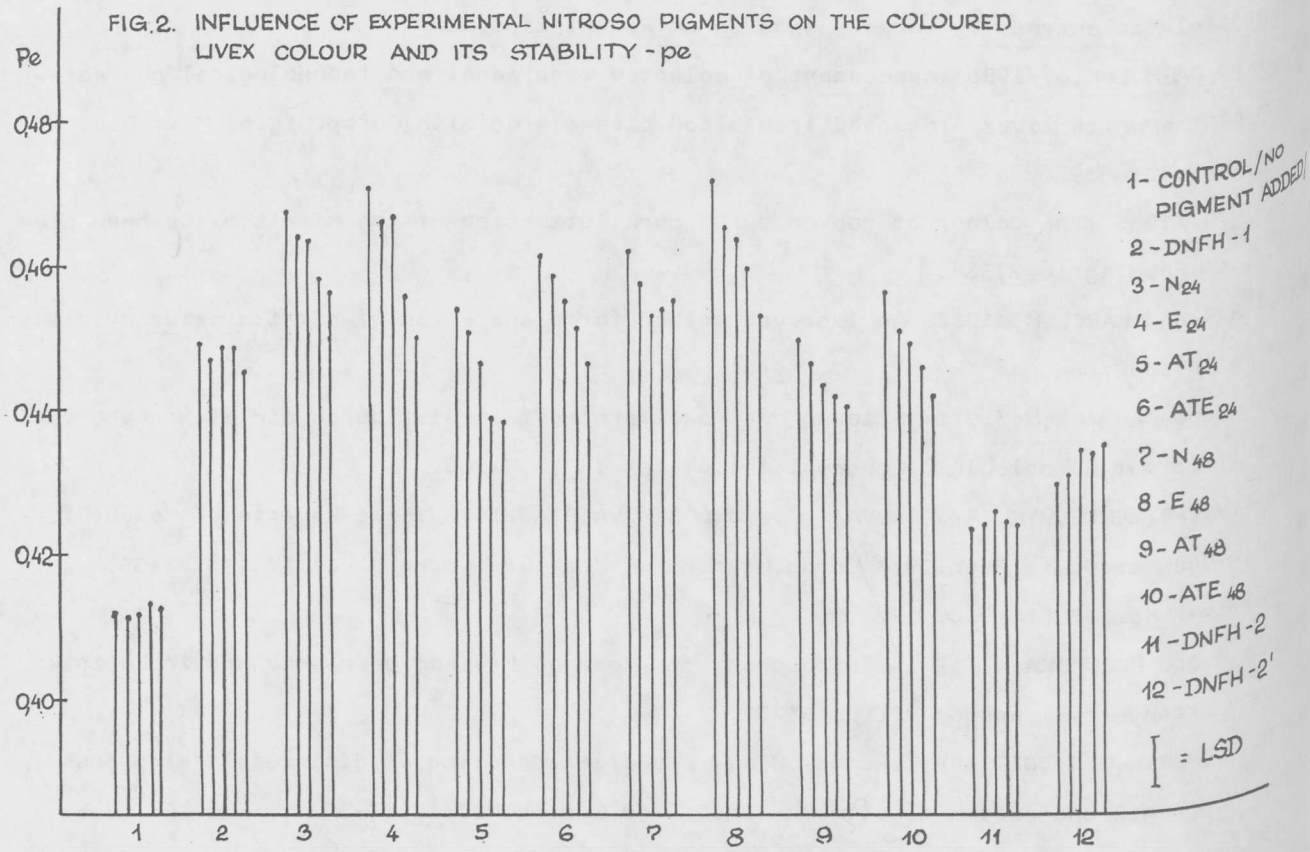
FIG.1. INFLUENCE OF EXPERIMENTAL NITROSO PIGMENTS ON THE COLOURED LIVEX COLOUR AND ITS STABILITY - λd



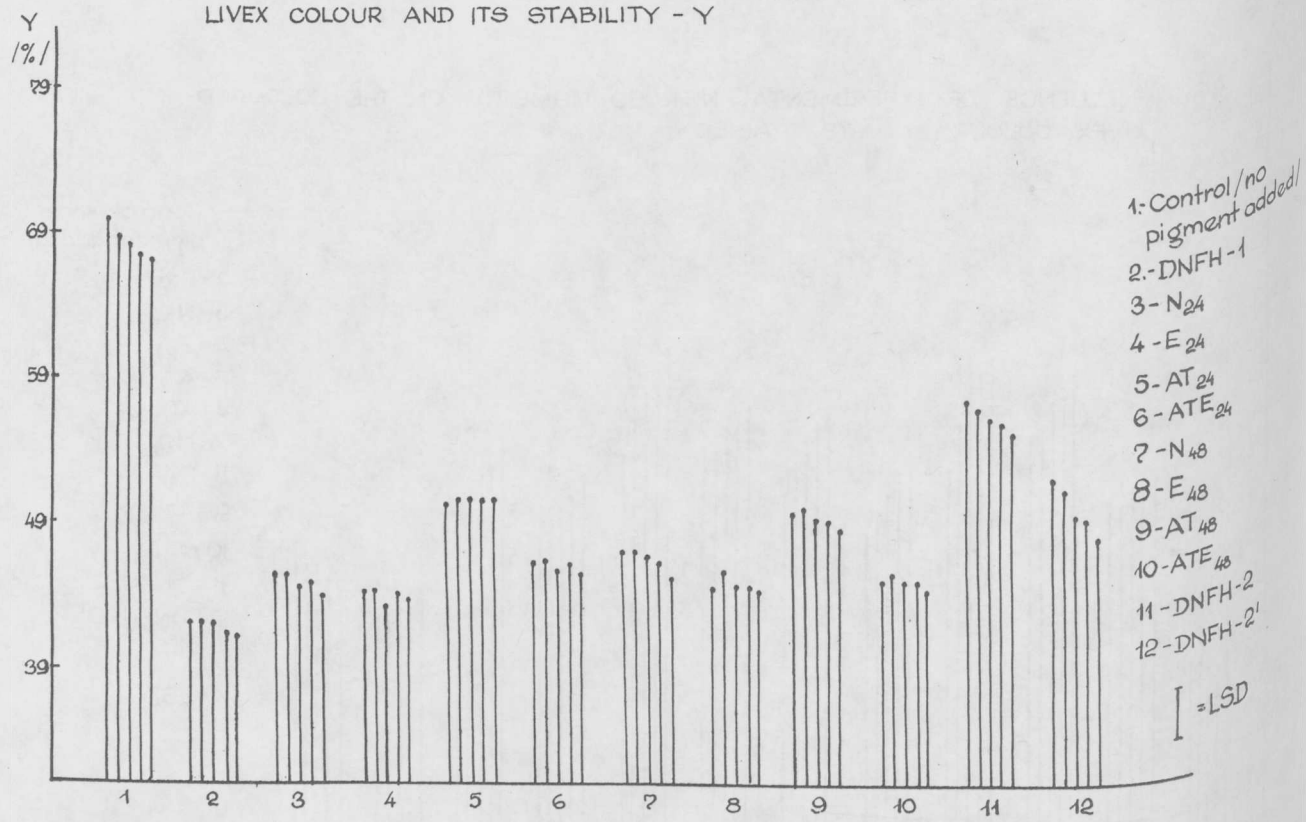
IIII values determined after : 0,1,3,6 and 12h illumination, respectively

FIG.2. INFLUENCE OF EXPERIMENTAL NITROSO PIGMENTS ON THE COLOURED LIVEX COLOUR AND ITS STABILITY - pe



- values determined after : 0,1,3,6 and 12h illumination, respectively

FIG.3 INFLUENCE OF EXPERIMENTAL NITROSO PIGMENTS ON THE COLOURED LIVEX COLOUR AND ITS STABILITY - Y



- values determined after : 0,1,3,6 and 12h illumination, respectively