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DECOLORATION OF BLOOD BY HEM OXIDATION

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SUMMARY

The erythrocyte fraction of animal blood is decolorised by liberation and separation of hem from hemoglobin at approx pH 2.5 and destruction of residual hem by incubation with 0.3 % hydrogen peroxide at 20° for approx 20 hours. The hydrogen peroxide has only little effect on amino acid composition and solubility of the globin which might be used to enrich the protein content of meat products.

INTRODUCTION

The blood from farm animals contains about 18 percent protein and can be regarded as a protein reserve equivalent to 6-7 percent of the lean meat in the carcass. To reap this reserve hygienic blood collecting equipment (Wismer-Pedersen 1979) has in recent years been installed in slaughter plants in various countries. Of the collected blood the plasma fraction is used as a food ingredient. The major protein content of the blood however rests in the erythrocytes which mainly are used as animal feeds because only small concentrations in food products impart an undesirable dark color.

The erythrocytes can be decolorised by removal of the hem group from hemoglobin by acidified acetone (Tybor et al. 1975) or absorption on caboxy-methyl-cellulose (CMC) (Sato et al. 1981, Autio 1983). Alternatively decolorisation can be achieved by oxidative destruction of the heme molecule (Bingold 1949, Oord & Wesdorp 1979, Borchers 1942, Mitsyk & Osadchaya 1970). Hydrogen peroxide is very efficient to destruct the heme molecule but when erythrocytes in the raw material hemoglobin is protected from the impact of hydrogen peroxide by the inherent catalase activity. Inactivation of catalase thus is a prerequisite for the process. In the earlier studies on decolorisation of hemoglobin with hydrogen peroxide the reaction took place at temperatures of 50-70°C. The catalase activity is inactivated at the high temperature but the decolorised globin is completely coagulated and devoid of functional properties. Catalase may however also be inactivated in an acid solution corresponding to pH 2 - 4.5 (Carlo-Bondi & Centamori 1954, Shpitsberg 1965) This paper deals with a method to decolorise erythrocytes by removal of heme from hemoglobin at low pH and destruction of residual heme in the supernatant globin solution by hydrogen peroxide. It thus combines the two above mentioned approaches to decolorise hemoglobin. For the sake of brevity the decolorised erythrocytes are in the following referred to as 'globin'.

MATERIALS AND METHODS

Materials

The raw material for the decoloration studies was the commercial erythrocyte fraction of swine blood, kindly supplied in flake-frozen condition by the STEFF-HOULBERG meat packing company, Ringsted. The erythrocytes were stored at -18°C until use.

Decoloration procedure

Erythrocytes were diluted with tap water to a protein concentration of about 7 % and agitated while 4N HCl was added slowly to give a pH of 2.5 - 3.5. After about 20 minutes the solution was centrifuged at 18000 G for 20 minutes in an International M25 centrifuge. The pH of the supernatant was then adjusted to 4-5 by addition of 2N NaOH and a 35 % hydrogen peroxide solution added to give a concentration between 0.04 and 0.35 %. The solution was incubated at temperatures between 4° and 40°C until the destruction of the heme molecule was complete. After incubation pH of the solution was adjusted to 5 and the protein isolated by freeze-drying. In some of the experiments residual hydrogen peroxide was eliminated by addition of ascorbic acid.

Model sausage

The effect of globin as a meat replacer was studied in an experimental sausage with a standard recipe consisting of 128 g lean pork, 42 g ice-water, 72 g soybean oil and 5 g NaCl. The emulsion was prepared essentially as described by Morrison et al (1971) The emulsion was filled into cylindrical cans (55 mm diameter, 35 mm high) which were heated in a water bath at 75°, 85° and 100°C for 30 minutes.

Analytical methods

Absorbance spectra of the erythrocyte solutions were recorded with a Shimadzu recording spectrophotometer UV-240. Iron content in globin was determined by atom absorption after destruction with nitric acid and the nitrogen content by the Kjelfoss technique. The amino acid composition of globin was determined as described by Mason et al (1980) Emulsion capacity was determined essentially as described by Franzen & Kinsella (1976) The sausage texture was determined with an Instron Material Testing Machine, Universal model 1140 equipped with a plunger of 35 mm diameter. The texture was measured as the force required by the plunger to press the sausage to 30 % of original thickness.

RESULTS AND DISCUSSION

Separation of globin and hem at low pH

When a solution of erythrocytes is acidified the heme molecule is released from the globin and may polymerize to various extent. On centrifugation polymerized heme together with cell debris and adhering hemoglobin precipitate. In table 1 the composition is given of the original erythrocytes and the two fractions after centrifugation. The erythrocyte fraction contains 98 % hemoglobin which consists of 96 % globin and 4 % hemin. The process reduces the iron content of the protein to 0.09 % or about a quarter of the original content. The iron content of lean pork is about 7 ppm or less than 1 percent of that in globin. Even replacement of 1 percent pork in a sausage emulsion with the globin preparation will thus double the pigment content. Further reduction in the pigment content is thus required if significant amounts of globin should be included in meat products without gross change in color. This reduction is effected by oxidation of the heme molecule.

Oxidation of the protophorphyrin ring

Figure 1 shows the effect of hydrogen peroxide incubation on the spectrum of the erythrocyte solution after centrifugation and adjustment of pH to 4. The absorption peak at 395 nm is believed due to absorption of hematin expellet from the globin at pH 2.5 and the peak at 275 nm due to monomeric heme associated with the globin (Polet & Steinhard 1969, Allis & Steinhard 1970) To the absorption peak at 275 nm might also contribute the amino acids tryphophan, tyrosine and phenylalanine which absorb light near 270 nm (Smith 1929) After incubation the two peaks have disappeared and the spectrum has become quite nondescript. The most significant

Table 1. Composition of centrifugate and precipitate from centrifugation of an acedified erythrocyte solution (pH 2.5)

	% N in dry matter	% Fe in dry matter	approximate composition calculated from N and Fe content
erythrocytes	16	0.328	98% hemoglobin (96% globin + 4% hemin)
supernatant from centrifugation	15.7	0.09	94% globin + 1% hemin
precipitate from centrifugation	12.6	1.7	65% globin + 20% hemin

feature of the spectral change appears to be the disappearance of the peak at 395 nm. The effect of the hydrogen peroxide most likely is a cleavage of the protophorphyrin ring into two fragments by oxidation of two opposite methen bridges (Fischer & Dobeneck 1940, Heikel 1958) Figure 2 shows that complete cleavage of the ring requires a hydrogen peroxide concentration of 0.2 - 0.4 percent. Of equal importance is complete inactivation of catalase activity which requires the impact of an acid environment corresponding to pH not above 2.5 for 30 minutes at 20°C, and removal of the cell debris, which in the present work was done by the centrifugation step. It was thus observed that some catalase activity remained in the precipitate. Like hemoglobin catalase is a heme protein. Inactivation of enzyme activity in the solution may be ascribed to denaturation of the protein. The oxidation process is temperature dependent especially with regard to completion of the reaction. This is illustrated in figure 3. After 24 hours the solution incubated at 4°C showed considerably more absorption around 395 nm and visually appeared considerably darker than the solutions at 20 and 40°C. After 48 hours the difference in absorption between the solutions at 4° and 40° is slightly reduced which indicates that the reaction at 4°C proceeds very slowly and requires extended periods to reach completion. A reaction temperature of 20°C is preferred. Higher temperatures will accelerate the reaction but might reduce the solubility of the globin.

Properties of the globin

Incubations of proteins with hydrogen peroxide might result in destruction of the sulfur containing amino acids, especially methionine, in particular at temperatures around 50°C (Cuq et al 1973, Rasekh et al 1972) In order to ascertain if destruction of amino acids occurs under the conditions described above the amino acid composition of globin before and after incubation was determined. The results are given in table 2. It appears that the content of sulfur containing amino acids is not reduced by the hydrogen peroxide incubation whereas the tryptophane and tyrosine content is reduced respectively to about 50 and 70 percent of original. The tryptophane content however is still above the FAO human (child) recommendation of 0.5 g tryptophan per 100 g protein (Arrogave 1974) The instability of tryptophane in acid environment is well known especially in the presence of oxidative substances (Olcott & Fraenkel-Conrat 1947) It may be concluded that the effect of the hydrogen peroxide treatment on the nutritive value of the globin appears insignificant. In figure 4 is shown the solubility of the globin in relation to pH. The solubility curves are typical for acid denatured globin. The hydrogen peroxide treatment has further reduced the solubility at pH 6-7 possibly due to formation of -S-S- bonds between peptides by oxidation of SH-groups. Experience with proteins in egg powders desugared by incubation with glucose oxidase and hydrogen peroxide in amounts corresponding to what have been used in these studies

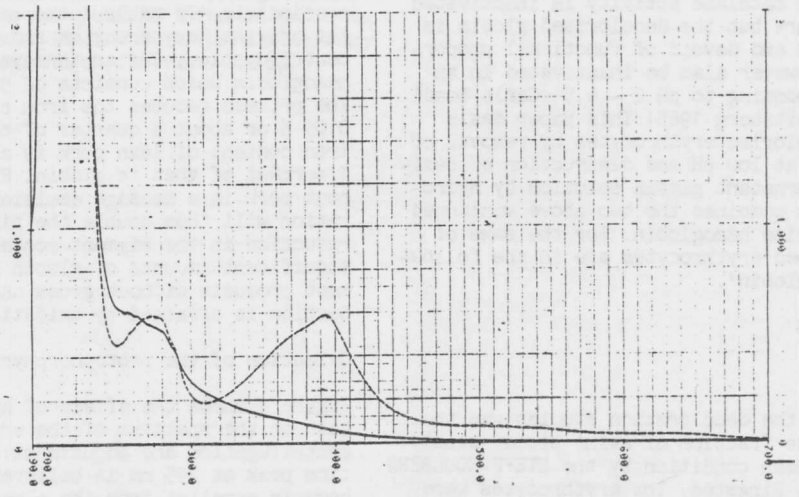

Figure 1. Absorbance spectra of erythrocyte solution before --- and after incubation with 0.35% H_2O_2 in 20 hours at 20°C. ———

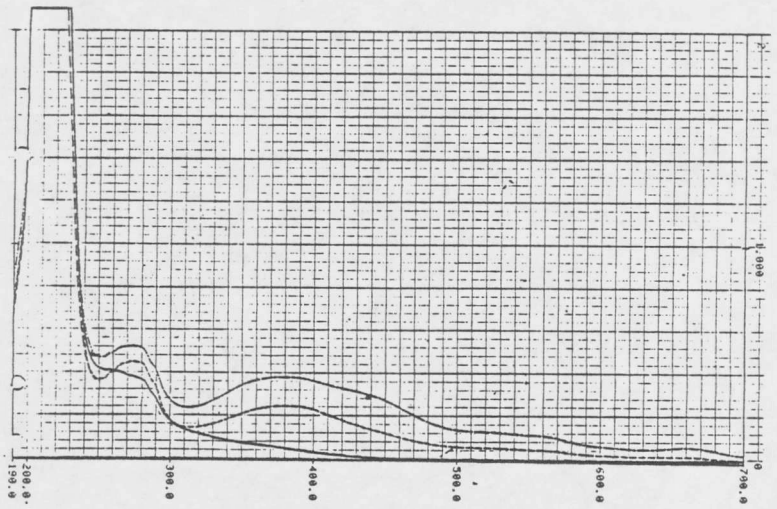

Figure 2. Spectra of erythrocyte solutions incubated with 0.35% ——, 0.04% -.-. and 0.008% H_2O_2 ---- for 20 hours at 20°C.

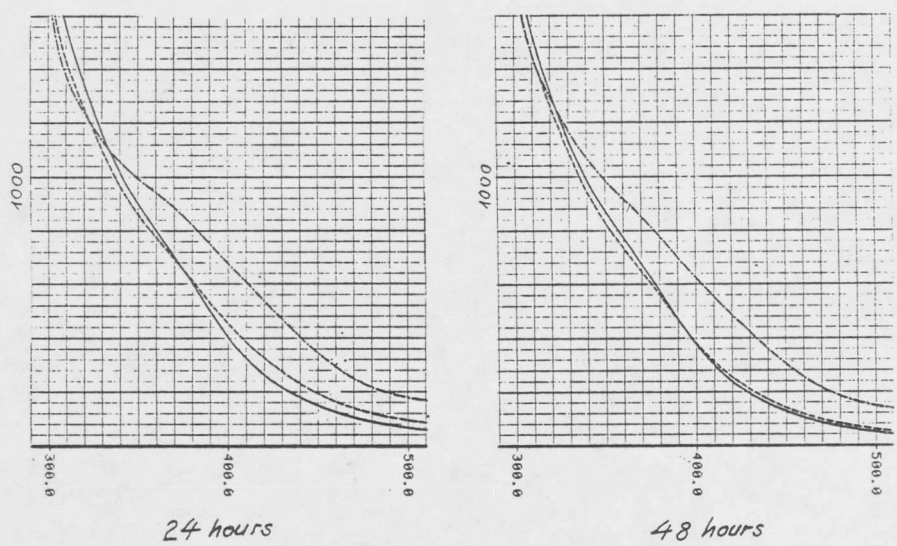

Figure 3. Effect of incubation temperature 40°——, 20°--- and 4°C-·-· on spectra of erythrocyte solutions incubated with 0.35% H_2O_2 after 24 hours and 48 hours.

Table 2. Effect of hydrogen peroxide treatment on aminoacid content of globin.

aminoacid	globin before H_2O_2 incubation gaminoacid/16 g N	globin after H_2O_2 incubation gaminoacid/16 g N
alanine	8.35	8.44
arginine	4.17	4.18
aspartic acid	12.07	12.25
cystein/cystine	0.638	0.641
glutamic acid	7.95	8.14
glycine	4.84	4.90
histidine	8.10	7.95
isoleucine	0.433	0.445
leucine	13.72	13.78
lysine	8.95	8.94
methionine	0.762	0.764
phenylalanine	6.95	6.79
proline	3.64	3.38
serine	4.66	4.66
threonine	2.97	3.00
tryptophane	1.73	0.906
tyrosine	2.11	1.48
valine	10.06	10.24
g aminoacid residues per 16 g N	87.56	86.41

Table 3. Effect of replacement of meat with blood proteins on shrinkage and texture of an emulsion sausage.

protein content in sausage	max. temperature during heat treatment	% shrinkage during heat treatment	texture of cooked sausage as compression force (kg)
Control with	75°C	2.6	3.4
10.37% meat protein	85°	6.0	3.9
	100°	12.7	3.9
9.38% meat protein	75°	4.1	3.3
1.06% globin "			
10.44% total protein	85°	9.3	3.7
	100°	13.6	4.1
7.46% meat protein	75°	3.5	2.4
2.09% globin "			
0.96% plasma "	85°	7.5	3.2
10.51% total protein	100°	12.3	4.2

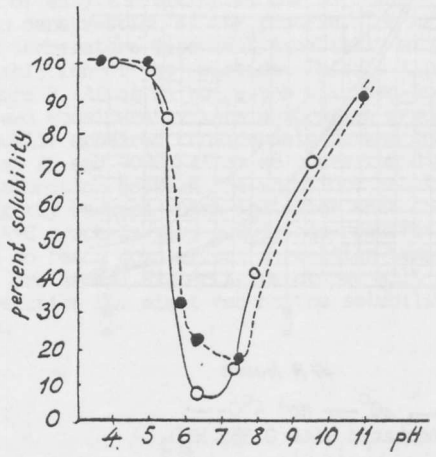

Figure 4. Solubility of globin in water in relation to pH

Before incubation with H_2O_2 ---●---●---

After incubation with H_2O_2 —○—○—

however not revealed effects on protein solubility (Kline et al 1954) In figure 5 is shown the emulsion capacity of globin in relation to meat proteins. In destilled water globin has a high capacity which however is reduced with increasing NaCl concentrations due to decreasing solubility of globin with increasing salt concentration (Autio et al 1984) At 0.6 N NaCl the emulsion capacity of globin is similar to that of the myofibrillar proteins. Although the globin at concentrations around 3-4 % is capable of forming gels on heating as reported by Autio et al (1985) for globin prepared by the CMC method, no gelling at lower concentrations could be detected in this study.

Application of the products from the decoloration process

Of interest for possible industrial use is the precipitate from the centrifugation of the erythrocytes and the decolorised globin. The precipitate contains 20% hemin (table 1) and appears suitable for isolation of hemin which besides dietetic use might be promising for preparation of polymer compounds with special properties like artificial hemoglobins (Dickerson & Cook 1981) and cyanide ion exchangers (Kokufuta, Watanabe & Nakamura 1981) The main product of the process, globin, may be used to enrich food products, especially meat products, with protein, or to replace meat in such products. In table 3 is shown the effect of replacing meat protein in an experimental sausage with globin, and globin + blood plasma in the same protein ratio as they occur in blood. The quality criteria are shrinkage during heating and texture of the cooked sausage. Substitution of 10 % of the meat protein by globin results in increased shrinkage during cooking especially at the lower heating temperatures. No effect on texture of the cooked product was apparent. The higher shrinkage is probably due to the lack of gel forming capacity of the globin. When the globin is combined with blood plasma which furnishes gel forming capacity, replacement of about 25 % of the meat is possible. Same shrinkage and texture of the sausage could thus be produced when the emulsion was heated to 100°C where the gel forming ability of blood plasma is maximum (Wismer-Pedersen 1980).

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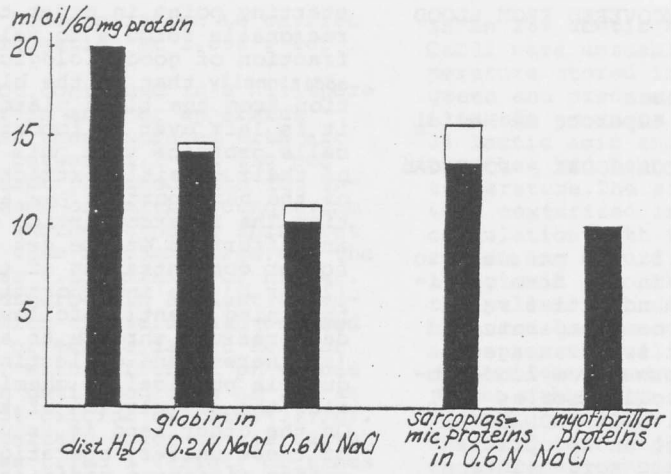

Figure 5. Emulsion capacity of globin at pH 5 compared to meat proteins at pH 6. Total bar indicates emulsion capacity; black portion of bar shows oil remaining in emulsion after cooking at 70°C in 15 min.

REFERENCES

Allis, J.W. & Steinhard (1970) Acid denaturation of carboxyhemoglobin. Protein unfolding without heme detachment. Biochemistry 9, 2286.

Arrogave, G. (1974) Amino acid requirement by age and sex In "Nutrients in Processed Foods. Part 2 Proteins (White, Fletcher, Ellis ed.) Publishing Science Group Action Mass. p.15.

Autio, K. (1983) A new method for globin and heme preparation from blood corpuscle concentrate. Proceedings 29. European Congress of Meat Research Workers II, p. 765.

Autio, K., Kiesvaara, M., Mälkki, Y. & Kanko, S.(1984) Chemical and functional properties of blood globin prepared by a new method. J.Food Sci. 49, 859.

Autio, K., Lyytikainen, H., Mälkki, Y. & Kanko, S. (1985) Penetration studies on blood globin gels. J. Food Sci. 50, 615.

Bingold, K. (1949) Entstehung des Pentdyopents und seine Bedeutung für den Hämoglobinstoffwechsel. Deutsche Arch. Klin. Med. 195, 413.

Borchers, C.W.A. (1942) DE-PS patent 744.055.

Carlo-Bondi & Centamori, M. (1954) Value of morphological structures on catalase activity. Riv. Biol. 46, 261 (cit CA 49, 2549).

Cuq, J.L., Provansal, M. Guilleux, F. & Cheftel, C. (1973) Oxidation of methionine residues of casein by hydrogen peroxide. J. Food Sci. 38, 11.

Dickerson, L.C. & Chien, J.C.W. (1981) Hemin copolymerization with vinyl monomers of potential in artificial hemoglobins. Polymer Preprints 22 (1) 134.

Fischer, H. & v. Dobeneck, H. (1940) Uber die Pentdyopent-Reaktion. Z.physiol. Chem. 263, 125.

Franzen, K.L. & Kinsella, J.E. (1976) Functional properties of succinylated and acetylated soy protein. J. Agric. Food Chem. 24, 788.

Heikel, T. (1958) A paper electrophoretic and paper chromatographic study of pentdyopent. Scand. J. Clin. & Lab. Investigation 10, 191.

Kline, L., Sonoda, T.T. & Hanson, H.L. (1954) Comparisons of the quality and stability of whole egg powders desugared by the yeast and enzyme methods. Food Technology 8, 343.

Kokufuta, E., Watanabe, H. & Nakamura, I. (1981) Preparation of copolymer of styrene with hemin and its functional property as cyanide ion exchanger. Polymer Bulletin 4, 603.

Mason, V.C., Bech-Andersen, S. & Rudemo, M. (1980) Z. Tierphysiol., Tierernährung u. Futtermittelkunde 43, 146.

Mitsyk, V.E. & Osadchaya, I.F. (1970) USSR patent 289, 804.

Morrison, G.S., Webb, N.B., Blumer, T.N., Ivey, F.J. & Haq, A. (1971) Relationship between composition and stability of sausage-type emulsions. J.Food Sci. 36, 426.

Olcott, H.S. & Fraenkel Conrat, H. (1947) J.Biol.Chem. 171, 583.

Oord, A.H.A. van den, & Wesdorp, J.J. (1979) Decoloration of slaughterhouse blood by treatment with hydrogen peroxide. Proceedings 25. European Meeting of Meat Research Workers, p. 827.

Polet, H. & Steinhard, J. (1969) Sequencial stages in the acid denaturation of horse and human ferrihemoglobins. Biochemistry 8, 857.

Rasekh, J., Stillings, B.R. & Sidwell, V. (1972) Effect of hydrogen peroxide on the color, composition and nutritive quality of FPC. J.Food Sci. 37, 423.

Sato, Y., Hayakawa, S. & Hayakawa, M. (1981) Preparation of blood globin through carboxymethyl cellulose chromatography. J.Food Technology 16, 81.

Shpitsberg, V.L. (1965) Isolation and some chemical properties of catalase from human erythrocytes. Biokhimiya 30, 801. (cit CA 63, 16672).

Smith, F.C. (1929) Proc. Roy. Soc. London B 104, 198.

Tybor, P.T., Dill, C.W. & Landmann, W.A. (1973) Effect of decolorization and lactose incorporation on the emulsifying capacity of spray-dried blood protein concentrates. J.Food Sci. 38, 4.

Wismer-Pedersen, J. (1979) Utilization of animal blood in meat products. Food Technol. 33 (8) 76.

Wismer-Pedersen, J. (1980) Vollausnützung von Schlachttierblut bei der Herstellung von Fleischprodukten. Fleischwirtschaft 60, 652.