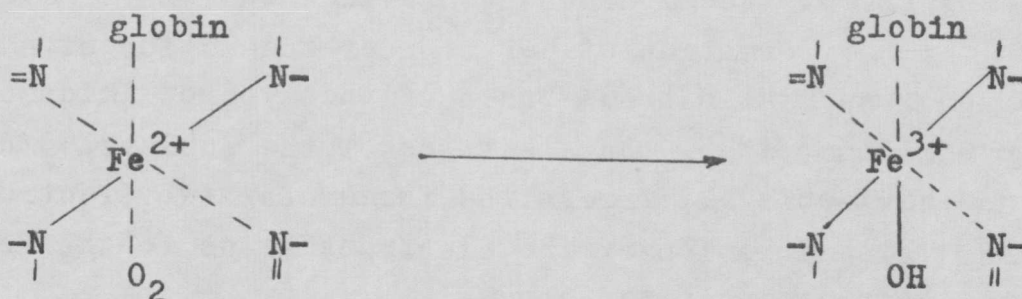


## SOME ASPECTS OF COLOUR FORMATION IN MEAT PRODUCTS

The natural (red) colour of meat fades during storage, and also during sublimation, salting and manufacture of meat products.

The chemical aspect of this process is connected with the dissociation of oxygen from oxymyoglobin and the formation of metmyoglobin.



A multitude of substances are proposed as additions to fresh meat products and food products made from them for preserving their marketable appearance, but none of them give the desired results owing to the formation of harmful byproducts or to a change in colour during storage.

It has been established that the processing of both fresh and salted meat products with strong electron-donor nitrous heterocyclic bases, in particular with imidazole, preserves the colour of meat products (1).

We became interested in the chemical nature of the bond of imidazole with hemin chloride obtained from ox-blood, and the preparation of imidazole-hemochromogen, a stable pigment that stabilizes the colour of meat products. With a view to the fact that in hemoglobin and myoglobin the bond of the iron in the heme with the protein part is due to the imidazole nitrogen of histidine, there should be no formation of undesirable byproducts when imidazole-hemochromogen is present.

It is general knowledge that strong ligand fields and the appearance of reverse pi-bonds with metals facilitate the high thermal and thermodynamic stability of iron complexes, while the presence of conjugated double bonds in a molecule of imidazole and similar heterocyclic bases leads to a blue colour of the comp-

lex compounds. It can be assumed that iron ( $\text{Fe}^{2+}$ ) forms an internal covalent diamagnetic iron-porphyrin coordination complex with imidazole, the latter having a sufficiently strong electron field to facilitate pairing of the electrons on the 3d orbital of iron. The mechanism of this process can be described as follows. The 3d orbital of iron has been paired electrons, and both 3  $\text{dz}^2$  coordination positions of the iron have a bond with the nitrogen of strong electron donor bases. The electrons in the electron shells of Fe are distributed as follows: 4  $\text{S}^2$  electrons of Fe give themselves up for the formation of  $\text{Fe}^{2+}$ . Under the action of a strong electron donor such as nitrous bases of the type of imidazole, purine, pyrimidine, etc., the electrons of the sublevel 3  $\text{dz}^2$  migrate to another sublevel, free in the former for two electrons of the ligand. This migration of the electrons leads to the formation of the complex indicated above.

The genetics of the absorption spectra of the oxidized and reduced forms of myoglobin and hemoglobin were studied, and also of the disodium salts of hemin chloride and imidazole-hemochromogen that we obtained.

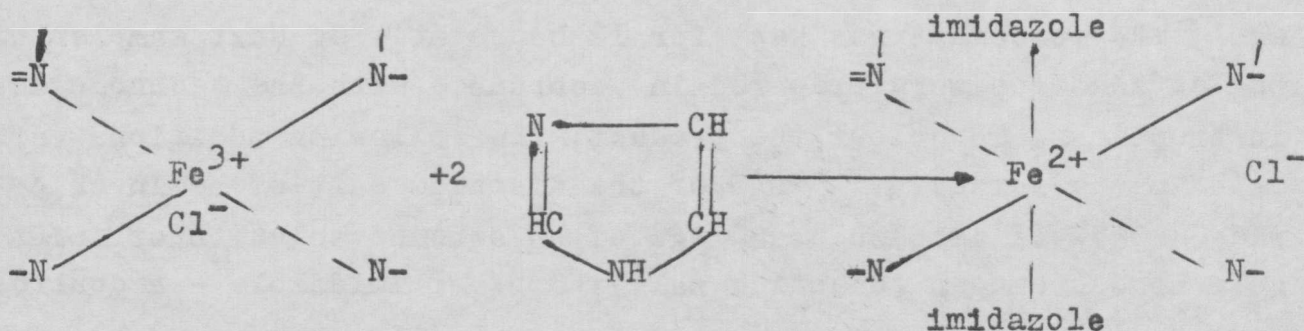
The hemin chloride was prepared according to the method proposed by Shalfeev (2). The following procedure is proposed for transferring in into a water-soluble state.

To a solution of sodium methylate prepared from 0.35 g of metallic sodium and 170  $\text{cm}^3$  of absolute methanol were added 2.1 g (0.035 mol) of hemin chloride and 45  $\text{cm}^3$  of pyridine. Ether was poured into the disodium salt of hemin chloride until precipitation was completed. The precipitate was filtered through a Buchner funnel, washed three times with ether (50  $\text{cm}^3$  a time) and dried during 24 hours in a vacuum desiccator. The yield of the disodium salt of hemin chloride was 1.5 g (75%).

Found: N - 7,84; Cl - 5,0%  $\cdot \text{C}_{34}\text{H}_{32}\text{O}_4\text{N}_4\text{FeClNa}_2$

Calculated: N - 8,02; Cl - 5,09%.

Preparation of the disodium salt of imidazole-hemochromogen.



We have proposed the following procedure to prepare the disodium salt of imidazole-hemochromogen: 0.5 g of imidazole was dissolved in 250 cm<sup>3</sup> of distilled water and 2.5 g of the disodium salt of hemin chloride were added; the solution acquired a dark red colour. The mixture was heated for 30 minutes on a water bath at 58-70°C and was filtered with suction. The filtrate was evaporated until dry at 30-40°C in a vacuum drier and kept until its weight remained constant. The disodium salt of imidazole-hemochromogen was obtained in the form of lustrous dark-violet crystals well soluble in water. The yield was 2,6 g (95%).

Found: N - 13,23; Cl - 4,24% · C<sub>40</sub>H<sub>38</sub>O<sub>4</sub>N<sub>8</sub>FeClNa<sub>2</sub>

Calculated: N - 13,46; Cl - 4,27%.

The absorption spectra of aqueous solutions of the substances being investigated were photographed with a Model Spekord self-registering spectrophotometer. The results are shown in the figure.

The data obtained show a hypsochrome shifting of the bands of the visible region of the spectrum for the disodium salt of imidazole-hemochromogen in the short-wave direction in comparison with the initial disodium salt of hemin chloride. This proves that the iron in the disodium salt of imidazole-hemochromogen is divalent (the absorption maximum is 542 and 578 nm).

We have also investigated the influence of the disodium salt of imidazole-hemochromogen on the colour of meat products. For this purpose samples of boiled sausages were prepared from beef of the highest grade after keeping it for 48 hours at 2-4°C. The meat was minced in a grinder. After the addition of 2,5% of

NaCl, the forcemeat was kept for 12 hours at 4°C. Next samples of boiled sausages were prepared in accordance with the technological instructions. To colour the product, the following additions were used in the forcemeat: 5 mg% of the disodium salt of hemin chloride + 0,5% of imidazole; 20 mg% of an aqueous solution of imidazole-hemochromogen (disodium salt); 0,5% of imidazole - a control sample.

The concentration of the pigment in the product was appraised by the method proposed by Hornsey (3). We introduced a modification to this method, consisting in that the water-acetone extract was measured at a wave length of 543 nm instead of 540 nm. The experiments were conducted three times. The results of the investigations are given in the following table.

T a b l e

Concentration of additions per 100 g of forcemeat	pH		Concentration of pigment (readings of spectrophotometer at wave length of 543 nm)
	before boiling	after boiling	
5 mg% of disodium salt of hemin chloride + 0,5% of imidazole	6,6	6,95	0,22
20 mg% of aqueous solution of imidazole-hemochromogen (disodium salt)	5,9	6,25	0,21
0,5% of imidazole - control sample	6,4	6,9	0,16

A glance at the table shows that samples which the disodium salt of imidazole-hemochromogen or imidazole in combination with the disodium salt of hemin chloride were added to had the best colour indices. Samples which only imidazole was added to were coloured less intensively.

As a result of our investigations we have: (1) developed a procedure for preparing a water-soluble form of hemin chloride by transferring it into the disodium salt; (2) determined the mechanism of the reaction between the disodium salt of hemin chloride and imidazole; (3) proposed a method for the preparation of the disodium salt of imidazole-hemochromogen, the latter stabilizing the colour of meat products; (4) determined the genetics of the absorption spectra of the disodium salt of hemin chloride, the

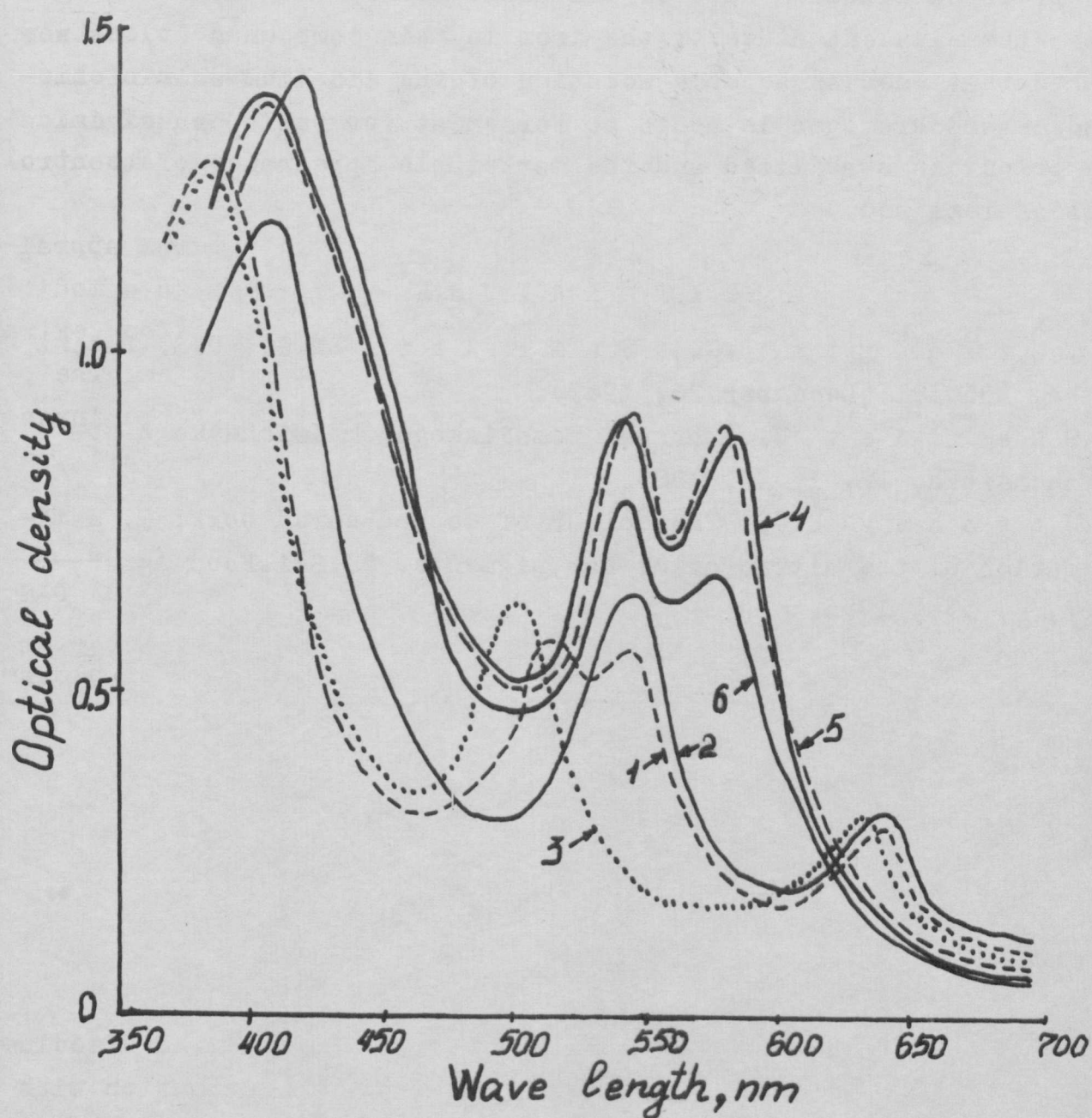


Fig. Absorption spectra of oxidized and reduced forms of myoglobin and hemoglobin, and the disodium salts of hemin chloride and imidazole-hemochromogen:

1 - disodium salt of hemin chloride; 2 - methemoglobin; 3 - met-myoglobin; 4 - oxymyoglobin; 5 - oxyhemoglobin; 6 - disodium salt of imidazole-hemochromogen

oxidized and reduced forms of myoglobin and hemoglobin, and also the prepared disodium salt of imidazole-hemochromogen, which indicates the divalent state of the iron in this compound (5) established that when an aqueous solution of the disodium salt of imidazole-hemochromogen is added to forcemeat for boiled sausages, the colour is stabilized and the marketable appearance of the product is retained.

#### L I T E R A T U R E

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