

DEPLETION OF NITRITE IN HEATED MEAT PRODUCTS DURING STORAGE

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The continuing decrease of the content of nitrite during heating and storage of meat products is a phenomenon which has not yet been explained satisfactorily. It might, however, have consequences for the keepability of heated meat products in view of the inhibitory action of nitrite on the outgrowth of bacterial spores after having been heated (1,2,3). Knowledge of the causes and mechanisms of the nitrite depletion was, therefore, considered to be important as an eventual means to show the way towards measures to reduce this loss as much as possible. Recent discussions about lowering the maximum level of nitrite allowed to be added to meat products, because of the possible formation of carcinogenic nitrosamines during processing or in the stomach after consumption, only add to the importance of an elucidation of the nitrite loss.

Only part of the disappearing amount of nitrite can be accounted for by the formation of nitrosomyoglobin and nitrate. Möhler (4) considers nitrous oxide to be a major product of nitrite breakdown in meat products. Walters (5) observed the formation of nitric oxide, nitrous oxide and nitrogen when a mixture of comminuted meat and nitrite was incubated under anaerobic conditions. We found the same gases in the headspace of an atypical heated meat product of pH 5.9 containing 0.5 % of sodium nitrite. To which extent these gases quantitatively correspond to the amount of nitrite disappeared is largely unknown, just as the mechanism of their formation.

The amount of nitrite lost by way of unknown reactions is larger as the heat treatment is intensified and the storage temperature is raised (6). The depletion is faster according as the pH of the product is lower. The depletion is often imputed to reactions between nitrous acid and proteins and other nitrogenous meat constituents. As far as we know, however, a thorough investigation to verify this assumption has not yet been carried out.

As a contribution to the solution of the problem of the nitrite depletion this paper communicates some results of a quantitative study of the effect of a number of factors on the depletion in heated meat products during storage. These factors are: pH, meat content, heat treatment, storage temperature, SH-groups, ferrous-ions, EDTA, level of added nitrite, sodium chloride concentration and application of vacuum.

2. MATERIALS and METHODS

The material of investigation consisted of finely chopped lean beef, emulsifiers and starch. After having been filled in round 200 g cans the emulsions were heated and subsequently stored generally at 18 °C. Polyphosphate, 4 % starch and in many instances also 2 % caseinate were used to avoid any jelly formation which is of utmost importance for a study of the reaction kinetics of the nitrite depletion.

The content of free nitrite was determined according to the ISO-method (7) with the exception that the extraction took place at room temperature by homogenizing in a Waring blender. Assay of the amount of protein-bound nitrite was carried out with a slightly modified version of method b of Mirna (8). After the addition of mercuric chloride the mixture was heated for half an hour on a boiling water bath and stirred occasionally. The zinc sulphate was added after cooling to room temperature. This modification yielded

somewhat higher results than the original procedure.

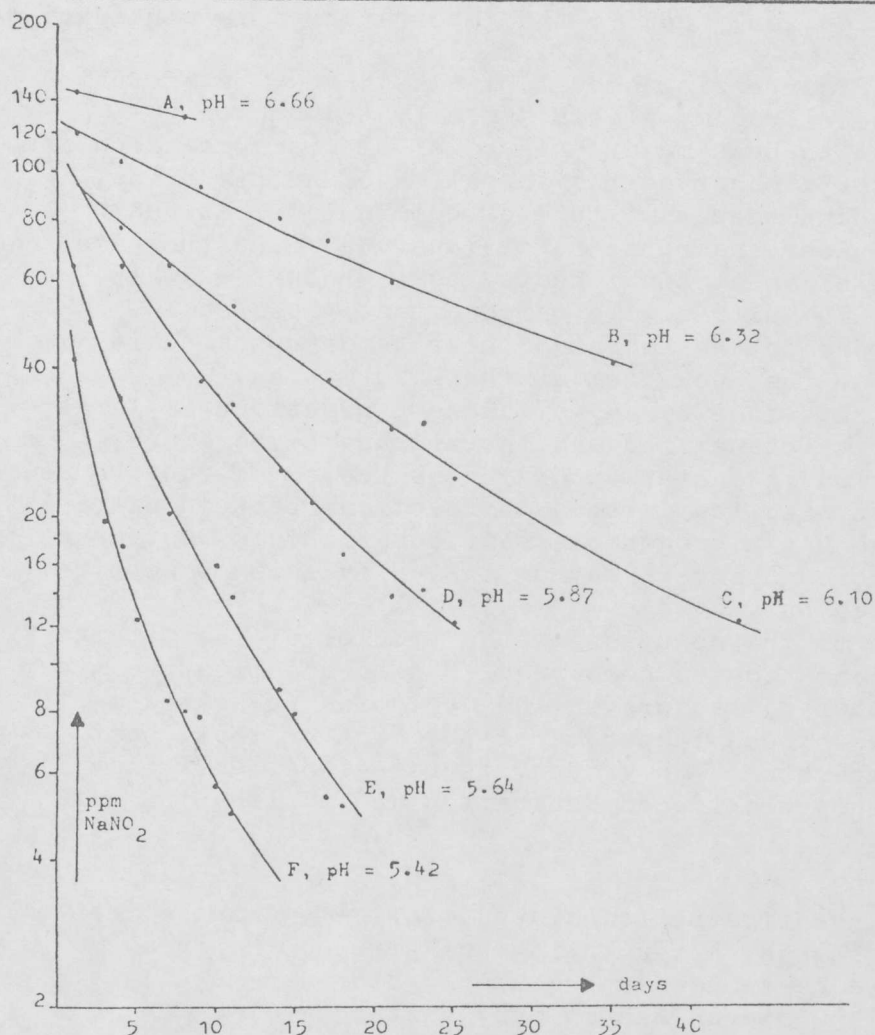
The content of pigment-bound nitrite was calculated from the extinction at 640 nm of an acetone-extract obtained according to Gantner (9). A molecular extinction coefficient of 4800 for acid hematin (10) was used and a 1:1 relation of NO and iron in the haem-pigment molecule was supposed.

3. RESULTS and DISCUSSION

3.1. pH-effect

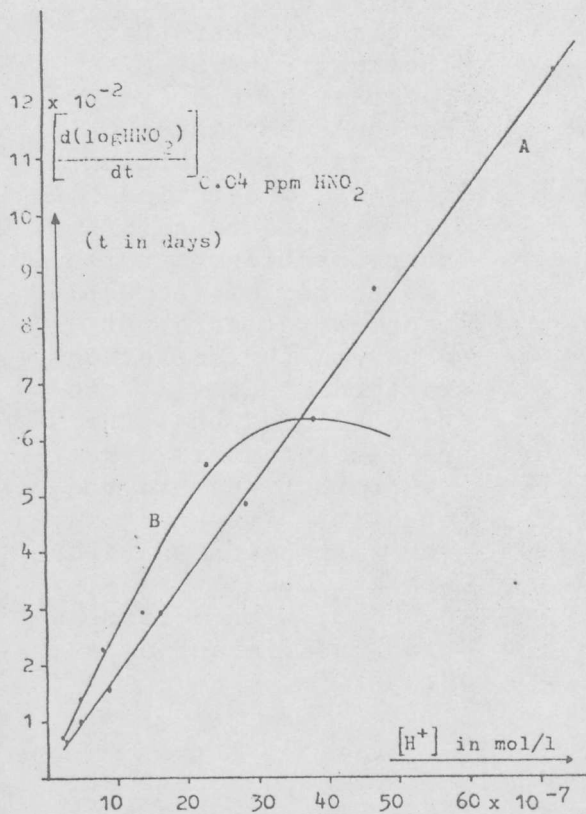
In a series of products of identical composition, which varied only in pH by addition of glucono-delta-lactone, the content of free nitrite was followed for some weeks' storage at 18 °C. The relation between the logarithm of the nitrite concentration and the storage time is convex with respect to the origin (fig. 1). Only at the higher pH-values linearity is approached. The order of the kinetics of the nitrite loss, therefore, is not 1 as often assumed, but lies between 1 and 2. Using the equilibrium constant $K_{\text{HNO}_2, 18^\circ\text{C}} = 5.0 \times 10^{-4}$

Fig. 1. Nitrite Depletion at various pH-values



(11) the nitrous acid concentration can be calculated from the sodium nitrite content, if the H^+ -concentration is supposed to be equal to $\text{antilog}(-\text{pH})$. The curves relating $\log [\text{HNO}_2]$ with storage time naturally have exactly the same course as those for the relation between $\log [\text{NaNO}_2]$ and storage time; they only shift with respect to one another. If their slopes at a fixed HNO_2 -concentration C are plotted against $[\text{H}^+]$ a straight line is obtained under certain conditions, which will presently be commented upon (fig.2, line A). At a given nitrous acid concentration C the logarithmic depletion rate is:

Fig. 2. Relation between Logarithmic Depletion Rate and Hydrogen Ion Concentration



$$V_c = \left\{ \frac{d \log \text{HNO}_2}{dt} \right\} [\text{HNO}_2] = C = k [\text{H}^+]$$

where t is the storage time and k is a concentration-dependent factor. At higher pH-values this factor is nearly constant over a broad range of concentrations (see fig. 1) so that k can be considered as being the depletion rate constant. The change of the HNO_2 concentration with storage time is in that case:

$$\begin{aligned} \frac{d[\text{HNO}_2]}{dt} &= k [\text{H}^+] [\text{HNO}_2] = \\ &= k [\text{H}^+]^2 [\text{NaNO}_2] \end{aligned}$$

It should be mentioned that in many cases, depending on the particular concentration C chosen, the linearity demonstrated in line A is lost at the highest H^+ concentrations. Curve B in fig. 2 is an example of such a case, derived from another experiment. This is a consequence of the fact that the change in the slope $\frac{d \log [\text{HNO}_2]}{dt}$ with storage time progressively increases with decreasing pH. In a low pH product the slope at a certain (low) HNO_2 concentration could, therefore, be even smaller than that at the same HNO_2 concentration in a product of higher pH.

The amount of protein-bound nitrite depends on the pH of the product. It increases as the pH lowers. There is also a slight initial increase with storage time, after which the level remains rather constant.

Table I: Concentrations of free and protein-bound nitrite (as ppm NaNO_2) after different times of storage at 18 °C

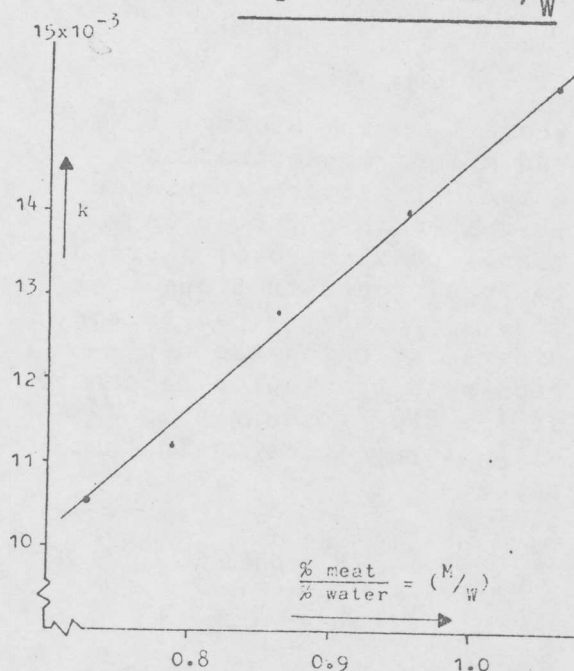
storage time in days	pH=6.32		pH=6.10		pH=5.87		pH=5.64		pH=5.42	
	free	bound	free	bound	free	bound	free	bound	free	bound
1	119	7.0	94.2	7.4	94.8	9.1	64.3	10.9	40.8	13.3
4	105	7.1	76.2	7.2	64.2	9.8	34.8	11.6	15.4	14.5
7	-	-	64.0	7.2	44.4	10.0	20.2	11.4	8.5	13.4
11	87.0	7.7	53.0	8.3	33.6	11.1	13.6	11.5	5.0	13.3
14	79.0	7.0	44.7	7.9	24.7	10.9	8.9	11.7	3.6	12.4
17	71.9	7.8	37.3	8.3	18.0	10.3	5.4	10.2	-	-

Table I demonstrates this behaviour. For comparison the concentrations of free nitrite are given as well.

3.2. Effect of meat content

Fig. 3 pictures the depletion rate of nitrite in a series of products with decreasing meat contents obtained by adding more water. As it may be assumed that the depletion reactions take place mainly in the water phase, the meat content divided by the moisture

Fig. 3. Variation of Rate Constant of Depletion with M/W



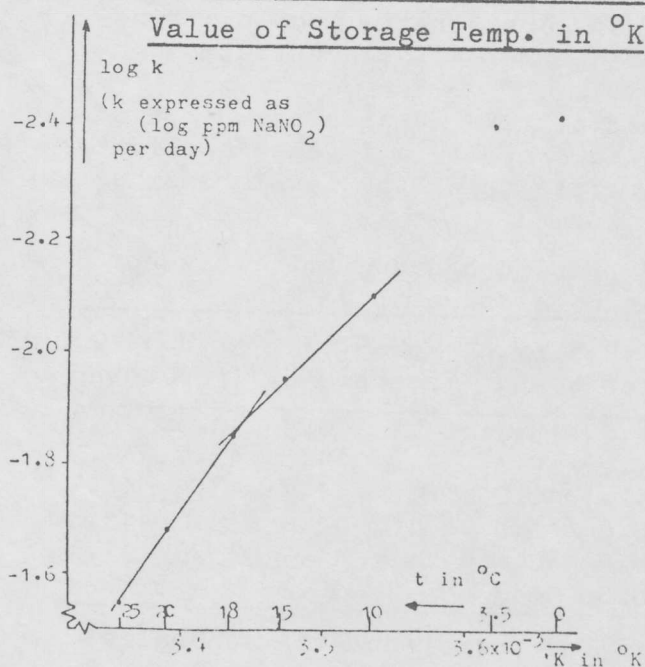
content, M/W , is plotted on W , the abscissa rather than the meat content M . Because the nitrite content decreased approximately according to first-order kinetics in the range of concentrations studied, the slopes of the depletion curves were put equal to the depletion rate constants. It can be concluded that the rate constant is directly proportional to the quantity M/W .

The regression equation was $k = 0.0151 M/W - 0.00048$, with a correlation coefficient of 0.996.

3.3. Effect of storage temperature

The temperature dependence of the rate constant of chemical reactions generally can be represented by the Arrhenius-equation: $\log k = \frac{-2.3 E}{RT} + C$. In fig. 4 the \log (depletion rate constant) is plotted against the reciprocal of the temperature in $^{\circ}K$. Evidently the Arrhenius-equation does not seem to

Fig. 4. Plot of $\log k$ against Reciprocal



apply to the reaction complex of the nitrite depletion. One value of the activation energy for the whole temperature range could not be calculated, therefore. The activation energies for the ranges 10-18 $^{\circ}C$ and 18-25 $^{\circ}C$, however, were calculated as being 2.2 and 3.3 kcal/mole resp. Remarkably, the temperature gradient of the depletion rate constant is considerably smaller at low temperatures (0-3.5 $^{\circ}C$) than at higher temperatures (15-18 $^{\circ}C$). This means that a certain temperature difference at low storage temperatures is far less critical for the nitrite depletion than at higher temperatures.

3.4. Effect of varying heat treatment

The depletion rate increases significantly with the intensity of the heat treatment applied in the preparation of the product.

Table II. Rate constants of nitrite loss after heating at different temperatures

Temp., °C	rate constant $k \times 10^3$
70	12.9 ± 1.7
80	12.1 ± 0.5
90	12.7 ± 0.2
100	14.2 ± 0.7
110	14.9 ± 0.7
120	17.9 ± 1.2

In table II the rate constants of the nitrite disappearance at 18 °C after a 70 minutes heating at various temperatures is shown. As yet, no reasonable explanation can be given for this effect.

3.5. Role of SH-groups

As cysteine was very reactive towards nitrite as compared with other amino acids (12, 13), it was thought that SH-groups - protein bound or not-might play a part in the mechanism of the depletion of nitrite. To investigate this the effect of a blocking of SH-groups on the depletion rate was established. Table III shows that the addition of mercuric chloride and the alkylating

Table III. Effect of SH-reagents on depletion rate and content of protein-bound nitrite

reagent	rate constant, $k \times 10^3$	protein-bound NaNO_2 (ppm)
-	16.0 ± 0.4	3.4
NEM, 18 mmole	12.3 ± 0.9	1.5
HgCl_2 , 18 mmole	9.1 ± 0.9	1.4

reagent N-ethylmaleimide (NEM) indeed suppress the depletion somewhat, but not to such an extent that the SH-groups can be concluded to play a very important part in the mechanism.

The content of protein-bound nitrite being lower in the products containing the reagents than in the control product is to be expected, as the formation of thio-nitrosogroups in the first is obstructed.

In another experiment it appeared that the addition of extra SH-groups in the form of 1 mmole reduced glutathione does not affect the depletion rate considerably either ($k = 0.0138$ vs 0.0126 for the control product).

3.6. Effect of EDTA and ferrous ions

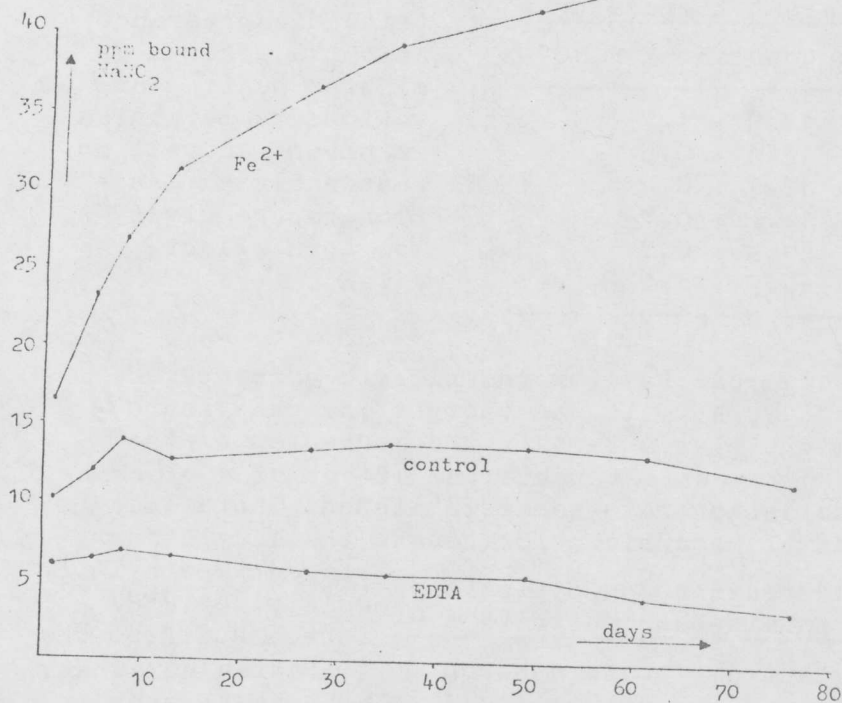
The possibility was considered, that metal traces affect the nitrite depletion by acting as electron transferring agents in the nitrite reduction. In the first place iron comes into consideration, as it occurs in free form in muscle tissue. It appears from table IV that extra ferrous-ions increase the depletion rate sig-

Table IV. Effect of added Fe^{2+} on nitrite depletion rate

addition	rate constant $k \times 10^3$	pigment-bound nitrite (ppm NaNO_2)
-	12.6 ± 0.5	11.4
EDTA, 2 mmole	12.6 ± 0.4	9.7
FeSO_4 , 1.2 mmole	16.8 ± 0.7	11.2
FeSO_4 , (corrected)	12.9 ± 0.5	-

nificantly indeed. In the same product, however, the concentration of protein-bound nitrite is gradually increasing during storage, whereas in the other products it remains rather stationary (Fig. 5). If, however, the falling of the concentration of free

Fig. 5. Change in concentration of protein-bound nitrite during storage



nitrite is corrected for this increase of protein-bound nitrite, the rate constant appears to be equal to that of the control product. This means that the greater depletion rate of free nitrite can be credited completely to the concomitant gradual increase of the protein-bound nitrite. In view of the fact that the depletion rates in the control product and the

products containing ethylenediamine tetraacetic acid (EDTA) are equal as well, the content of endogenous ferrous ions is apparently too low to have any significant influence. The amount of protein-bound nitrite in the EDTA product, on the other hand, is significantly lower than in the control product (fig. 5). This means that the protein-bound nitrite not only consists of nitrite bound in the form of thionitroso-groups (8). The endogenous iron, that is adsorbed by the proteins, evidently binds nitrite in ferrous nitrosyl coordination complexes. The protein-bound nitrite in the EDTA-product, which decreases after a certain storage period, then could be ascribed exclusively to thionitroso-groups. The initial increase and the gradual decline afterwards of the amount of this kind of bound nitrite could possibly imply an explanation for the phenomenon of the curved depletion courses pictured in fig. 1. A slowly attained equilibrium could exist between free and SH-bound nitrite which is subsequently delivering free nitrite when the level of the last has become low after prolonged storage.

Table IV shows furthermore, that EDTA has an unfavourable effect on the formation of the nitroso pigment, which is in accordance with the observations of others (14).

3.8. Other factors

Finally, a number of factors should be mentioned which have been established to have no noticeable effect on the nitrite loss. They are: level of added nitrite, content of sodium chloride and the application of vacuum to the chopper and during the filling of the cans.

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