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CONVERSIONS OF NITRATE AND NITRITE AND THE APPEARANCE OF GREEN DISCOLOURATION IN CURED PORK MEAT

By

A. K. Iskandaryan

According to established standards, the permissible content of nitrate in cured pork meat is 200 mg %, of nitrite - 20 mg %. If the nitrate and nitrite contents of cooked cured pork exceed these standard norms the meat is considered inedible. This is why the question of the accumulation of nitrate and nitrite in meat cured under normal conditions at a temperature of +3 to +4°C has been to a certain degree accentuated.

Quite the opposite is true of literature on the accumulation of nitrate and nitrite on pork cured at higher temperatures (hastened methods of curing). This question has not been studied at all. This may be explained by the fact that the hastened process of curing pork meat - i.e., curing meat under conditions that lead to a simultaneous acceleration of normal colouring and "flavour" - has but recently been developed ("USSR Meat Industry", N 6, 23, 1953. N. Drozdov and A. Iskandaryan).

However, even when normal curing methods are applied, at temperatures of +3 to +4°C, violations of the normal temperature cycle are at times observed, when the temperature is somewhat heightened; thus the question of the accumulation of nitrate and nitrite in meat at heightened temperatures acquires some practical interest even for normal curing conditions. The literature on the question contains some conjectures to the effect that in such cases (particularly for nitrate curing) the accumulation of nitrite in the meat may exceed the permissible level (20 mg %). (Pfizenmeyer, Teitschr. f. Untersuchung der Lebensmittel, 1923, 45, 192; Gorovetz-Vlasova. L. Experimental Study of Bacon Curing, Trade Literature Publishing House, 1931; Vvedensky B., Lavrova L., Pavolv D., "USSR Meat Industry", 1931, 8, 31.)

Contrary to the above-mentioned authors, Ries, Meyer and Miller consider that a higher curing temperature cannot lead to the accumulation of toxic doses of nitrite (Zeitschrift f. Untersuch. der Lebensmittel, 55, 325-353, 1928). However, they also note that if the storage of cured meat is not properly controlled the nitrate may be converted into nitrite.

According to Maassen (Arb. aus dem Genussamt 18, 21-27, 1902) the fact that Ries, Meyer and Miller found only traces of nitrite in meat that was cured in brines containing 2 per cent of nitrate may be explained by assuming that the growth of the denitrifying microbes depends on the nitrate concentration.

As to the possibility of the further reduction of nitrate-nitrite in the curing process - there is an indication of such a fact by L. Jensen (Microbiology of Meats, 1945), who found qualitatively the presence of hydroxylamine analysis in very old curing brines.

In the present work the study of the conversion of nitrate to nitrite in the process of curing meat and the study of the accumulation of these substances was performed by a quantitative method we developed for finding nitrate-nitrite and hydroxylamine

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in their/presence. (Fig. No.1). This method is based on photo-colorimetric analysis of the azo dye that is formed by the interaction of nitrite first with sulphanilic acid and then with ∝-naphthylamine (Gries' reaction); nitrate is defined by its quantitative reduction into nitrite through the agency of zinc dust. Such a reduction leads to the formation of subsequent reduction stages, - to hydroxylamine. Therefore reduction is followed by re-oxidizing the reduction products to nitrite with iodine in an acetate solution in the presence of sulphanilic acid (Blom's reaction). The superfluous iodine is removed by thiosulphate, upon which the resultant diazonium salt combines with the ∝-naphthylamine, and the intensity of the azo dye is established colorimetrically.

When determining nitrate in the presence of nirite and hydroxylamine the latter two must first be completely eliminated from the solution. This is attained by oxidizing hydroxylamine into nitrite, and then eliminating the entire nitrite content by adding sulphanilic acid in the form of gaseous nitrogen obtained through heating diazonium salts.

Hydroxylamine is determined by the same Blom reaction, but first the nitrite is removed from the solution by the above-described method.

Quantitative analysis is interfered with by the presence of chloride ions, but this interference may be eliminated by diluting the solution under investigation 1000-fold.

For the determination of nitrate, nitrite and hydroxylamine by this method in cured meat 5-6 g of cold-ground meat is triturated in a mortar for 20-25 minutes with 20-25 ml of water cooled to +10°C; then the mixture is quantitatively transferred to a 100 ml flask and water is added up to the mark. The flask is agitated 10 to 15 minutes and the liquid filtered through a paper filter. 10 ml of the filtrate or of the brine are placed in a 100 ml flask where the proteins are precipitated by means of 2 ml of a 5 per cent solution of zinc sulphate and 2 ml of 0.3 N barium hydroxide; after water has been added up to the mark the solution is filtered. One sample of this filtrate is used for determining nitrite, another - after the nitrite has been destroyed in it - for determining hydroxylamine. The nitrate content is determined in a third sample, from which the nitrite and hydroxylamine have been removed.

Our first series of experiments was conducted on pork chunks weighing 200 to 250 g, taken from a single carcase and cured for 10 days at a temperature of +18 to +20°C. The meat and brine ratio was 1:1. Sugar was not added. The brines contained 25 per cent NaCl and 2.0, 1.0 and 0.5 per cent nitrate. Parallel experiments were conducted with 0.05 per cent nitrite in the brine.

The cured meat was cooked for 45 minutes at a temperature of 83 to 85°C. Upon cooling (for two hours) the sample was carefully minced at a low temperature, well mixed and weighed in batches for investigation.

The results obtained by one such series of experiments are tabled below (Table No. 1). This table shows that in the pork cured at +18 to +20°C in nitrate-containing brine no more than 6.4mg % of nitrite accumulated, a quantity that does not exceed the permissible limit (20 mg %). Even when there was as much as 2 per cent of nitrate in the brine the nitrate accumulating in the meat did not exceed 195 mg %.

If we compare the amount of nitrite found in pork meat cured in an 0.5 per cent nitrate brine with its amount at 0.05 per cent

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of nitrite in the brine, it will be seen that the amount of the nitrite accumulated in both cases is approximately the same.

The results drawn up in Table No. 1 also demonstrate that at a high nitrate concentration (2 per cent) in the brine, notwithstanding the accumulation of approximately 200 mg % of nitrate in the meat, the amount of nitrite is at a minimum (about 0.55 mg %). On the contrary, a low concentration of nitrate (0.5 per cent) in the brine leads to the accumulation of the highest amount of nitrite in the meat - approximately 6.5 mg %

TABLE NO.1

No. of parallel tests	Nitrate or Nitrite content in brines in per cents.	Cured cooked meat	
		Nitrate in mg %	Nitrite in mg %
1	(Nitrate	195.0	0.55
2	(2.0	194.5	0.56
3	(Nitrate	115.0	2.52
4	(1.0	117.0	2.51
5	(Nitrate	40.0	6.40
6	(0.5	3.94	6.32
7	(Nitrate	none	5.40
8	(0.05	none	5.42

In these experiments the presence of hydroxylamine in the cured pork meat was not discovered, although it had been discovered in the brine in noticeable quantities throughout the entire process. Evidently, the further-reaching reduction processes occur chiefly in the brines, with the participation of microflora. However, it also is possible that in the meat the nascent hydroxylamine is rapidly engaged in further reactions and therefore not discovered.

In order to study the dynamics of the formation of hydroxylamine in brines during the curing process two forms of brine were prepared. The first contained 18 per cent of sodium chloride and 0.5 per cent of sodium nitrate against the weight of the brine; the second contained a similar amount of sodium chloride and 0.05 per cent of sodium nitrite against its weight. Sugar was not added.

Chunks of pork weighing 300 to 350 g that had undergone preliminary ultraviolet irradiation were cured in these brines, in sterilized exsiccators at a temperature of +18 to +20°C. The brines were analysed for hydroxylamine at intervals of 3, 5, 7 and 10 days.

The results of the tests for hydroxylamine in the nitrate brine are drawn up in Table No. 2, for the nitrite brine - in Table No. 3.

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TABLE NO. 2.

ACCUMULATION OF HYDROXYLAMINE IN NITRATE BRINE

No. of parallel tests	Curing period in days.	Quantity of hydroxylamine in mg %	Discovery of nitrite
1	3	0.0392	Discovered
2	3	0.0387	"
3	3	0.0388	"
4	5	0.0176	"
5	5	0.0179	"
6	5	0.0176	"
7	7	0.0112	"
8	7	0.0112	"
9	7	0.0114	"
10	10	0.0549	"
11	10	0.0552	"
12	10	0.0554	"

TABLE NO. 3.

ACCUMULATION OF HYDROXYLAMINE IN NITRITE BRINE

No. of parallel tests	Curing period in days	Quantity of hydroxylamine in mg %	Discovery of nitrate*
1	3	0.0248	Not discovered
2	3	0.0251	" "
3	3	0.0255	" "
4	5	0.0432	" "
5	5	0.0428	" "
6	5	0.0424	" "
7	7	0.0336	" "
8	7	0.0340	" "
9	7	0.0342	" "
10	10	0.0264	" "
11	10	0.0268	" "
12	10	0.0266	" "

* In order to establish the possible oxidation of nitrite into nitrate in the brines we applied our method of determining nitrates, nitrites and hydroxylamine in their joint presence.

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The results drawn up in tables No. 3 and No. 2 make it evident that the formation and accumulation of hydroxylamine in brine sets in from the very first days of curing under heightened temperature conditions, and that it does not disappear from the brine during the entire curing period. The same experiments were the means of establishing that the reduction of nitrate with the formation of nitrite and hydroxylamine takes place in the brine even in the absence of sugar (see Table No.2). On the other hand, the oxidation of nitrite into nitrate under these conditions is not observed (see Table No.3.).

In the tests for discovering hydroxylamine while curing pork under normal conditions (+3 to +4°C) in brine containing 18 per cent - sodium chloride, 0.5 per cent sodium nitrate and 0.5 per cent sugar we limited ourselves to purely qualitative observations.

For these experiments we cured chunks of pork weighing 300 to 350 g in brine that contained 18 per cent sodium chloride, 0.5 per cent sodium nitrate and 0.5 per cent sugar against the weight of the brine. The ratio of the meat and brine was 1:1. The brine was analysed for hydroxylamine over intervals of 15 and 20 days. The results obtained are tabled below.

TABLE NO.4.

THE DISCOVERY OF HYDROXYLAMINE IN BRINES KEPT AT TEMPERATURES OF +3 to +4°C.

No. of parallel tests	curing period in days.	Discovery of hydroxylamine	Discovery of nitrite
1	15	Discovered	Discovered
2	15	"	"
3	15	"	"
4	20	"	"
5	20	"	"
6	20	"	"

The results drawn up in Table No. 4 show that hydroxylamine is formed in the course of the curing process under normal temperature conditions.

Contrary to the statements of L. Jensen (Microbiology of Meat, 1945) to the effect that the formation of hydroxylamine in brines under normal curing conditions occurs only after 60 to 80 days of curing our experiments show (see Tables 2, 3 and 4) that hydroxylamine forms from the very first days of the process. Besides this, our experiments prove that Jensen's assertion that the formation of hydroxylamine occurs only after the complete disappearance of nitrite from the brine to be wrong.

Our further experiments proved that a sufficient accumulation of hydroxylamine in the brine may cause a green discoloration of the meat.

For these experiments we took pork chunks weighing 300 to 350 g that contained some measure of fat. The sodium chloride concentration in the brines was 18 per cent, the hydroxylamine content - 0.0005 per cent, 0.005 per cent and 0.05 per cent against the weight of the brine. To obtain these hydroxylamine concentrations in the brines, corresponding amounts of hydroxylamine hydrochloride were dissolved in the solution of sodium chloride. Sugar was not added. The curing temperature was +18 to +20°C. The pieces of

pork, placed in exsiccators, were sterilized by ultraviolet rays and then covered with the prepared solutions. For each concentration of brine four tests were made. The first test sample was cured 3 days, the second - 5 days, the third - 7 and the fourth - 10 days.

The results are presented in Table No. 5.

TABLE NO.5*

THE FORMATION OF A GREEN DISCOLORATION OF THE MEAT BY HYDROXYLAMINE.

The product under investigation	0.0005% NH_2OH			0.005% NH_2OH			0.05% NH_2OH		
	Curing period in days								
	5	7	10	5	7	10	5	7	10
Muscle tissue:									
1)Before cooking	grey		green	grey		green	dark-green		
2)After cooking	"		"	"		"	"		
Back fat (with no traces of blood).	white			white			white		

* Note: Data for the third day of curing for all concentrations of hydroxylamine: muscle tissue - gray, fat - white.

The experiments in which pork chunks were cured at lowered temperatures (+3 to +4°C), with the same concentrations of hydroxylamine, sodium chloride and the same curing periods showed that the green discoloration acquired by the muscle tissue at +3 to +4°C is more intense than that acquired at +18 to +20°C. The fat is white.

The data expressed in Table No. 5, as well as the results obtained at curing temperatures of +3 to +4°C, prove that hydroxylamine, when its accumulation in the brine is sufficient, leads to an abnormal discoloration of the meat, namely, grey and grey-green hues. The higher the hydroxylamine concentration - the faster and more intensive the discoloration of the meat to grey and green.

Table No.5 also shows that hydroxylamine-containing brine does not cause the back fat to acquite a green colour.

CONCLUSIONS

1. It has been shown that the accumulation of nitrate and nitrite in pork meat cured at a temperature of approximately +18°C in brine containing 2.0, 1.0 and 0.5 per cent of nitrate, does not exceed the permissible norms. When the brine contains 2 per cent of nitrate, the nitrate content in the pork attains its ultimately permissible limit, approximately 200 mg %.

2. At high nitrate concentrations in the brine (2 per cent) the nitrite content in the pork is at its lowest level (about 0.55 mg %); contrariwise, at low nitrate concentrations in the brine (0.5 per cent) a much greater amount of nitrite accumulates in the meat - approximately 6.5 mg %.

3. It has been established that in curing pork meat at +18°C in the absence of sugar, under conditions of comparative aerobiosis, processes not of oxidation, but of reduction take place; the oxidation of nitrite to nitrate does not occur.

4. It has been found that the formation and accumulation of hydroxylamine under both normal and heightened temperature conditions begins within the very first days of treatment, and that the hydroxylamine does not disappear from the moment of its appearance in the brine.

5. It has been found that the appearance of a green and greyish-green discoloration of the cured pork is connected with the formation of hydroxylamine during the reduction of nitrate and nitrite in the course of the curing process.

A definitely noticeable green discoloration appears in the meat at hydroxylamine concentrations of 0.0005 to 0.005 per cent in the brine. At the same time the colouring of the fatty tissue does not change.

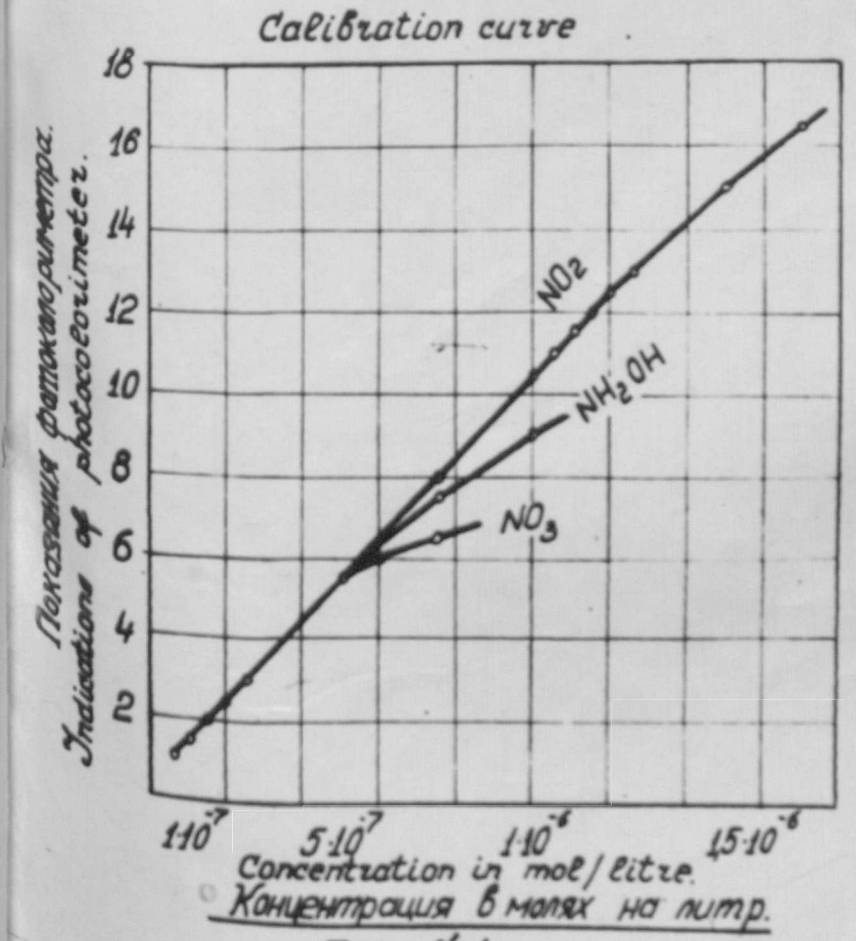
Calibration curve
Показания фотоколориметра.
Indications of photocolorimeter.
18
16
14
12
10
8
6
4
2
NO2
NH2OH
NO3
1·10⁻⁷
5·10⁻⁷
1·10⁻⁶
1,5·10⁻⁶
Concentration in mol/litre.
Концентрация в молях на литр.

Fig. №: 1