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## **ДОСЛІДЖЕННЯ ЗРОСТАННЯ І ТОКСИНОУТВОРЕННЯ ПАЛИЧКИ БОТУЛІЗМУ У ВАРЕНИХ КОВБАСАХ, ЩО МІСТЯТЬ БАРВНИК ІЗ КРОВІ**

**Т.Л. Колесник, А.О. Колесник**

*Досліджено вплив різних концентрацій нітриту натрію, барвника та їх комбінацій на розмноження збудника ботулізму в м'ясному фарші та продукцію його токсину в умовах зберігання готового продукту. У ході дослідження доведено, що нітрит натрію має антиботулінову активність залежно від його концентрації в рецептурі варених ковбас і умов зберігання готової продукції. Зниження вмісту нітриту натрію до 1,5 г і його використання разом із 2% барвника не погіршує мікробіологічних показників якості варених ковбас щодо зростання і токсиноутворення *S. botulinum*.*

**Ключові слова:** варена ковбаса, паличка ботулізму, токсиноутворення, барвник, нітрит натрію, ковбасний фарш, готовий продукт, сорбат, умови зберігання.

## **ИССЛЕДОВАНИЕ РОСТА И ТОКСИНООБРАЗОВАНИЯ ПАЛОЧКИ БОТУЛИЗМА В ВАРЕННЫХ КОЛБАСАХ, СОДЕРЖАЩИХ КРАСИТЕЛЬ ИЗ КРОВИ**

**Т.Л. Колесник, А.А. Колесник**

*Исследовано влияние различных концентраций нитрита натрия, красителя и их комбинаций на размножение возбудителя ботулизма в мясном фарше и продукцию его токсина в условиях хранения готового продукта.*

*В процессе исследования доказано, что нитрит натрия имеет антиботулиновую активность в зависимости от его концентрации в рецептуре вареных колбас и условий хранения готовой продукции. Снижение содержания нитрита натрия до 1,5 г и его использование в сочетании с 2% красителя не ухудшает микробиологические показатели качества вареных колбас относительно роста и токсинообразования *S. botulinum*.*

**Ключевые слова:** вареная колбаса, палочка ботулизма, токсинообразование, краситель, нитрит натрия, колбасный фарш, готовый продукт, сорбат, условия хранения.

## THE RESEARCH OF GROWTH AND TOXIN FORMATION OF BOTULISM BACILLI IN BOILED SAUSAGES CONTAINING COLOR AGENT FROM BLOOD

T. Kolesnyk, A. Kolesnyk

*The effects of different concentrations of sodium nitrite, color agent, and their combinations on the reproduction of the botulism causative agent in mincemeat and products of its toxin in storage conditions of the finished product are investigated.*

*The object of the study is sausage mincemeat of «Stolovaya» variety with addition of various concentrations of sodium nitrite and its combinations with the coloring agent and sodium sorbate. Sausage mincemeat with different concentrations of the ingredients was inoculated with spores of *C. botulinum* at the rate of  $10^3$  microbial bodies per 1 g. During researches the conditions of development of *C. botulinum* in boiled sausages were simulated. Control sample is sausage mincemeat without studied additives, which was inoculated with spores of *C. botulinum*, and subjected to heat treatment similar to the test samples.*

*It is shown that sodium nitrites have anti-botulinus activity in dependence on their concentration in the recipe of boiled sausages and storage conditions of finished products. The decreasing of sodium nitrates content to 1,5 g and its use in combination with 2% of the coloring agent does not make worse the microbiological indices of quality of boiled sausages regarding to growth and toxin formation of *C. botulinum*. At the same time in the sausage mincemeat with traditional concentrations of sodium nitrite (7,5–5,0 g) and in the sausage mincemeat which is recommended by us (1,5 g) in combination with the coloring agent (2%), reliable suppression of toxin formation of *C. botulinum* is only ensured by compliance with sanitary and hygienic requirements for storage (storage at low positive temperatures).*

**Keywords:** *boiled sausage, botulism bacillus, toxin formation, coloring agent, sodium nitrite, sausage mincemeat, finished product, sorbate, storage conditions.*

**Statement of the problem.** The coloring agent from blood of slaughtered animals (carboxyhemoglobin) is developed for the reducing the amount of sodium nitrite which is used for coloring of boiled sausages. Color formation of sausages is achieved by introducing to the recipe of coloring agent (2%) in combination with sodium nitrite in an amount of 1.5 g per 100 kg of meat raw material. The complete elimination of sodium nitrite from recipe of boiled sausages is not possible, because the sodium nitrite has antibacterial activity.

Sodium nitrite has an inhibiting effect on the development of various types of microorganisms and toxin formation, in particular, on the

accumulation of aflatotoxin. The inhibitory action of nitrites on growth and toxin formation of *C. botulinum* has great importance.

In connection with the above mentioned, the studies of growth and toxin formation of botulism bacillus in sausages with reduced amount of sodium nitrite are very important [1].

**Review of the latest research and publications.** The significant problem of use of nitrites in food processing industry is evaluation of their antimicrobial activity and therefore determination of the acceptability of reducing their doses in food products.

The ability of nitrites have an inhibitory effect on the development of various types of microorganisms (*Salmonella*, *Staphylococcus aureus*, fungi) and toxin formation, in particular, the accumulation of aflatotoxin is recognized by most researchers. The inhibitory effect of nitrites on the growth and toxin formation of *C. botulinum* has great importance.

Different doses of nitrites, which may have an inhibitory effect on growth and toxin formation of *C. botulinum* and other microorganisms are pointed out (from 80 to 150 mg/kg) [2].

The effect of the residual content of sodium nitrites in model suspensions from pork during storage up to 6 months, as well as preserving additives in pasteurized meat on *C. botulinum* was investigated. It is shown that the residual amounts of nitrites are not directly related to the ability to inhibit the growth of *C. botulinum* of A and B types. The information about the existence of nonproteolytic strains of *C. botulinum*, which, although are more sensitive to the effects of heat, salt and nitrites, but able to grow and accumulate toxins at temperature below 10°C is particular important.

Considerable amount of researches are devoted to the use of antioxidants, since these substances delay the process of accumulation of nitrosamines through the intensification of the formation of nitric oxide and its compounding with myoglobin. Thus, it is shown that the concentration of 8-hydroxyquinoline in an amount of 1000 mg/g inhibits toxin formation within 60 days of storage at 27°C, even under the inoculation of 8000 cells/g. However, this amount of antioxidant causes the color changes of mincemeat color, but under the combination of antioxidant in an amount of 200–500 mg/kg and 40–80 mg/kg of nitrite the inhibitory effect on the growth of *C. botulinum* toxin formation without discoloration of the samples is marked.

Sorbic acid in an amount 0,2–0,26% to meat in combination with 40 mg/g of nitrite and 550 mg/g of ascorbic acid reduce the toxin formation of *C. botulinum* and microbial spoilage. Sorbitol antimicrobial effect intensifies with increasing of salt concentration from 2,5 to 3,5%, at pH value below 6,0 and at low temperature storage.

The process of introducing of ascorbic acid into the mass of mincemeat under the study of growth and toxin formation of botulinum

bacillus in sausages, color formation in which is ensured by the introduction of coloring agent from the blood of slaughtered animals in combination with reduced concentration of sodium nitrite should be studied [3; 4].

In the USA method for reducing the content of residual nitrite and nitrosamines in meat while anti-botulinus action by using the mixture from salt, nitrite, polyphosphate and special additives with ascorbic acid and its salts was patented [5].

The tendency to increase the binding of nitrite with mioglobin by acidification of the medium led to the investigation of the effect of organic acids on the accumulation of nitrosamines and toxin formation of *C. botulinum*.

The cycle of works are devoted to the use of phosphates in the production of meat products for inhibiting the growth of *C. botulinum*. Sodium acid pyrophosphate in mixture with sodium nitrite effect on the growth and toxin formation of emulsions in the beef and pork sausages; the maximum delay of toxin formation (12–18 days) is caused by combination of 0,4% sodium acid pyrophosphate, 40 mg/kg sodium nitrite and 0,26% of potassium sorbate. The authors suggest that sodium acid pyrophosphate does not effect on the spores germination, but it is effective inhibitor of toxin formation.

These properties of phosphates, we take into account for substantiation of the necessity to introduce them in the recipe of boiled sausages with the coloring agent from the blood of slaughtered animals [6; 7].

**The purpose and objective of the article** is investigation of the effects of different concentrations of sodium nitrite, color agent, and their combinations on the reproduction of the botulism causative agent in mincemeat and products of its toxin in storage conditions of the finished product.

**Presentation of the research material.** The object of the study is sausage mincemeat of «Stolovaya» variety with addition of various concentrations of sodium nitrite (30,0; 15,0; 7,5; 5,0; 1,5 g) and its combinations with the coloring agent (2%) and sodium sorbate (0,2%).

Sausage mincemeat with different concentrations of the above mentioned ingredients was inoculated with spores of *C. botulinum* at the rate of  $10^3$  microbial bodies per 1 g.

Control sample is sausage mincemeat without studied additives, which was inoculated with spores of *C. botulinum*, and subjected to heat treatment similar to the test samples.

The research results which simulate conditions of development of *C. botulinum* in boiled sausages are presented in table 1.

Data presented in table 1 show that in the cold-storage conditions ( $t = +4 \pm 1,0^\circ\text{C}$ ) for 3 and 5 days toxins formation of botulism bacilli is completely suppressed regardless of the content in mincemeat of the sodium

nitrites, sodium sorbate, coloring agent and their combinations. The same situation is in the control non-nitrite samples.

The growth of botulism bacilli in the sausage mincemeat is observed under the same storage conditions ( $+4 \pm 1^\circ\text{C}$ ). The intensity of the growth of botulism bacilli depend on the concentration of sodium nitrite in the mincemeat.

Table 1

**The growth and toxins formation of botulism bacilli in sausage mincemeat with different color-forming additives (storage at temperature  $4 \pm 1^\circ\text{C}$ )**

The composition of color-forming additives in the sausage mincemeat		Storage, three days		Storage, five days	
Name	Concentration	Growth (MPN)	Toxins formation (UA <sub>50</sub> )	Growth (MPN)	Toxins formation (UA <sub>50</sub> )
NaNO <sub>2</sub>	30,0	absent	0	нет	0
NaNO <sub>2</sub>	15,0	$1,2 \times 10^2$	0	$1,2 \times 10^2$	0
NaNO <sub>2</sub>	7,5	$2,0 \times 10^1$	0	$2,0 \times 10^1$	0
NaNO <sub>2</sub>	5,0	$2,0 \times 10^1$	0	$2,0 \times 10^1$	0
NaNO <sub>2</sub>	1,5	$2,0 \times 10^1$	0	$2,0 \times 10^1$	0
NaNO <sub>2</sub> + sodium sorbate 0,2% + coloring agent 2%	1,5	$2,2 \times 10^{11}$	0	$2,2 \times 10^1$	0
NaNO <sub>2</sub> + 2% coloring agent	1,5	$2,2 \times 10^1$	0	$2,2 \times 10^1$	0
Sodium sorbate 0,2% + coloring agent 2%		$2,2 \times 10^1$	0	$2,2 \times 10^1$	0
Coloring agent – 2%		$2,2 \times 10^1$	0	$2,2 \times 10^1$	0
Sausage mincemeat without additives (control sample)		$2,2 \times 10^1$	0	$2,2 \times 10^1$	0

It is noted that during the storage of the finished sausage mincemeat at temperature of  $4^\circ\text{C} \pm 1$  complete inhibition of growth of *C. botulinum* (MPN = 0) is observed only under the concentration of sodium nitrite 30,0 g. Sodium nitrite concentration levels of 7,5–1,5 g hardly retard the growth of *C. botulinum* (MPN =  $2,0 \times 10^1$  –  $2,2 \times 10^1$ ), that prove the growth indices in the control non-nitrite samples (MPN =  $2,2 \times 10^1$ ).

Research results of growth and toxin formation of botulism bacilli in the sausage mincemeat which is stored after the heat treatment at temperature of  $22 \pm 1^\circ\text{C}$ , in conditions which are not in accordance with the requirements for storage of boiled sausages (the temperature is not higher

than 6°C, relative humidity in the range of 95%), for 5-days (period which is twice as long than permissible shelf life of boiled sausages in refrigerated state) are presented in the table 2.

Table 2

**The growth and toxins formation of botulism bacilli in sausage mincemeat with different color-forming additives (storage at temperature 22 ± 1°C)**

The composition of color-forming additives in the sausage mincemeat		Storage, three days		Storage, five days	
Name	Concentration	Growth (MPN)	Toxins formation (UA <sub>50</sub> )	Growth (MPN)	Toxins formation (UA <sub>50</sub> )
NaNO <sub>2</sub>	30,0	0,6×10 <sup>2</sup>	1,72	4,6×10 <sup>2</sup>	10,0
NaNO <sub>2</sub>	15,0	0,6×10 <sup>2</sup>	1,72	5,8×10 <sup>2</sup>	10,0
NaNO <sub>2</sub>	7,5	2,2×10 <sup>2</sup>	3,19	6,9×10 <sup>2</sup>	10,0
NaNO <sub>2</sub>	5,0	3,1×10 <sup>2</sup>	5,62	8,0×10 <sup>2</sup>	10,0
NaNO <sub>2</sub>	1,5	4,0×10 <sup>2</sup>	5,62	8,6×10 <sup>2</sup>	10,0
NaNO <sub>2</sub> + NaNO <sub>2</sub> + sodium sorbate 0,2% + coloring agent 2%	1,5	4,0×10 <sup>2</sup>	3,16	8,5×10 <sup>2</sup>	10,0
NaNO <sub>2</sub> + 2% coloring agent	1,5	4,0×10 <sup>2</sup>	3,16	8,8×10 <sup>2</sup>	10,0
Sodium sorbate 0,2% + coloring agent 2%		4,6×10 <sup>2</sup>	5,62	10,2×10 <sup>2</sup>	17,7
Coloring agent – 2%		6,8×10 <sup>2</sup>	10,0	14,2×10 <sup>2</sup>	31,6
Sausage mincemeat without additives (control sample)		6,9×10 <sup>2</sup>	10,0	16,2×10 <sup>2</sup>	31,6

Data presented in table 2 show that the growth and toxin formation of *C. botulinum* of A, B and E serotypes in the finished sausage mincemeat during storage at 2 ± 1°C were observed in all studied samples, in both test and control samples.

At the same time, there is definite dependence of growth intensity and toxin formation on content and the concentration of sodium nitrite:

– the maximum growth and toxin formation in control non-nitrite samples and in samples which contain coloring agent perse (MPN = 6,8×10<sup>2</sup>; UA<sub>50</sub> = 10) were observed after three days of storage at temperature of 22 ± 1°C; pronounced growth and toxin formation delay compared with control sample (HBЧ = 0,6; EД<sub>50</sub> = 1,72) is observed only in the samples which contain concentrations of sodium nitrite which are not used in

Ukraine during the boiled sausages production (30,0–15,0 g); growth and toxin formation of *C. botulinum* in sausage mincemeat containing both used concentrations of sodium nitrite (7,5–5,0 g) and recommended concentrations of sodium nitrite (1,5 g) are not significantly different among themselves by activity (for example,  $UA_{50} = 3,19 - 5,62$  at  $UA_{50} = 10$  in the control sample);

– the growth and toxin formation of *C. botulinum* in all studied sample were marked after five days of storage at temperature of  $22 \pm 1^\circ\text{C}$ . Thus, some delay in growth and toxin formation compared with the control (non-nitrite) samples was observed in all test samples which contain nitrite.

It should be noted that the assessment of microbiological processes in boiled sausages, which are stored 5 days at temperature of  $22 \pm 1^\circ\text{C}$ , hasn't practical significance, since, by this time test and control products lose their necessary consumer qualities (spoilage symptoms are found out).

**Conclusions.** Thus, it is shown that sodium nitrites have anti-botulinus activity in dependence on their concentration in the recipe of boiled sausages and storage conditions of finished products.

The decreasing of sodium nitrates content to 1,5 g and its use in combination with 2% of the coloring agent does not make worse the microbiological indices of quality of boiled sausages regarding to growth and toxin formation of *C. botulinum* of A, B and E serotypes which vegetate in Ukraine. At the same time in the sausage mincemeat with traditional concentrations of sodium nitrite (7,5–5,0 g) and in the sausage mincemeat which is recommended by us (1,5 g) in combination with the coloring agent (2%), reliable suppression of toxin formation of *C. botulinum* is only ensured by compliance with sanitary and hygienic requirements for storage (storage at low positive temperatures).

#### Список джерел інформації / References

1. Переработка и использование побочных сырьевых ресурсов мясной промышленности и охрана окружающей среды : справочник / под ред. А. Б. Лисицына. – М. : ВНИИМП, 2000. – 230 с.

Lisitsyna, A.B. (ed.) (2000), *Processing and use of by-product raw materials of the meat industry and the environment [Pererabotka i ispolzovanie pobochnykh sur'evukh resursov maysnoy promuschlennosti i okhrana okrugaushei sredy]*, VNIIMP, Moscow, 230 p.

2. Спиричев В. Б. // Вопросы питания. – 2006. – № 5. – С. 45–54.

Spirichev, V.B. (2006), *Problems of nutrition [Voprosu pitaniya]*, No. 5, pp. 45-54.

3. Чапуу, М.К. (2004), *Br. Med. G.*, Vol. 308, pp. 1081-1082.

4. Блажевич Н. В. Космическая биология / Н. В. Блажевич, В. Б. Спиричев. – 2002. – № 2. – С. 34–40.

Blageevich, N.V., Spirichev, V.B. (2002), *Astrobiology [Kosmichna biologiy]*, No. 2, pp. 34-40.

5. Безнітритне виробництво ковбасних виробів: перспективи розвитку : монографія / Л. В. Молоканова, А. А. Квасніков, О. О. Орешина, Я. А. Попова. – Донецьк : Ноулджд, 2014. – 192 с.

Molokanova, L.V., Kvasnikov, A.A., Oreshyna, O.O., Popova, Ya.A. (2014), *Nitrates production of sausages: perspective of development* [Beznitrytne vyrobnytstvo kovbasnykh vyrobiv: perspektivu rozvytku: monohrafiia], Knowledge, Donetsk, 192 p.

6. Баженова Б. А. Формирование окраски вареных колбас с биологически активной добавкой / Б. А. Баженова, Г. Н. Амагзаева, М. Б. Данилов // Мясная индустрия. – 2011. – № 12. – С. 46–48.

Bazhenova, B.A. (2011), “Formation of color of cooked sausages with a biologically active additive”, [“Formirovanie okraski varennykh kolbas s biologicheskoi aktivnoy dobavkoy”], *Meat industry*, Vol. 11, pp. 46-48.

7. Козлова Т. А. Исследование влияния пищевых красителей природного происхождения на физико-химические свойства вареных колбасных изделий / Т. А. Козлова // Russian Journal of Agricultural and Socio-Economic Sciences. – 2012. – No. 2 (2). – С. 34–39.

Kozlova, T.A. (2012), “Investigation of the effect of food dyes of natural origin on the physicochemical properties of cooked sausage products” [“Issledovanie vliyaniya pishchevyykh krasiteley prirodnoho proishozhdeniya na fiziko-himicheskie svoystva varennykh kolbasnykh izdeliy”], *Russian Journal of Agricultural and Socio-Economic Sciences*, No. 2 (2), pp. 34-39.

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