PE4.120 An attempt to employ Staphylococcus carnosus ATCC 51365 in the intensification of meat curing process 445.00

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***Abstract*. The conducted studies showed that during curing of meat, 10-40% of the added nitrite might be a subject of oxidation to nitrates. In raw products, nitrates may be reduced by denitrifying bacteria [4]. In case of heat-treated products, it is not possible due to a relatively low initial number of denitrifying bacteria.**

It may be supposed that the limitation of available nitrite, being caused by its conversion into nitrate during meat curing process may have a significant effect on stability of colour of meat product, which are heat treatment.

The aim of the work was to employ the strain of denitrifying bacteria which produce nitrate reductase enzyme in the intensification of meat curing process.

The submitted results constitute the first stage of the work aimed at determination of the effectiveness of sodium nitrate and nitrite reduction by *Staphylococcus carnosus* ATCC 51365 strain.

In the work, the effect of temperature, time and quantity of the added strain and of sodium chloride on the effectiveness of sodium nitrate and nitrite reduction was examined.

The obtained results of the studies indicate the potential possibility of employing the discussed strain in meat batters in order to intensify the process of curing, with the appropriately modified process of production of meat product.

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Index Terms: nitrites, nitrates, meat products, Staphylococcus carnosus

I. INTRODUCTION

At present, in the European Union countries, including Poland, it is allowed to employ nitrite curing in meat products, subject to heat treatment [2, 8]. Nitrite is introduced to a product in a form of curing salts, mixture of edible salt with sodium or potassium nitrite (NaCl – 99.4%; Na/KNO_2 – 0.6%) [8, 12].

The basic reaction of the curing process, in result of which the characteristic colour of cured meat is developed, is nitrosylmyoglobin formation. During the discussed reaction, nitrogen oxide replaces water molecule (or oxygen, depending on the form of color's occurrence), being joined to iron in heme part of myoglobin, forming nitrosylcomplex in which iron remain on +2 degree of oxidation. The resulting nitrosylmyoglobin, as affected by heat treatment, is converted to nitrosylmyochromogen [7, 14]. One of the stages of nitrosomyoglobin formation includes reactions, leading to nitrogen oxide production from the added nitrites [7]. During curing of the meat, the substances are added with the aim to increase the effectiveness of the process. They may be classified into two categories: pH-lowering substances (citric acid, and glucono-delta-lactone) and reducing substances (ascorbic acid and its salts, and iso-ascorbic acid and its salts). The reducing substances accelerate considerably curing, causing reduction of nitrite to nitrogen oxide [10, 11]. In the studies of Cassen et al. on balance of nitrite, added to the meat during curing process, it was found that only 5 -15% nitrite are bound with meat colors (myoglobin and haemoglobin), 1-10% are converted into nitrates, 5-20% remain as a free form, 1-5% are released as gas, 1-15% are bound with –SH groups, 1-15% with proteins and 1-15% with fats [1, 4]. On the ground of the studies conducted by Dederr, concerning nitrate and nitrite residues in meat products, Honikel found that the amount of nitrite which may be converted into nitrates may be higher and constitute 10 – 40% [4]. In connection with many competitive reactions in meat, it is necessary to employ few times higher additive of nitrite during curing process than it would result from the amount bound by muscular dyes [1]. In raw products, the nitrates, resulting from the added nitrites may be reduced by denitrifying bacteria [4]. In case of the products subjected to heat treatment, when taking the relatively low initial count of denitrifying bacteria, it is not possible.

Improvement of hygiene level during slaughter of animals and carcass dressing and also, lower and lower temperatures, employed in manufacturing rooms, restrict development of favourable microflora, occurring in the meat [9].

It may be supposed that limitation of available nitrite, being caused by its oxidation to nitrate during meat curing process may have a significant effect on colour stability of meat product, which are heat treatment.

The aim of the work was to try to employ *Staphylococcus carnosus* ATCC 51365 strain, producing nitrate reductase in order to intensify meat curing process in the heat-treated products. The submitted results constitute the first stage if work aimed at determination of the effectiveness of sodium nitrate and nitrite reduction by the strain *Staphylococcus carnosus* ATCC 5136. In the study, the effect of temperature, time and amount of the strain added as well as of sodium chloride on the rate of sodium nitrate and nitrite reduction, was examined.

II. MATERIAL AND METHODS

In the studies, there was employed the strain *Staphylococcus carnosus* ATCC 51365 (The American Type Culture Collection), producing enzyme – nitrate reductase, being isolated from dry sausage. The experiment was carried out in a model system. Liquid medium TSB (Difco, USA) with the following composition was used (g/l): 17g of bio-trypcase, 3g of bio-soyase, 5 g of sodium chloride, 2.5 g of dipotassium phosphate, 2.5 g of dextrose and 970 g of water (pH=7.30).

To determine the effect of temperature on the effectiveness of sodium nitrate and nitrite reduction by *Staphylococcus c*., the following experiment was carried out: To the test-tubes with the liquid TSB medium (9 ml), *Staphylococcus c*. ($1.5*10^6$ CFU/g) and nitrate or sodium nitrite (100 mg/l) were introduced. Incubation was conducted at temperatures of 4, 10, 12, 15, 20, 30, 40, 42 and 45^{o}C during 24h. The examined culture was not added to the control samples. After incubation, the content of sodium nitrate and nitrite and the number of *Staphylococcus c*. in the samples, was determined. The experiment was performed in two repetitions.

In order to determine the effect of the incubation time and of the level of *Staphylococcus c*. addition on the degree of sodium nitrate and nitrite reduction, the following experiment was carried out: To the test-tubes with the liquid TSB medium (9 ml), three different levels of *Staphylococcus c*. (sample 1 –$1.7* 10^6$ CFU/g; sample 2 – $3.4* 10^6$ CFU/g and sample 3 – $5.1*10^6$ CFU/g) and sodium nitrate or nitrite (100 mg/l) were introduced. Then, the samples were incubated for 0, 2, 4, 6 and 24h at temperature of 30^{o}C. After incubation, the content of sodium nitrate and nitrite and the count of *Staphylococcus c*. bacteria in the tested samples were determined. The examined culture was not added to the control samples. The level of sodium nitrate and nitrite was determined before and after incubation. The experiment was carried out in two repetitions.

In order to determine the effect of the 2% addition of sodium chloride on the rate of sodium nitrate and nitrite reduction and on the *Staphylococcus c*. growth, two parallel series of the tests were carried out. In the first series, to the test tubes with TSB medium (9 ml, sodium chloride content in medium equal to 0.5%), the examined strain ($3.1*10^6$CFU/g) and sodium nitrate or nitrite (100 mg/l) were added. The samples were incubated for 0, 2, 4, 6 and 24 h at temperature of 30^{o}C. In the second series of the tests, sodium chloride was added to the test-tubes so as to obtain its 2% content in the medium. After incubation, the level of sodium nitrate and nitrite and the bacterial counts of the given strain were determined in the samples. The experiment was conducted in two repetitions.

The cultures used in the tests were stored in glycerol solution at temperature of -18^{o}C. Before each experiment, the culture was thawed in a liquid TSB medium during 20h at temperature of 30^{o}C. For particular experiments, the specified amount of culture from the second subculture was collected.

In the study, the following analytical methods were employed: determination of nitrites and nitrates – HPLC; determination of the number of *Staphylococcus c*. bacteria – plate count method.

III. RESULTS AND DISCUSSION

On the ground of the obtained results, sodium nitrate reduction by *Staphylococcus c*. strain in the samples, incubated at temperatures of 20, 30, 40, 42 and 45^{o}C was found (Tab.1). In the samples which were incubated at 30, 40 and 42^{o}C, the highest 100% degree of reduction was obtained. The mean content of the resulting residual nitrates in the discussed samples was contained within the range of 25.0 – 33.0 mg/l. In the samples which were incubated at 4, 10, 12 and 15^{o}C, any activity of the culture was not recorded (Tab.1).

Tab.1. Effect of temperature on degree of sodium nitrate reduction by *Staphylococcus carnosus* ATCC 51365 strain during 24h incubation

S	S.c.	Incubation temperature [^oC]	*$NaNO_2$ [mg/l]	*$NaNO_3$ [mg/l]
C	-	30^{o}C	0.0	80.4
1	+	4^{o}C	0.0	80.4
2	+	10^{o}C	0.0	80.0
3	+	12^{o}C	0.0	79.3
4	+	15^{o}C	0.0	83.9
5	+	20^{o}C	12.7	44.9
6	+	30^{o}C	25.0	0.0
7	+	40^{o}C	27.8	0.0
8	+	42^{o}C	33.0	0.0
9	+	45^{o}C	57.2	19.8

S - number of sample
+/ addition of *Staphylococcus c.* on the level of $1.5*10^6$ CFU/g
-/no additive, */ determined mean values

The studies revealed that *Staphylococcus c*. strain reduced sodium nitrite in the samples, incubated at 20, 30, 40 and 42^{o}C. In the samples which were incubated at temperatures of 30^{o}C, the highest, 65% degree of reduction was found. In the samples incubated at 4, 10, 12 and 15^{o}C, any reduction of sodium nitrite was not recorded (Tab.2).

Tab. 2. Effect of temperature on degree of sodium nitrite reduction by *Staphylococcus c*. ATCC 51365 during 24h incubation

S	S.c.	Incubation temperature [^oC]	*$NaNO_2$ [mg/l]	*$NaNO_3$ [mg/l]
C	-	30^{o}C	110.2	0.0
1	+	4^{o}C	105.9	0.0
2	+	10^{o}C	105.8	0.0
3	+	12^{o}C	102.8	0.0
4	+	15^{o}C	100.0	0.0
5	+	20^{o}C	69.5	0.0
6	+	30^{o}C	39.5	0.0
7	+	40^{o}C	68.8	0.0
8	+	42^{o}C	57.9	0.0
9	+	45^{o}C	98.5	0.0

S - number of sample
+/ addition of *Staphylococcus c.* on the level of $1.5*10^6$ CFU/g
-/no additive, */ determined mean values

In the incubated samples, the number of bacteria of Staphylococcus c. strain was examined. The increase of the bacterial count of the discussed strain in the samples incubated at 20, 30, 40 and 42^{o}C was found. In the samples which were incubated at 30 and 40^{o}C, the highest increase was recorded (10^8 CFU/g). In the samples incubated at temperatures of 4, 10 and 15^{o}C for 24h, any increase of bacterial count was not observed. The decrease in the number of bacteria to the level of 10^4 CFU/g was found at temperature of 45^{o}C during 24h incubation. Reduction of nitrates by Staphylococci is connected with the production of enzyme – nitrate reductase, the maximum synthesis of which is observed during exponential growth of bacteria [5, 6, 13]. Reduction of nitrites is connected with the production of nitrate reductase by the cells and, as it is given by Neubauer and Gotz, it occurs only and solely in anaerobic or oxygen-poor environment, with the simultaneous access of nitrite or nitrate [5]. The same authors express the opinion that it is probable that the utilization of nitrates and nitrites by Staphylococci as acceptors of electrons in respiratory chain may lead to generation of nitrogen oxide. On the ground of the results contained in Tab.1 and 2, we may state that effectiveness of culture's action is greater in relation to sodium nitrate as compared to sodium nitrite. It has been confirmed by the studies of Gotterup, Olsen et al. who studied the relationship between nitrate/nitrite and activity of reductase of different strains of *Staphylococci* [2].

Tab. 3. Effect of the time of incubation at 30°C and the amount of the added *Staphylococcus carnosus* ATCC 51365 on the rate of sodium nitrate reduction

Incubation time [h]	S	*Staphylococcus c. [CFU/g]	*$NaNO_2$ [mg/l]	*$NaNO_3$ [mg/l]
0	1	[a]$2.3*10^6$	0.0	81.5
0	2	[a]$4.6*10^6$	0.0	81.1
0	3	[a]$6.9*10^6$	0.0	80.3
2	1	$4.3*10^6$	0.0	80.0
2	2	$1.0*10^7$	0.0	81.9
2	3	$1.6*10^7$	0.0	81.9
4	1	$2.1*10^7$	0.0	82.8
4	2	$4.2*10^7$	18.8	65.6
4	3	$8.6*10^7$	31.8	45.0
6	1	$6.6*10^7$	45.1	3.4
6	2	$2.1*10^8$	54.9	0.0
6	3	$3.6*10^8$	8.1	0.0
24	1	$3.1*10^8$	4.2	0.0
24	2	$3.3*10^8$	11.1	0.0
24	3	$4.3*10^8$	11.2	0.0

S – number of sample, */ determined mean values, [a]/the amount of the added *Staphylococcus c.*

Based on the conducted studies, it may be stated that the time of incubation and the amount of the introduced strain has a significant effect on the rate of sodium nitrate reduction by *Staphylococcus c*. strain. The first reduction of sodium nitrate was recorded after 4 hours of incubation at 30°C in samples 2 and 3 (Tab.3). In sample 3, in which the initial amount of culture in the test tube was the greatest, i.e. $6.9 *10^6$ CFU/g, the degree of sodium nitrate reduction was the highest one. After 4h of incubation at 30°C, the discussed strain reduced sodium nitrate from 80.3 mg/l (determined added value) to 45.0 mg/l. After 6h of incubation, the total amount of the introduced sodium nitrate was reduced. In sample 1 where the initial amount of the discussed strain was the smallest, i.e. $2.3* 10^6$ CFU/g, any reduction of nitrate was not found after 4h of incubation. After 6 hours of incubation, the reduction of sodium nitrate to the level of 3.4 mg/l was recorded. In the incubated samples, the growth of *Staphylococcus c*. strain was observed (Tab.3).

The conducted studies showed that the amount of the added strain and incubation time had a significant effect on the rate of sodium nitrite reduction by *Staphylococcus c*. strain (Tab.4). The first reduction of sodium nitrite was observed after 6 h of incubation in all the samples. In sample 1, where the initial amount of the strain was the smallest, i.e. $1.7 * 10^6$ CFU/g, the lowest degree of sodium nitrite reduction was found. After 6 hours of incubation, at temperature of 30°C, the discussed strain reduced sodium nitrite in sample 1 from 105.6 mg/l (determined added value) to the level of 77.1 mg/l. The highest degree of reduction after 6 h of incubation was recorded in sample 3 in which the initial amount of the strain was the greatest, i.e. $5.1 * 10^6$ CFU/g. In the discussed case, the strain reduced the added sodium nitrite to the level of 53.6 mg/l. After 24 h of incubation, the lowest sodium nitrite content: 1.6 mg/kg was determined in sample 3. In the incubated samples, the growth of *Staphylococcus c*. strain was observed (Tab.4).

Tab. 4. Effect of the time of incubation at 30°C and the amount of the added *Staphylococcus carnosus* ATCC 51365 on the rate of sodium nitrite reduction

Incubation time [h]	S	*Staphylococcus c. [CFU/g]	*$NaNO_2$ [mg/l]	*$NaNO_3$ [mg/l]
0	1	[a]$1.7*10^6$	105.6	0.0
0	2	[a]$3.4*10^6$	105.6	0.0
0	3	[a]$5.1*10^6$	105.6	0.0
2	1	$4.6*10^6$	104.3	0.0
2	2	$7.4*10^7$	100.8	0.0
2	3	$1.3*10^7$	105.3	0.0
4	1	$2.0*10^7$	104.0	0.0
4	2	$2.7*10^7$	92.9	0.0
4	3	$6.6*10^7$	100.4	0.0
6	1	$1.3*10^7$	77.1	0.0
6	2	$1.3*10^8$	60.3	0.0
6	3	$3.0*10^8$	53.6	0.0
24	1	$3.8*10^8$	14.2	0.0
24	2	$3.8*10^8$	8.3	0.0
24	3	$4.3*10^8$	1.6	0.0

S – number of sample, */ determined mean values, [a]/the amount of the added *Staphylococcus c.*

The studies revealed the effect of the addition of sodium chloride in the quantity of 2% on inhibition of the growth of *Staphylococcus s*. bacteria, as observed after 2h and 4 h of incubation at 30°C. The number of bacteria, being determined in the samples with the addition of sodium chloride and sodium nitrite (100 mg/l $NaNO_2$+2% NaCl) was lower as compared to those ones obtained in variants with lower sodium chloride content (100 mg/l $NaNO_2$+0.5% NaCl). It indicates the action which inhibits growth of *Staphylococcus c*. bacteria, exerted by sodium nitrite and chloride in the employed concentrations. After 24h of incubation, the number of bacteria in the both

samples was similar. On the other hand, any significant differences in the degree of reduction of the added sodium nitrite in the samples with 0.5 and 2% of sodium chloride were not found.

The studies conducted for the samples which contained 100 mg/l $NaNO_3$+2% NaCl and 100 mg/l $NaNO_2$+0.5% NaCl revealed the effect of higher sodium chloride content on the growth of *Staphylococcus c*. bacteria and on the degree of reduction of the added sodium nitrate. In the samples, containing 2% of sodium chloride and incubated at 4h and 6 h at 30°C, the degree of sodium nitrate reduction and lower count of bacteria was lower than in variants with 0.5% addition of sodium chloride. After 24h of incubation at temperature of 30°C, the number of *Staphylococcus c*. bacteria and the degree of reduction in both samples was found on a similar level.

IV. CONCLUSION

On the ground of the obtained results it was found that *Staphylococcus carnosus* ATCC 51365 strain reduced sodium nitrate and nitrite. The rate of sodium nitrate and nitrite reduction was dependent on the time, temperature and of the amount of the added strain. Reduction of sodium nitrate and nitrite and the growth of the examined strain in the samples, incubated at 20, 30, 40 and 42°C was observed. The rate of reduction was the greatest at temperatures of 30, 40 and 42°C. Any reduction of sodium nitrate and nitrite at temperatures of 4, 10 and 15°C was not recorded. The minimal time necessary for occurrence of sodium nitrate reduction was equal to 4 hours and in case of sodium nitrite – 6 hours. The highest rate of sodium nitrate and nitrite reduction was found in case of the greatest amount of the introduced culture – 5.1 * 10^6CFU/g. It was stated that the examined strain reduced more rapidly nitrate than nitrite. The inhibition of bacterial growth was observed at the addition of 2% sodium chloride and the content of sodium nitrite or nitrate in the quantity of 100 mg/l. Any effect of the 2% addition of sodium chloride on the rate of sodium nitrate reduction was not found.

The obtained results of the studies indicate the potential possibility of employing the examined strain in meat batters with the aim to intensify the curing process at appropriately modified process of production of meat product.

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