NITROSOMYOGLOBIN FORMATION IN CROUD BEEF AFTER ADDITION OF NICOTINAMIDE ADENINE DINUCLEOTIDE

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Summary

Nitrosomyoglobin formation in ground beef after addition of nicotinamide adenine dinucleotide/ NAD / is studied.The described stimulating effect of NAD can not be influenced by the addition of substrates of citric acid cycle,but depends on pH and concentration of the co-factor.NAD-ase activity of meat limits rapidly the accelaratig effect of NAD.

FORMATION DE NITROSOMYOGLOBINE DANS LA VIANDE HACHÉE DE BOEUF APRÈS L'ADDITION DE NAD

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Résumé

On a étudié la formation de nitrosomyoglobine dans la viande hachée de boeuf après l'addition de nicotinamide adénine dinucléotide. L'effet stimulant constaté du NAD n'a pas été influencé par l'addition de substrats du cycle citrique acide, mais par le pH et par la concentration du coenzyme. L'activité destructrice du NAD dans la viande a rapidement limité son effet accélérant.

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NITROSOMYOGLOBINBILDUNG IN RINDERHACKFLEISCH NACH ZUSATZ VON NAD

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Zusammenfassung

Es wurde die Bildung von Nitrosomyoglobin in Rinderhackfleisch nach einem Zusatz von Nikotinamid Ademin Dinukleotid untersucht. Während der festgestellte stimulierende Effekt des NAD durch einen Zusatz von Substraten des Citric acid cycle's nicht beeinflusst wird, ist dieser vom pH und von der Konzentration des Koenzymes abhängig. Die NAD-zerstörende Aktivität des Fleisches beschränkt schnell den beschleunigenden Effekt des NAD.

ОБРАЗОВАНИЕ НИТРОЗОМИОГЛОБИНА В СМОЛОТОЙ ГОВЯДИНЕ ПОСЛЕ ДОБАВЛЕНИЯ НАД

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Резюме

Исследовано образование нитрозомиоглобина в смолотой говядине после добавления НАД (никотинамид аденин диноклеотида). Установленный эффект НАДа не влияется добавлением субстратов лимонно-кислого цикла, но зависит от pH и концентрации кофермента. Разрушающая НАД активность мяса быстро ограничивает ускоряющий эффект НАДа.

NITROSOMYOGLOBIN FORMATION IN GROUND BEEF AFTER ADDITION OF NICOTINAMIDE ADENINE DINUCLEOTIDE

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The participation of mitochondrial enzymes and co-factors in the reducing acitivity of post mortem muscle has been considerd many times. Watts et al. /1966/ established that NAD /nicotinamide adenine dinucleotide/ accelerates the metmyoglobin reducing activity of ground meat when nitrite is used as an oxidizing agent. Their next work demonstrates that the rate of reduction of NAD to NADH controls the over-all rate of reduction of metmyoglobin and the reducing activity can be influenced by the addition of various substrates /Saleh, 1968/. In model tests Koizumi & Brown /1971/ observed rapid formation of nitrosomyoglobin from metmyoglobin and nitrite in the presence of reduced NAD /or NADPH/ and flavins. They suggested the NADH-FMN system may participate in the formation of curing pigment by means of reduction of metmyoglobin to the deoxy form.

In the course of studies on the nitrosomyoglobin formation in ground beef we investigated the stimulating effect of NAD and the conditions under which it could be realized.

Materials and Methods

1. Chilled for 48 hours beef from the semitendinosus derived from well rested animals was used. All meat was trimmed of external fat and connective tissue, ground twice and mixed before each experiment. Ten-grams portions were weighed out and placed in Thunberg tubes. After the addition of 0.5 mg sodium nitrite, various quantities of NAD and other additives /final volume of 3 ml/ the sample was mixed thoroughly and the air was evacuated for 2 min. Tubes were placed in a water bath at 37°C for 30 min. All solutions to be added were adjusted to pH of the meat when necessary.

2. Nitrosomyoglobin was determined by the method of Mirna & Schütz /1972/. For determination of nicotinamide adenine dinucleotide was used the method of Klingenberg /1965/.

Results and Discussion

Under the described conditions we confirmed the findings of Watts et al. /1966/, that the addition of 0,5 µmol NAD per g ground beef markedly enhanced formation of nitrosomyoglobin. The results from more than 10 different experiments ranged from 20% to 100% acceleration. We made no attempts to elucidate what the differences between various batches of meat depended on. It is surely, however, that our findings were not pH-dependant - pH of meat varied from 5,4 to 5,6 in all experiments.

Following the scheme of Saleh & Watts /1968/ we tested various substrates of the citric acid cycle for their effect on the nitrosomyoglobin formation in the presence of NAD. We found no effect after the addition of malate, fumarate, succinate and glutamate in quantities ranged from 50 to 150 mg%. The tested additives had no effect in the absence of NAD either.

Our results contradict the stimulating effect described by Saleh & Watts /1968/ and support the assumption that the reduction of NAD to NADH is not an obligatory step for increasing nitrosomyoglobin formation. This is supported by the fact that there are no difference between samples treated with NAD or NADH in equal molar quantities - both of them accelerated curing pigment formation to the same extent.

In an attempt to shed more light on the stimulating effect of NAD we investigated the influence of pH and concentration of NAD. Different meat patterns with initial pH 5,5-5,6 were adjusted to pH 6,0 and 6,5 with K_2CO_3 solution. The nitrosomyoglobin content was measured after 30 min at 37°C treatment in samples with and without NAD 0,5 µmol per g ground beef/. The results from a typical experiment are presented in Table 1:

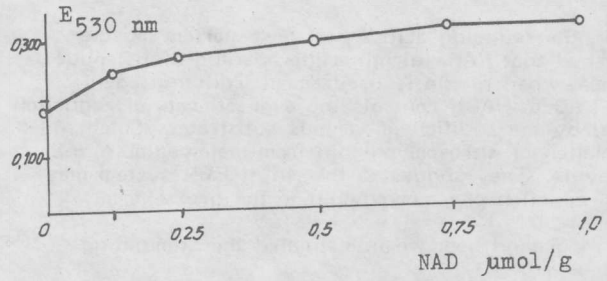
Table 1.

pH	NAD added µmol/g	$NaNO_2$ added mg%	NOMb formation E 530 nm
5,5	0	5	0,160
	0,5	5	0,315
6,0	0	5	0,220
	0,5	5	0,250
6,5	0	5	0,230
	0,5	5	0,220

It is shown that the NAD-stimulating effect is pH-dependant - from a marked stimulation at pH 5,5 to no effect at pH 6,5. It must be underlined that in the control samples /without NAD/ more nitrosomyoglobin was formed at pH 6,0 and 6,5 than at pH 5,5,

The influence of the concentration of NAD on the nitrosomyoglobin formation is shown on fig. 1.

Figure 1.



The increase of NAD concentration up to 0,75 µmol/g increases the quantity of curing pigment formed for 30 min. at 37°C. The highest effective concentration of NAD does not differ significantly from the one, naturally present in muscle at the time of slaughter /0,60 - 0,70 µmol per g, according to Atkinson & Follet, 1971/. Further addition of NAD is not effective. It is well known that NAD-destroying enzymes present in meat, which are responsible for the loss of NAD after slaughter /Severin, 1963/. To verify our supposition for the non-enzymatic action of NAD we added 0,5 µmol/g ground beef and stored it for 24 hours in +2°C /Table 2/.

Table 2.

Hours	Sample	E 530 nm	NAD content µmol/g
0	Control	0,355	0,29
	With NAD	0,400	0,79
2	Control	0,350	0,21
	With NAD	0,400	0,59
4	Control	0,280	0,18
	With NAD	0,370	0,43
24	Control	0,220	0,09
	With NAD	0,255	0,14

It is shown that the decrease of NAD content, following the action of NAD-ases, lowers its stimulating effect. The possible product of the NAD-ases action, nicotinamide, has no effect in equal molar quantities.

The mechanism of the described action of NAD is not clear at present. Further investigations are needed to shed light on it.

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