BUDAPESTI CORVINUS EGYETEM

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THESES OF DOCTORAL DISSERTATION

Effect of different nitrite-concentrations in meat products

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PhD SCHOOL/PROGRAM

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INTRODUCTION

Sodium nitrite is probably the most important additive in meat industry having been used since more than 200 years. Exact history of its use is not known. Common salt used for preservation of meat was likely contaminated with salpetre (potassium nitrate). Meat prepared with this substance stayed nice pink in color and became characteristically flavored. This effect of salpetre was recognized in the 1800-s and they began to make use of it. Polenski in 1891 detected that nitrate can be transformed to nitrite as a result of bacterial metabolism, while Lehman in 1899 stated that characteristic pink color of cured meat is caused by nitrite not by nitrate. Haldene discovered in 1901 that this pink color is formed as a result of a reaction between nitrogen oxide and meat pigments. These findings contributed to direct applications of nitrite making nitrate-nitrite transformation superfluous. Since nitrite *per se* is poisonous, it can be used only in form of curing salt, which is a 99,5% NaCl and 0,5% $NaNO_2$ blend. This way overdosage can be avoided.

Nitrite has several functions. It is bacteriostatic and sporostatic, inhibits growth of microbes, mainly that of *Clostridium botulinum* (causing botulism) by inhibiting outgrowth of its spores, exhibiting thus important food safety feature. In addition it plays a decisive role in characteristic pink color formation of meat products, has antioxidative effect, inhibits rancidity, prevents formation of WOF (Warmed Over Flavor). It has a favorable effect on sensory value, first of all on flavor, in formation of characteristic cured meat flavor. In higher concentrations nevertheless it has a disadvantageous, health risk property: on the effect of inadequate technological and culinary practice nitrosamines can be formed from nitrite. For this reason its use is strictly regulated.

Since scientific and practical interest for nitrite has been ever since vivid, and since information misleading the consumers can be heard and read rather often, I considered very important to launch and evaluate a series of experiments, that investigate the effect of different nitrite concentrations on chemical, microbiological and sensory characteristics with heat treated and non heat treated meat products equally.

AIM OF EXPERIMENTS

Aim of my thesis was the investigation of the effect of different nitrite concentrations while considering food safety and quality.

Main steps of my investigations were as follows:

1. First regulations referring to use of nitrite were reviewed in connection with both traditional and organic products.
2. In preliminary experiments effect of different meat additives on nitrite and nitrate was studied:
   - Sodium nitrite and nitrate content of meat raw materials, seasonings and constituents of plant origin were determined.
   - A dry sausage model without curing salts were prepared, with powdered vegetable of high nitrate content and with nitrate reducing microorganisms as well as effect of smoking – indispensable with Hungarian dry sausage technology – on nitrite and nitrate content was studied, too.
   - Change in nitrite content of curing salt ("nitrite salt") during storage in function of time was also determined.
   - Residual sodium nitrite and nitrate content with meat products of domestic manufacture was reviewed.
3. In accordance with the aim of my experiments heat treated models (Bologna sausage) and non heat treated models (dry sausage) were prepared with different nitrite concentrations (0, 50, 100, 150 mg/kg) following the domestic technology. Bologna sausage was stored for 90 days at 4 °C and 12 °C, while dry sausage was stored for 43 days at 4 °C without packaging as well as vacuum packed and in MAP. Following parameters were measured during storage:
   - Chemical: pH, moisture, nitrite and nitrate content, total pigment content, nitroso pigment content, reddening %, rancidity
   - Microbiological: total aerobic count
   - Color: lightness, red color intensity, hue and stability of color during light exposure
   - Texture: hardness, chewing energy need and elasticity
   - Sensory: color, odor (aroma), flavor, texture and overall acceptance

   In addition to these parameters challenge tests were carried out with Bologna sausage inoculated with *Enterococcus faecalis* and dry sausage with *Listeria monocytogenes*.

4. A reaction kinetic model was elaborated in order to study the changes of different parameters during storage.
5. Probability of nitrosamine formation in meat products was calculated.

RESULTS

Sodium nitrite can be considered with good reason as the most important additive of meat products. Many scientific data support the view that use of sodium nitrite is indispensable in manufacturing meat products thanks to its food safety and food quality (color forming, antioxidative and sensory) influencing effect. In case of failure of GMP, health risk may arise because its toxicity and the possibility of nitrosamine formation. In my thesis study of such effects was aimed at. As a result of my preliminary and main experiments the following conclusions and suggestions were made:

1. **Interpretation of regulations**

   Regulations referring to use of nitrite and nitrate are difficult to interpret, they are too complicated. It would be reasonable to establish a uniform regulation – without typical, hardly identifiable national products – dividing meat products to heat treated and non heat treated ones. Added nitrite should not be regulated since it cannot be controlled anyway. Residual nitrite content of final product is 50 mg/kg sodium nitrite, including nitrite and nitrate content, yet expressed in sodium nitrite content.

   The 80 mg/kg sodium nitrite limit valid for organic meat products should remain, because no effective additive or process is available for its substitution as for now.

2. **Nitrite and nitrate content of ingredients (meat, seasonings, vegetables)**

   – These ingredients also contain nitrate. Source of meats' nitrate content is animal feed and water. Nitrate content of beef is higher than that of pork or poultry. Also seasonings have nitrate content, the highest being in paprika (red pepper), as a consequence nitrate content of meat product can be elevated by 15 mg/kg. High nitrate content is typical for flavor enhancers, too. Product development in meat industry involves application of vegetables in an ever increasing manner aiming at healthier nutrition thanks to their high fibre content. At the same time they increase nitrite content of the product (e.g. addition of 15% chive increases nitrite content of meat product by 150 mg/kg).

- Part of nitrate may be transformed to nitrite during manufacturing in presence of bacteria. For this reason residual level of nitrite may be higher than legal limit, notwithstanding the fact that no extra nitrite was added. It can also be a problem with the authorities that nitrate is detectable in meat product without addition any amount of nitrate.
- By the help of nitrate reducing bacteria significant amount of nitrite can be formed from vegetables of high nitrate content making thus possible production of meat products, chiefly dry sausage with no additives. In my experiments nitrate reducing ability of *Staphylococcus carnosus* was tested. Sausages prepared with this method were of lower grade, were more pale and less characteristic in flavor. This phenomenon can be explained by the fact that concentration of nitrite formed during ripening was not sufficient for developing a proper color and flavor. As further disadvantage the allergenic effect of powdered vegetables with high nitrite content, like in my experiments, can be mentioned.
- Affected by intensive smoking nitrate can be detected on the surface of dry sausages which migrates into deeper layers. Part of it may be transformed to nitrite resulting the presence of nitrite and nitrate with no addition of any of those.
- Nitrite concentration of curing salt (nitrite salt) in depots does not change significantly during storage, remaining in accepted range, yet it is influenced by relative humidity. Higher relative humidity causes increasing moisture resulting in lower relative sodium nitrite concentration.
- In my experiments it was stated that sodium nitrite and nitrate content of domestic meat products remains below legal limit.

3. Effect of amount of added nitrite on characteristics of meat products

In my main experiments different amounts of sodium nitrite were added to heat treated and non heat treated meat products, and food safety (microbiological) as well as food quality characteristics (chemical, color, texture, sensory) were analyzed during storage. Bologna sausage models were stored for 90 days at 4 and 12 °C, dry sausage models were stored for 43 days at 4 °C without packaging as well as vacuum packed and in MAP (CO_2:N_2=30:70).

3.1. As a result of different nitrite concentrations conclusions as follows could be made:

- Concentration of sodium nitrite in meat batter decreased significantly starting immediately after addition and is further reduced during heat treatment. The higher the initial concentration the higher its residual value, evidently. This concentration decreases until

50^{th} day of storage when reaching a steady state. This fact is important to know in case of comparative tests, since values will differ depending on timing of measurement (being in unstable range results are not comparable). During manufacturing part of nitrite is transformed to nitrate, no such transformation takes place in final product. The higher the initial nitrite content the higher the nitrate formed. Nitrate content in final product decreases during storage until day 50, and similarly to nitrite the system becomes stable with a limit.

- As experiments showed amount of nitrite above a certain level does not influence the amount of total pigment: same amount is formed affected by 50 or 150 mg/kg sodium nitrite. The same is true for the content of nitrosopigment. Both pigment contents are reduced somewhat until the 34^{th} day of storage reaching then a stable value, reddening % is this way constant during storage. Bologna sausage without added nitrite also contains nitrosopigment explained by the fact that nitrite content of meat itself makes a slight nitrosopigment formation possible.
- When analyzing rancidity, evidently no nitrite effect was detected in Bologna sausage stuffed in oxygen-proof casing.
- In case of suitable pasteurizing heat treatment added nitrite amount did not influence microbiological status of meat product. This was found in challenge test, too, since *E. faecalis* (10^5 CFU/g) inoculated into meat batter was inactivated also with the mildest heat treatment applied (F_{70}=53,5 min).
- Analyzing the results of color measurements it was stated, that Bologna sausage without added nitrite was the most pale, the least red in terms of red color intensity and color. When increasing nitrite concentration color of the product turns red and deeper. No differences were found between Bologna sausage samples with 100 and 150 mg/kg added nitrite. Color characteristics hardly change during storage. As it was shown 50 kg/kg added nitrite is sufficient for proper color formation.
- During measurement of texture it was stated that samples with nitrite were harder and more elastic. Nitrite content, storage temperature and time did not influence texture.
- Bologna sausage samples without added nitrite proved sensoric objectionable mainly because of their greyish color, but other characteristics (aroma, flavor, texture, overall acceptance) turned out of lower grade. Increasing of amount of added nitrite on the other hand did not cause significant improvement above a certain level in these characteristics. Accordingly 100 mg/kg of added nitrite is fully effective for ensuring proper sensory

characteristics of Bologna sausage. Values of sensory characteristics gradually decreased during storage resulting in loosing of mainly flavor characteristics, the chief sensory property. It was stated that flavor deteriorations takes place also without lipid oxidation, caused by different (mainly protein) degradation. This phenomenon can be detected already at day 30 during 90 days storage, sensory value is halved by then. This should be taken into consideration by the food chains that require production of items with a shelf-life of longer-than-ever. Temperature elevation contributes to further deterioration of quality.

Results of subjective sensory analysis are in good agreement with that of instrumental analysis.

3.2. As a result of different added nitrite content in dry sausage the following conclusions can be made:

- Ratio of sodium nitrite in meat batter and in final product does not depend on the added amount. 35-38% in batter while 70% in final product broke down. Sodium nitrite content in final product decreased significantly during storage by day 15 (1-4 mg/kg) and stayed there till the end of storage regardless of initial nitrite content and way of packaging. From added nitrite nitrate was formed very rapidly, the higher the initial nitrite the higher the nitrate content which further increased during ripening. During storage the value was stable till day 29 and started to decrease afterwards (nitrite practically disappeared by day 19). As expected nitrite content of samples with no added nitrite was lowest, it was reduced to 10 from initial 15 mg/kg. Even if no nitrite was added to dry sausage model nitrate was detectable in final product and during storage. No difference was found in initial nitrate content in meat batter no matter 50 or 100 mg/kg nitrite was added. Residual nitrate level reached the value of sausage without added nitrite, *i.e.* 10 mg/kg. On the contrary initial nitrate concentration in sausage was two times higher than that of the samples with 50 or 100 mg/kg, and it was two times higher (28 mg/kg) at the end of storage. Way of packaging had no influence on nitrite breakdown.
- Total pigment content of nitrite-less sausage was the lowest as expected for its lowest nitrosopigment content. Total pigment contents of sausages with 100 and 150 mg/kg added nitrite did not differ. In case of dry sausages 100 mg/kg initial nitrite content is necessary for saturating the hem. The extent of reddening % depended on the added nitrite, as expected.
- Sausages without packaging became rancid during storage. Rate of this change depended on nitrite content: most rancid was the nitrite-less while least rancid was the sample with

highest added nitrite. Prepacked sausages with nitrite became rancid till day 19 of storage, when a steady state condition was found with similar rancidity as with nitrite-less samples.

- Amount of added nitrite did not affect total count of sausages explainable by the fact that more sensitive microbes are inactivated while more resistant ones survive and grow compensating these two processes each other. Total count of sausages increases during storage regardless the way of packaging. This process lasts to day 36 till steady state. Reduction of *Listeria monocytogenes* count in artificially inoculated sausage attained 2 orders of magnitude in samples without added nitrite while 4 orders of magnitude with nitrite containing sausages. This shows the importance of nitrite use in terms of food safety.
- As for color characteristics color of sausages in visible range differed from each other. Samples with no and with 50 mg/kg nitrite were rather grey, while those with 100 and 150 mg/kg were nice red. The higher the initially added nitrite the nicer red was the color, explainable because of more intensive reddening, yet there was no difference between samples with 100 and 150 mg/kg, in other words 100 mg/kg is sufficient for proper color formation. Differences in color remained till the end of storage in unpacked samples, but equalized when prepacked. Red color intensity during light exposure decreased no matter what the nitrite content was. This reduction depended on storage time. The older the product the lower the initial red color intensity and the more rapid was the reduction to constant value.
- There was no difference in hardness between the samples without and with 50 mg/kg added nitrite. Similarly no difference was detected between samples with 100 and 150 mg/kg, these were nevertheless harder than former ones.
- Sausages without added nitrite were worst in all sensory characteristics. Increase of nitrite until 100 mg/kg improved all the characteristics. This was not remarkable in case of color and texture, while hardly ever were found differences in terms of aroma, flavor and overall acceptance between samples with 100 and 150 mg/kg. This means that with dry sausages 100 mg/kg added nitrite is sufficient for proper sensory characteristics. With increasing storage time sensory features became lower similarly as with heat treated sausages, mainly flavor and aroma deteriorate, which are main sensory characteristics. By day 29 of storage the value of these characteristics is reduced to 25% of initial value.

Way of packaging plays an important role in preserving sensory value. Unpacked sample looses moisture for which reason its color deepens, texture becomes harder, its aroma, flavor and overall acceptance is reduced. Vacuum packaging proved as best method, products stored this way received highest scores for flavor, aroma and overall acceptance. This can be considered as important statement because food chains prefer MAP to vacuum packed products claiming this as consumers' demand. They are doing so in spite of the several disadvantages of MAP – in addition to worse sensory effects – such as undetectable damage of package making oxygen infiltration possible that causes spoilage of microbiological and chemical nature. As further disadvantages larger volume and higher cost can be mentioned. It has an advantage, too: individual slices can be separated easier. Keeping all these in mind vacuum packaging of meat products can be considered as a better way in order to preserve the sensory characteristics in addition to microbiological status, which consideration was supported by experimental results as well.

3.3. Reaction kinetic characteristics *i.e.* types of reactions of different properties were determined.

3.4. Cancerogenic nitrosamines can be formed only at low pH-value and at high temperature. The concentration of nitrite necessary for the formation of the highest amount of DMNA ever detected is by 15 000 less than that of the nitrite concentration applied in practice when manufacturing a meat product. If no nitrite is added nitrite content of meat is also 60 times as high as necessary for formation of DMNA. For this reason reduction of added nitrite (even if by 50%) makes hardly ever sense.

Results received in my experiments can be successfully applied during production of traditional and organic meat products as well as in activities of authorities and retail.

THESES

1. It was stated that affected by smoking nitrate is formed on the surface which migrates into the product. Part of this nitrate is transformed to nitrite due to the activities of bacteria, as a consequence of which nitrite and nitrate can be detected in intensively smoked products without added nitrite.

2. Nitrite content in Bologna sausage is reduced continuously until day 50 of storage when it stops and the system becomes stable. This limit value in case of sausage with 100 mg/kg is 37 mg/kg. This fact has great significance in comparative tests, since changes in nitrite concentration in unstable period during storage makes the comparison not possible.
3. In case of GMP – proper heat treatment or ripening and drying resp. – nitrite has no influence on microbiological status, on total count. By the help of artificial inoculations (challenge tests) it was proved that with cooked sausage the mildest heat treatment applied is sufficient for inactivation of apathogenic *E. faecalis*. At the same time 4 log reduction in *L. monocytogenes* count can be achieved in products with added nitrite, on the contrary to 2 log reduction in sausage sample without added nitrite for which reason use of nitrite in production of dry sausages can not be omitted mainly not for food safety reasons.
4. Quality features of cooked and dry sausages (sensory characteristics) can be ensured by 100 mg/kg added sodium nitrite.
5. Sensory characteristics of meat products deteriorate continuously during storage even in gas tight containers. Color is fading, intensity of aroma and flavor decreases. Organoleptic scores for Bologna sausage are halved by day 30 of storage and to 25% with dry sausages. This should mean that manufacturing of meat products with very long shelf-life does not serve the interest of consumers since not only sensory value is reduced but also more additives are necessary.
6. Best choice of packaging for dry sausages is vacuum packaging. It has been proven that quality characteristics can be most efficiently preserved in such packaging.
7. Reaction kinetic model was established for changes of heat treated and non heat treated sausages and constants were determined. Knowing this model probability and extent of changes can be planned.

PUBLICATIONS

ARTICLES IN JOURNALS

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R & D PROJECTS

International project participant

1. Fermentált szárazárugyártó szektor fejlesztése továbbképzések útján, az innováció figyelembevételével (MEAT-TIPS)
2. Technológiai transzfer és innovációs megoldások és beavatkozások a Dél-Kelet-Európai régiók agrár-élelmiszer szektorában (TECH.FOOD)
3. Új, funkcionális húskészítmények kifejlesztése (NUTRAMEAT)
4. Regionális élelmiszerek Európában és jelentőségük a XXI. Századi Európában (LOCALFOOD)
5. Hagyományosan érlelt kolbászok biztonsága: védőkultúrák és bakteriocinek kutatása (SAFETYSAUSAGE)
6. Innovatív képzés a kelet- és közép-európai húsipari középvezetők részére (EASTMEAT)
7. Szárazkolbászok fermentálása és érlelése (CUREM II)