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- Food chemistry
- Biotechnology, microbiology
- Physical property of food
- Food quality and safety
- Health
- Food nanotechnologies
- Food processes
- Economics and management
- Automation of food processes
- Food packaging

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Analysis of the process of formation of n-nitrosodimethylamine in brewer’s malt

Vitalii Shutiuk¹, Oleksandr Bessarab¹, Temenuzhka Haralanova², Sergii Vasylenko¹

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Abstract

**Introduction.** The article deals with the issues of carcinogenic substances formation in the manufacture of beer. It is shown that the basic technological process, that influences the N-NDMA accumulation in beer, is brewer’s malt drying. The determining factor, influencing the N-NDMA content in malt, is the concentration of nitrogen dioxide at the entrance to malt layer.

**Materials and methods.** In the course of research, drying conditions typical for dryers of «Latvian Academy of Agriculture» type, one-deck dryers and apparatuses on malt manufacturing in combined way were secured. The measuring of nitric oxides was carried out by the means of gas- analyzer of 645 HL 20 type, the principle of operation of which is based on chemiluminescent method of determination of nitric oxide.

**Results and discussion.** More intensive absorption of nitrogen dioxide were observed during the first stage (constant speed) of drying due to the large number of free moisture presence in malt – a good nitrogen dioxide absorbent. The concentration of nitrogen dioxide in the drying agent has the greatest impact on intensification of N-NDMA formation processes in malt, that is recorded in all experiments.

That is why in order to achieve guaranteed quality of the product, one should establish the limit of concentration of NDMA in malt equaling 15 mcg/kg. Drying of malt with drying agent with concentration of nitric dioxide up to 0.4 mg/m³ guarantees concentration of NDMA lower than this limit. This concentration should be limiting in the field of development of modern heating vent systems.
Introduction

Manufacturing method of many food substances involves adding various chemical substances to raw material and inters, as well as complicated heat treatment. At that, together with useful qualities, a relatively small concentration of reagents and additives remaining in products may have toxic and carcinogenic ones. Besides, harmful substances may be generated with the help of heat treatment.

Since carcinogenic substances, which products contain may cause formation of cancerous growth within an organism of a human; the topical task is extraction of carcinogenic substances from food [3]. Nowadays the problem of extraction of carcinogenic nitrosamines (NA) from food substances, which were discovered during the 1970-ies with the help of thermochemiluminescent method of NA determination in products, is of utmost importance [6].

Until now, the NA entry to human organism with various drinks is not taken into account [1]. For the first time NA was found in beer in 1979 in Germany. Researches ascertained that mostly N-nitrosodimethylamine (NDMA) was found in beer, in 70...75 % ale samples and in 80...100 % stout samples.

Analysis of numerous beer samples in 35 countries displayed that the product contained mostly NDMA, sometimes even high concentration of it (up to 68 mcg/l). Analysis of NDMA content during technological process of beer manufacturing showed that its accumulation in malt (up to 300 mcg/l) occurs at the drying stage with the use of gas-air bearer, containing nitric oxides [7, 8].

Analyzed material made it possible to determine the average consumption of NA with different products per person in various world regions (Fig.1.). E. g. in Germany an average citizen consumes 7, 0…10, 0 mcg NA per week [2].

![Fig.1. Average consumption of beer and juice in the world per person, l/year, 2011](image)

In view of the fact that nitrosamines brought in with beer constitute a considerable part of the general consumption of NA by a person, in a range of countries limiting value of NDMA in malt and beer is recommended. This limiting value is not scientifically valid. It defines the possible limiting value of NA consumption with beer; specific part is quite high and totals 64% [4, 5].
Research of NA percentage in food substance has been carried out since 1969; the first beer samples were analyzed in 1979. Analysis of the samples elicited in them mainly NDMA. N-nitrosodimethylamine is a virulent carcinogenic substance, included together with some other nitroso-substances by International Agency for Research on Cancer (IARC) to the first category of substances, which are carcinogenic to humans. The impact of this carcinogenic substance on human organism is interrogated in scientific works [1, 3, 7, 8].

**Materials and methods**

In the course of research, drying conditions typical for dryers of «Latvian Academy of Agriculture» type, one-deck dryers and apparatuses on malt manufacturing in combined way were secured (vide table).

<table>
<thead>
<tr>
<th>Number of variant</th>
<th>Duration of the process of malt drying</th>
<th>Total duration of drying, h</th>
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<td>Duration of drying with the temperature of drying agent, h</td>
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<td></td>
<td>55 °C</td>
<td>65 °C</td>
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<tr>
<td>1</td>
<td>5,0</td>
<td>3,0</td>
</tr>
<tr>
<td>2</td>
<td>10,0</td>
<td>6,0</td>
</tr>
<tr>
<td>3</td>
<td>18,0</td>
<td>8,0</td>
</tr>
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* – transition from 65 to 85°C.

Concentration of nitric oxides in drying agent totaled: 0,1; 0,3; 0,6 mg/m³. In course of carrying out an experiment, the following parameters were measured and controlled:
- expenditure of drying agent;
- temperature of drying agent under the malt layer and out of it;
- humidity of lower and upper malt layer;
- the quantity of damp and dry malt, the thickness of a layer;
- percentage of nitrites, nitrates and NDMA in lower and upper layers.

The measuring of nitric oxides was carried out by the means of gas-analyzer of 645 HL 20 type, the principle of operation of which is based on chemiluminescent method of determination of nitric oxide. The main point of it lies in the fact that reaction between nitric oxide (NO) and ozone (O₃) results in receiving an excited molecule of nitrogen dioxide, because of which luminescence takes place:

\[
\text{NO} + \text{O}_3 \rightarrow \text{NO}_2^{(\text{hv})} + \text{O}_2 ,
\]

\[
\text{NO}_2^{(\text{hv})} \rightarrow \text{NO} + \text{hv} ,
\]

where by (hv) photon of light, got during luminescence, is conventionally denoted.

Detected radiation is registered by photomultiplier tube and by the value of output current the concentration of NO in an analyzed sample is calculated. To calculate the nitrogen dioxide NO₂ concentration, which together with NO is present in drying agent, NO₂ should be transformed into NO. Reduction of nitrogen dioxide is realized in converter with the help of catalyst, heated to the temperature of +200 °C. Since in this condition radiation from the total concentration of nitrogen oxides is detected, concentration of NO₂ is estimated through difference between the value of electric signal proportional to concentration of sum of oxides NO₂ and, correspondingly, NO.
Percentage of nitrites in samples was estimated with the help of the method in accordance with state standards of Ukraine 4948:2008 “Fruits, vegetables and products of their processing. Methods of estimating the percentage of nitrates”. To estimate the percentage of N-nitrosamines methodics instructions on methods of inspection МУК 4.4.1.011-93 “Estimating of volatile N-nitrosamines in food raw material and foodstuff” were used (Ratified by Federal Service for Supervision of Consumer Rights Protection and Human Welfare in 22.12.1993). Quantitative estimation of NDMA percentage in a sample was carried out with the standard solution of NDMA.

Sampling was carried out in upper and lower layers. A concentration of nitrites, nitrates and NDMA was estimated separately in every sample. At the end of drying all the malt was mixed in order to estimate the average indexes.

To value air pollution with nitric oxides concentration of oxides in air was estimated, which equaled 0.02...0.08 mg/m³. During rush hours because of heavy traffic NOₓ concentrations rose up to 0.1...0.12 mg/m³, while nitric dioxide concentration did not exceed 0.05 mg/m³. Samples, selected during intermediate stages of drying, were completely dried by the air, heated in electric heating coil, in which concentration of NOₓ did not exceed 0.02 mg/m³.

Because of fluctuation of NOₓ concentration in environment, while drying correction of nitric oxide in drying agent has been carried out. With prescribed expenditure of drying agent V (m³/h), nitric dioxide concentration in drying agent at the input to layer C_{NO₂}^{in} and at the output of layer C_{NO₂}^{out} the following parameters were estimated:

- amount of nitric dioxide (mg), put into layer per time unit on the basis of 1 kg of malt:
  \[ C_{NO₂} = \frac{1}{G_e} C_{NO₂}^{in} V, \text{ mg/h}; \]  

- amount of nitric dioxide, absorbed by 1 kg of malt per time unit:
  \[ G_{NO₂}^{abs} = \left( C_{NO₂}^{in} - C_{NO₂}^{out} \right) V, \text{ mg/h}; \]  

- amount of nitric dioxide, put into the layer during all drying period per 1 kg of malt:
  \[ \bar{C}_{NO₂} = \frac{1}{G_e} \int_0^\tau C_{NO₂}^{in} V \, d\tau, \text{ mg}; \]  

- amount of nitric dioxide absorbed by 1 kg of malt during the whole drying period:
  \[ \bar{G}_{NO₂}^{abs} = \frac{1}{G_e} \int_0^\tau \left( C_{NO₂}^{in} - C_{NO₂}^{out} \right) V \, d\tau, \text{ mg}. \]

Since nitric dioxide is present in the air, concentration of nitric oxide in drying agent was recorded in order to ascertain its influence upon NDMA formation in malt.

**Results and discussion**

Results of experiments are displayed in table 2, which presents parameters of malt drying of 20 h duration with feeding of nitric oxides in drying agent and without it.
Fig. 2. Malt drying with drying agent, containing nitric oxides:

- Without feeding; feeding of NO₂ with concentration, mg/m³:
  - a – 0.1;
  - b – 0.2;
  - c – 0.3;
  - d – 0.6.
Results of experiments show that the process of drying characteristic of real-life environment was reproduced. It can be assumed from dependence of temperature change and humidity on time, as well as good quality of malt.

It was established that nitric oxide, containing in drying agent, penetrates the malt, almost not being absorbed. A small change of nitric oxide concentration in drying agent at the output of the layer, as a rule, occurring at the final drying stage, can be explained by partial NO₂ reduction, oxidation of nitrogen in the air during the period of increase in temperature of drying agent and less intensive sorption of NO₂ by the malt layer with relatively low humidity of it (Fig.2, a-f).

Change in concentration of nitric dioxide in drying agent while its penetrating the malt layer is indicative of absorption of nitric dioxide by malt layer. More intensive sorption of NO₂ occurred during the first stage of drying that can be explained by the presence in malt a large quantity of free moisture, which is a good absorber of nitric dioxide. During this stage, absorption of nitric dioxide occurs throughout the whole layer. At that, driving force is the difference of NO₂ concentrations in drying agent and on the surface of the malt.

During the second stage of drying, in which humidity of malt is less than maximal hygroscopic one, zone of moisture evaporation moves to seeds and the intensity of absorption of nitric dioxide lowers. During the drying intensity of absorption of nitric dioxide is insignificant, that can be explained by little amount of moisture in the layer. Insignificant growth in difference of concentration of nitric oxide in drying agent while its penetrating the malt layer at the end of drying is not indicative of the growth in intensity of NO₂ sorption by the malt layer during this period and can be explained by its partial reduction to NO, which is confirmed by growth in concentration of NO at the output of the drying agent from the layer.

During the first stage of drying up to 85% of nitric dioxide, absorbed by malt during the whole drying process, is absorbed. With duration of drying of 12, 20 and 30 h, this period lasts, correspondingly, first 7…9, 12…14, 17…19 h.

The amount of absorbed nitric dioxide equals 35…50 % of its total quantity, put into the layer during the whole drying process. Absorption is realized through moisture with formation of nitrous acid (HNO₂) and products of its dissociation. It is confirmed by the growth in concentration of nitrites and nitrates in the malt during its drying.

Tracing the dynamics of changing of the quantity of nitrites and nitrates in the malt, one should admit that germs of malt contained 1…7 mg/kg of nitrites and nitrates. It can be explained by their ingress in the malt with process water during the soak and with air during the growing of malt. During the process of drying of malt, the growth in concentration of nitrites and nitrates was observed, the intensity of their accumulation becoming higher together with increase of concentration of nitric dioxide in drying agent at the input to the layer. Concentration of nitrites and nitrates in dried malt equaled 2…9 mg/kg. Percentage of nitrites was 4…5 times lower than percentage of nitrates, that can be explained by instability of nitrites. Concentrations of nitrites and nitrates in malt are several times higher than the ones, which are needed to form NDMA in prohibitive amounts (estimated at mcg/kg). By the growth in concentration of nitrites and nitrates increased the amount of NDMA.

Concentration of nitric dioxide in drying agent influences the intensification of formation of NDMA in malt, which was observed during all the tests. Increase of concentration of nitric dioxide in drying agent from 0,1 to 0,6 mg/kg leads to 6…7 time the amount increase in concentration of NDMA in malt. Percentage of NDMA in malt at the same time increases to 8…15 mcg/kg (Fig.2, b-f) and is the highest permissible amount.
according to “The state sanitary regulations and rules “Medical requirements to the quality and safety of food substances and foodstuff” Order of Ministry of Health Protection of Ukraine № 1140 (29.12.2012).

Results of the tests on drying of malt with air without batching of nitric oxides in drying agent (malt being cooked using distilled water) nevertheless display the formation of nitrites, nitrates and NDMA in malt, which means that the layer of malt absorbed nitric oxide from the air. Thus, during the drying of malt in industry with clean air, which lasted 20 h, the increase of NDMA concentration in malt from 0.8 to 1,3 mcg/kg was observed (Fig.2, a).

Research of NDMA formation depending on technological modes of malt drying proved that decrease in duration of malt drying from 30 to 12 hours lowers the concentration of NDMA in malt not more than to 1.6 times and does not lose the task of radical decrease in concentration of NDMA in malt.

When the temperature of malt increases, accumulation of NDMA in it passes more intensively. It can be explained by activation of dissociation processes of NA with increase in temperature to more than 60 °С.

Humidity of malt also influences the formation of NDMA. Sorption of nitric oxides intensifies when the humidity of malt increases. Besides, humidity intensifies reactions of nitrosation of amines, in consequence of which increases concentration of NDMA in malt.

Conclusion

All things considered, among the factors, influencing the amount of NDMA in malt, the most important one is concentration of nitric dioxide at the input to the layer of malt. Limiting values of amount of NO₂ in drying agent should be conditional upon concentration of NDMA in malt, defined by hygienic regulations. That is why in order to achieve guaranteed quality of the product, one should establish the limit of concentration of NDMA in malt equaling 15 mcg/kg. Drying of malt with drying agent with concentration of nitric dioxide up to 0,4 mg/m³ guarantees concentration of NDMA lower than this limit. This concentration should be limiting in the field of development of modern heating vent systems.

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