Bacterial Starter in Horseflesh Sausages Production

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SUMMARY: Volatile fatty acids, volatile carbonyl compounds as well as ethers and sulphides prove to be aroma and flavor producing in cooked, cooked-smoked and half-smoked sausages. Methods for obtaining volatile aroma producing substances from the horseflesh sausage products have been developed. The experiments have shown that pure cultures L. plantarum and Str. diacetilactis produce volatile carbonyl compounds, their content growth being observed in the process of frying, cooking and smoking six sorts of sausages tested. But the total amount of volatile carbonyl compounds and especially their separate components quantitative ratios depend upon sausage sort. Isobutyric, butyric, crotonic, butylacetic aldehydes, diacetyl, acetone, diethylketon were prevailing in finished cooked-smoked sausages. Formic, acetic, valeric, and butylacetic acids were predominating in all sorts of sausages involved.

INTRODUCTION: Biotechnological and chemical processes play an important part in the sausage products processing. Finished products quality is determined by the character of these processes, and regulating biotechnological and chemical processes, it is possible to improve the finished product quality and to choose the necessary production technology. In this relation the technology where bacterial starter and some other additives could be used, is considered to have the future.

MATERIALS and METHODS: To prepare the exploratory run of half-smoked and cooked-smoked sausages the mixture of pure cultures Lact. plantarum (strain 70) and Str. diacetilactis (strain 137) was added to the sausage meat. Mixture ratio made 1: 1, that is 10 million cells per 1 gr of sausage meat. To prepare mother starter a portion of lab starter was introduced into sterilized nonfat milk, then kept in the thermostat for 16-18 hours at 26-28 degrees C until acidity made 80-85°T. Bulk starter was obtained by adding slowly 2-3% of mother starter into agitated sterilized milk. The milk was ripened in the thermostat for 10-12 hr at 24-26 degrees C. and then cooled till 4-6 degrees C. The starter obtained had clean sour milk flavor and pleasant aroma, there was no whey deposit, acidity made 90-100 ° T. Microscopic analysis gave positive results. To cook the exploratory run of Alatauskaya top-grade cooked sausage the bacterial meat starter was used, making 10% of the total sausage raw, to prepare the latter fresh-killed meat whose temperature was not less than 28 degrees C, was cured, trimmed and chopped; grinder plate holes being 2mm in diameter. Then 25% of cold water, 25% of flaky ice, 5% of salt,0,0125% of sodium nitrite have been added to the weight of the raw and pure bacterial starter in ratio 10mln cells per 1 gr of the raw, and the mass was processed in the chopper where it was kept for 48 hr at 4-5 degrees C.

Volatile carbonyl compounds and fatty acids recoveries were produced in the multipurpose vacuum apparatus; they were analysed and identified by gas chromotograph.

Control and exploratory run sausages having been forced, shrinked, cooked, smoked and dried, the probe has been taken. To determine the total bacteria amount in the sausaged studied, seeding was done in meat-pepton agar (MPA), thermostating for 48 hours at 28-30 degrees C. Lactic acid bacteria amount was determined by seeding in a hydrolyzed agar with further 48hr curing at 28-30 degrees C. Escheichia colis was investigated by the Endo agar seeding and Kessler medium seeding with thermostating for 48 hours at 37 degrees C. Proteus was detected by Shukevich method. The data obtained have shown that in Alatauskaya top-grade cooked-smoked sausage containing pure bacterial culture, the amount of lactic acid bacteria was increasing rapidly and has made $4,4*10^7 - 4,9*10^7$ per 1 gr sausage after forcing and shrinkage. After frying and especially cooking these bacteria amount rapid decrease (0,9*10 per 1 gr) was caused by a high temperature. During smoking and further drying lactic acid bacteria content was decreasing in small amounts ($0,6*10^7-0,5*10^7$ per 1 gr). After forcing and shrinkage the exploratory run sausages contained 2 times less microorganisms than control run sausages did ($3,7*10^7$ and $2,1*10^7$ per 1 gr respectively) and this can be explained by the slowing effect of the lactic acid bacteria. Aroma-forming bacteria in the exploratory and control run sausages have been as $2,6*10^4$ against $1,8*10^4$ per 1 gr after forcing and shrinkage. In the exploratory and control runs of Alatauskaya top-grade cooked sausage as well as in first-grade horseflesh half-smoked sausage lactic acid bacteria and other microorganisms content changes have been the same as in Kazakhstanskaya sausage. But still there are some differences observed. For instance, after forcing and shrinkage, Kazakhstanskaya cooked-smoked sausage contained a higher amount of lactic acid bacteria than Alatauskaya or horseflesh half-smoked sausage did ($4,4*10^7$ - $4,9*10^7$ and $3,7*10^7$ - $4,2*10^7$ per gr, respectively). This may be explained by a higher muscular tissue content and low fat content of Kazakhstanskaya sausage when it is compared with Alatauskaya and horseflesh sausages. Higher lactic acid bacteria content causes higher lactic acid content and rapid decrease of pH in Kazakhstanskaya exploratory run sausage in comparison with the control run sausage (483,3÷498,6 mg% against 480,2÷486,4 mg%). This seems to be the main explanation for the rapid decrease in the balance microorganisms content in the exploratory run sausages.

Lactic acid bacteria influence on volatile carbonyl compounds and fatty acids content growth.

The total amount of volatile carbonyl compounds produced by pure culture of lactic acid bacteria in nonfat milk in 12-24 hours at 22 degrees C has made 2,03± 0,05 mg per 100 gr dry substance against 0,84±0,04 mg per 100 gr of sterilized nonfat milk and, thus increased by 250,62%. The temperature having been increased up to 26 degrees C, the compounds amount has increased by 2,09±0,05 mg per 100 gr which is more than that of sterilized nonfat milk by 258,02%. Further temperature increase didnt cause any noticeable change of volatile compounds content. Total amount of volatile fatty acids made 13,18±0,05 mg per 100 gr at 22 degrees C, which is by 172,06% more than in sterilized nonfat milk. It increased till 13,40±0,06 mg per 100 gr temperature being 26 degrees C. This growth has been observed during all series of experiments and has proved to be reliable. The data taken show that pure cultures Str. diacetilactis (strain 137) and Lact. plantarum (strain70) do produce volatile carbonyl compounds and fatty acids. It has been established that pure bacterial cultures produce maximum of volatile substances when incubated at 26 degrees C. That is why bacterial starter produced at the mentioned temperature was used in the experiments made. In Alatauskaya top-grade cooked sausage carbonyl compounds content increases rapidly both in the exploratory and control runs during frying, while in the process of cooking the increase is not high because high temperatures decompose carbonyl compounds. If we take carbonyl compounds content after forcing as 100% in the exploratory run sausages after frying this content will increase up to 172,73% and up to 181,82% after cooking. In the control run this increase will be 170,31% and 178,13%, respectively. The total amount of carbonyl compound in the finished exploratory run sausages and control run ones is nearly the same (1,21 mg per 100 gr and 1,17 mg per 100 gr, respectively). When degustated both run sausages have got flavor and aroma marks from 9 to 12, but the exploratory run sausages have got higher marks than those of control run for appearance, consistency and cutcolour. Exploratory run sausages yield on charge has made 118÷120%. Hence, bacterial meat starter improves flavor and aroma. consistency, appearance and changes moisture-keeping properties of cooked sausage meat.

RESULTS and DISCUSSION: Total amount of carbonyl compounds in horseflesh and Kokchetauskaya first grade half-smoked sausages rapidly increases in the process of frying. This growth may be caused by the carbonyl compounds accumulation, the latter being the effect of Maillard reactions. Further temperature increase causes carbonyls decomposition or changes them to other substances, therefore at this stage of sausage production carbonyls content growth is not high. Then because of the sausage surface absorption of the smoke carbonyl compounds and as the result of the reaction between smoke components and sausage protein, carbonyl compounds content increases. Such changes have been observed during cooked-smoked sausages production as well, but their quantitative ratio was different. However, volatile carbonyl compounds content in the exploratory run was higher during all production stages than that of the control run. For instance, if we assume that carbonyl compounds content after forcing was 100%, then in the exploratory run of the horseflesh half-smoked sausage the increase has made 121,79% after shrinkage, 187,1% after frying; 206,4% after cooking; 247,4% after smoking and 253,4% after drying; in the control run the increase has made 113,92%; 175,95%; 299,11%; 232,91% respectively. Kazakhstanskaya cooked-smoked sausage carbonyl compounds content growth in the exploratory run has been after shrinkage - 122,99% (control run-144,77%), after frying - 193,11% (that of control run-175,0%), after cooking - 196,55%(control run-181,82%) , after smoking - 241,10%(control run-219,30%), after drying -249,43% (control run-227,3%). So we can draw a conclusion that lactic acids bacteria pure cultures when added to the sausage, cause an increased accumulation of volatile carbonyl compounds, thus improving flavor and aroma of finished products. It should be pointed out that in all experiments carbonyl content coefficient was not high and the results obtained, proved to be reliable. All this testifies to the fact that this method of carbonyl compounds recovery is a reproduced one. After shrinkage, acrolein, methylacrolein and hexen2-al-1 contend is slightly decreasing while the amount of other components is fairly increasing. All components content and especially that of isobutyric (36,86 mg eq. $*10^{-4}$) and crotonic (51,48 mg eq$*10^{-4}$) aldehydes, diacetyl (5,14 mg eq$*10^{-4}$) increases in the process of frying. The process is observed during further production stages and in finished products diacetyl content makes 7,55 mg eq*10 (which is 4 times more), acetoin content increases 8 times (8,92 mg eq$*10^{-4}$), isobutyric aldehyde content is $45,74*10^{-4}$, that of crotonic aldehydes-$59,26*10^{-4}$, methylethylketon content is $30,99*10^{-4}$ and capronic aldehydes content makes 16,91 mg eq$*10^{-4}$. Thus the above components seem to be most important for the full-bodied specific aroma of the finished product. Hence, we shouldn't neglect other volatile carbonyl compounds since they all influence on aroma and flavor of the product. Control run sausages contained all carbonyl compounds detected in the exploratory run sausages, but their content was considerably less, and this proves lactic acid bacteria to be very effective as aroma producing substances. Acrolein (19,87 mg eq*10^{-4}), isobutyric and crotonic aldehydes were prevailing in Kazakhstanskaya sausage after forcing. Shrinkage caused the decrease of acrolein, methylacrolein and 1 pentanolon. When frying, cooking and smoking have been performed, carbonyl compounds content increased and in the finished product their amount exeeded that of horseflesh half-smoked sausage. This could be caused by the raw quality and the production technology used. In Kazakhstanskaya cooked-smoked sausage isobutyric, butyric, crotonic and capronic aldehydes, diacetyl, acetoin, diethylketon were prevailing. In the same sausage control run containing no pure cultures of lactic acid bacteria, carbonyl compounds content was considerably lower than that of the exploratory run. The above mentioned carbonyl components were prevailing among 13 components detected in Kokchetauskaya half-smoked and cooked-smoked sausages. In Alatauskaya top-grade cooked sausage the total amount of volatile fatty acids has been constantly increasing. In the exploratory run sausage these acids content has made after forcing 8,50 mg per 100 gr, after frying -9,96 mg per 100 gr, after cooking-10,62 mg; in the control run sausages acids content has made 9,80 mg per 100gr after frying and 10,28 mg per 100 gr after cooking. In Kokchetauskaya horseflesh half-smoked sausage volatile fatty acids content growth after frying has been the result of fat hydrolysis reaction. However further production stages are characterized by a low growth value. This could be explained by changing a certain amount of free fatty acids to complex ethers, the latter being the result of the reaction between the acids and sausage alcohols. For instance, volatile fatty acids content growth in horseflesh sausage has made 10,06 mg per 100 gr after shrinkage; 10,75 mg after frying; 11,03 mg after cooking; 11,98 mg after smoking; the latter value may be the result of the sausage surface absorption of volatile fatty acids of the smoke. More rapid but the same character changes have been observed in Kokchetauskaya sausage.

CONCLUSION: Nature and quality of the raw may be supposed to influence on volatile fatty acids content. The experimental data have shown volatile fatty acids content growth in Kazakhstanskaya and Kokchetauskaya cooked-smoked sausages in their exploratory run to be higher than that of the control run. For example, in Kazakhstanskaya exploratory run sausages these acids content after shrinkage increases by 103,45%; after frying by 117,84%; after cooking by 118,68%; after smoking by 133,14%, while in the control run the acids content increases only by 129,70% after smoking. Some volatile acids qualitative composition both in the exploratory and control runs is the same and contains 9 fatty acids among which formic, acetic, valeric and capronic acids are predominating. Each acid content rapidly increases in the process of frying and after cooking acids containing carbon atoms up to C4 are reducing. For instance, Odesskaya sausage after frying contained formic acid 0,0303 mg per 100 gr, which is by 105,94% more than its content after forcing; however, after cooking formic acid content has made 0,0268 mg-eq. Further growth observed has been the result of smoking. Acetic and butyric acids both in the exploratory and control runs have undergone similar changes, their content being higher in the exploratory run sausages. Thus, the present paper has investigated the dynamics of 9 volatile fatty acids in different sausages, their content increasing rapidly in the process of drying and smoking, these growth values being always higher in the exploratory run sausages than in control runs. We may conclude that bacteria starter stimulates some volatile acids content growth and improves sausage flavor and aroma.