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EXAMINATION OF THE MICROBIOLOGICAL STABILITY AND SENSORY PROPERTIES OF HALF DRY SAUSAGES MANUFACTURED USING OF STARTER CULTURES AND SUGAR MIXTURES

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1. INTRODUCTION

The main conditions of producing food, including fermented half dry sausages are the following:

- Excellent basic material – up-to-date quality standards in production – microbiologically and toxicologically uncontaminated material.
- Products with constant excellent organoleptic properties, long shelf-life and economical production

To operate the production based on the traditional experiments is not sufficient to reach and ensure the goals mentioned above.

The use of modern principles (such as chemical production regulation, meat classification systems, Leistner obstacle principle), predictive microbiology, the HACCP, and other quality control systems is unavoidable. The producer needs as complete information as possible concerning the production. It is necessary to carry out basic experiments through a complete series of examinations – either on an international level.

The traditional production of half dry products has undergone major changes, concerning engineering and technology, in the past 20 years.

Besides OHKI, our Institute undertook a crucial role together with the predecessor of the Ringa Meat Industry Ltd., the Győr-Sopron County ÁHV in the domestic commercial contribution of raw fermented half dry products.

Our previous experiments:

- Dry product fermentation experiments with "Moson" dry sausage
- The possibilities of applying nitrite salt mixture with different admixtures (cystein-cystin, ascorbate, the effect of complexes with $Fe^{2+}$for sausage bar reddening)
- The effect of bowel diameter on dry sausage fermentation and sensory properties.
- Observation of water and salt migration between the sausage bar's core and bark during the fermentation process.
- Chemical and content change examinations were carried out during fermentation with the "Moson" dry sausage.
- We examined the changes in acid No., iodine No., peroxid No. during fermentation.
- We carried out first production experiments with the half dry sausages using traditional technology (without climatization equipment).
- We carried out production experiments with half dry sausages using import (SAGA I, SAGA II) starter cultures.
- We made 20 different experimental products using home starter cultures.
- We examined the 20 products' chemical, microbiological, sensory properties, and the amino acid composition of the final product.
- We examined the possibilities of using starter cultures isolated from domestic dry sausages.
- We created starter culture concentrates and examined their usability in the production of plant and animal fodder, or meat industry products.

Aims

The development of raw, fermented half dry sausages taken into consideration the up-to-date food production aspects:

- Examination of the effect of fast pre-cooling on the microbiological state of pig carcasses.
- The effect of processing (deboning) on for the microbiological state of the meat raw material.
- The choice of the starter culture and the quantity and quality of the sugar source applied.
- Examination of the effect of the raw pig meat (pork) and white meat from different weight classes on the product's sensory properties.
- Evaluation of the results of the production experiments carried out with various mixed cultures.
- Examinations for recording the aroma profile of the half dry sausages.

The ripening time of the traditionally produced half dry products and cured hams can be reduced using the results of the experiments, even new products can be introduced.

2. MATERIAL AND METHODS

2.1. The scene and time of research

The experiments took place in the Department of Food Technology and Microbiology, Faculty of Agricultural Sciences at Mosonmagyaróvár, Pannon University of Agricultural Sciences, (today known as the Institute of Food Science, Faculty of Agricultural Sciences, University of West Hungary). The production trials were carried out at Győr- Sopron Country, Győr ÁHV Factory (legal successor: Ringa Inc.).

The object of the experiment was a fermented sausage, called "Alpesi". We worked using the prescribed basic and secondary materials. We manufactured bulks of 6 kg (10 bars) in the laboratory model experiments. We used 100 kg of final product in each phase of the production experiment according to Table 1. After taking samples and factory and official quality control, the final products were sold.

Table 1.

Materials used for 100kg final product ("Alpesi" raw fermented sausage)

Nomination	Quantity (kg)
Pig trimmed meat	50.00
Pig chopped meat	60.00
Industrial bacon S. VII:	20.00
Total meat basic material	130.00
White pepper	0.40
Garlic powder	0.03
Coriander	0.06
Glucose	0.65
Nitrite salt mixture	3.30
Starter culture	1 box
Filling weight	134.44

The experimental production was carried out according to the prescriptions of the factory (Figure 1).

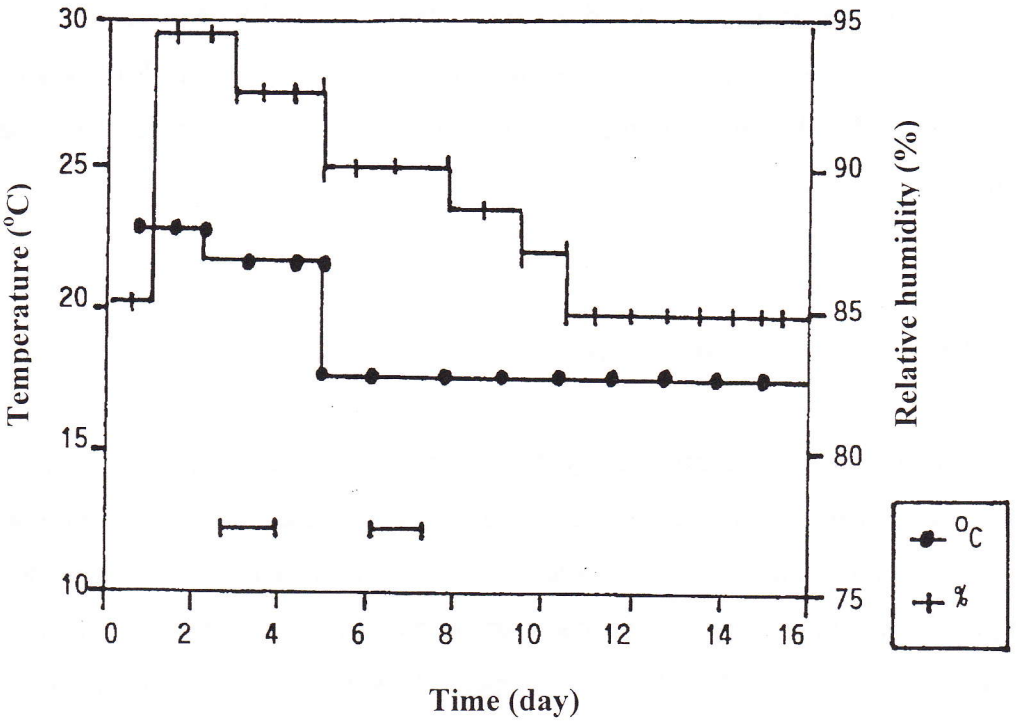

Figure 1 The temperature, relative humidity and smoking time in functional relationship with the fermentation time in the OHKI climatic

2.2 Examination of chemical composition

We applied methods generally used in the meat industry for the examination of chemical composition. The examination of water content was carried out according to MSZ 5874/4/4-80 (two parallel sample mixed with chempure silica sand, dried at 105±1 °C, until uniformity of weight). The water content is expressed in terms of mass percentage. We determined the salt content on the basis of the MSZ 5874/7-75 prescriptions (titration of chloride ions with $AgNO_3$, in the presence of K_2CrO_4 indicator).

We applied acidobutirometry for the determination of fat content (MSZ 5874/2-85).

The protein content was calculated according to MSZ 5874/8-78. The pH values of culture media and half dry sausages were measured directly with Radelkis OP-211/1, or HANNA digital pH meters, with combined glass electrode, 2 parallels from homogenized samples.

2.3 Microbiological examinations

Only bacteriologically adequate media and reagents were applied. For better comparison of the results only clearly declared medium components and analitically pure chemicals or powder soils were used. The powder soils were prepared according to the producer's instructions. Ion exchanged pure water was used for this, so it didn't contain any components which would affect the growing of microorganisms.

The number of mesophilic, aerobic, facultative anaerobic microorganisms (total plate count) was determined as follows. Decimal dilution series was made from the sample, and 1-1ml were taken from each dilution and pipetted into Petri dishes, and then appr. 18ml of 45 °C non-selective Plate-Count agar was poured onto it and stirred with constantly rotating movement (pour plate method). The solidified plates turned upside down were incubated for 72h at 30 °C water aerobic conditions. Only the plates containing 20-300 colonies were taken into consideration. The total plate count was calculated according to the following formula:

$$\bar{c} = \frac{\Sigma c}{n_1 + 0{,}1 \cdot n_2} \text{ d, where}$$

$\bar{c}$ weighted mean of colony count
Σc is the sum of all colonies counted on all dishes retained
n_1 is the number of dishes retained in the first dilution
n_2 is the number of dishes retained in the second dilution
d is the dilution factor corresponding to the first dilution.

Finally, the result was given in normal form referring 1 gram of the sample.

For the determination of the number of *Staphylococci* decimal dilution series was made from the sample, and 0,1ml were taken from each dilution and spread on the surface of selective Baird-Parker agar containing egg yolk emulsion. The Baird-Parkeragar contains sulphamethazin, 5% lithium-chloride and potassium tellurite for supressing the escorting microflora, glycin and pyruvate for selective stimulation of *Staphylococci*. The plates were incubated for 24-48 h at 37 °C under aerobic circumstances. After incubation the convex, black with white edge colonies, typical of the genus *Staphylococcus* were counted. Only the plates containing 1-150 colonies were taken into consideration. The *Staphylococcus* count was calculated according to the formula given above.

Coliform bacteria belongs to the Enterobacteriaceae family. They are capable of decomposing the lactose within 24-48h at 30 °C, producing acid and gas. To determine their count, the pour-platemethod was applied. The VRBL agar contains crystal violet and bile acid salt to supress the escorting microflora. The plates were incubated for 24h at 30 °C under anaerobic circumstances, and then the typical red colonies were counted. Only the plates containing 10-200 colonies were taken into consideration. The result was calculated as above.

The enumeration of lactose decomposing bacteria was carried out by plate pouring on differential China-blue lactose agar. The plates were incubated for 24-48h at 30 °C, and then the blue colonies– indicating lactose decomposition - were

counted. The colonies unable to decompose lactose remained colourless. Only the plates containing 10-200 colonies were taken into consideration. The final result was calculated according to the formula given above.

The determination of lactose decomposing streptococci count was also carried out using the pour-plate methodon M17 agar. This agar contains sodium-ß-glycerophosphate for increasing the buffer capacity, thus helping the reproduction of streptococci.

Elective MRS agar was used for the isolation of the genus *Lactobacillus*, which contains polisorbate-, acetate-, magnesium-, and mangan that help the reproduction of lactobacilli. The count of lactobacilli was also determined by the pour-plate method. Both in the case of the M17 and in that of MRS agar only the plates containing 10-300 colonies were evaluated. The final result was givenas above.

2.4 Sensory evaluations

The sensory evaluations were performed by institute and factory experts. For the sensory tests of the 'ready for cut' final products, the colour, smell, aroma, consistency and taste complex evaluation was carried out according to Mihajlova et al. (1988), and Roca and Incze (1989), since there is no standard sensory evaluation prescription available.

The various samples were given a code, and then a panel of 5 referees assessed judged the samples independently using a 5point rating scale.

The results of the sensory evaluations were analysed by ANOVA.

2.5 Record of the aroma profile (map)

20g from the 2cm inner core of the salami was homogenized with 50ml water in Euro-turrax stirrer at a rotation speed of 27000min^{-1}. The pulp thus obtained was distilled, and 50ml was gathered from the distillation. The components in the distillation were enriched by thesolid phase extraction (SPE) method. 20ml distillation was extracted with 200mg charge of LiChrolut RP-18. The material adsorbed on the surface of the charge was eluated with 3ml aceton. From this solution, 1 µl was injected into the GCMS device (Finnigan MAT GCQ system).

Measuring conditions:

Injector 250 °C splitless 1 min

Carrier gas: Helium 40 cm/sec

Column DB-5 MS 30 m* 0,25 mm 0,25 µ

Temperature programme: 40 °C 1 min 10 °C/min to 300 °C 300 °C 5 min

MS parameters: ion source 175 °C

Full scan 50-400 AMU

3. RESULTS

3.1 Examination of the effect of fast pre-cooling on the microbiological quality of pig carcasses

I started to examine the possibility of decreasing the microbial number in meat raw material by the microbiological qualification of the pig carcasses after slaughter. I determined sampling failure at modified tampon rub off on skinned or unskinned body surface (correction factors: 3,29 and 2,72). I marked the regions showing average microbial contamination on the half carcasses. Evaluating the results with two-way ANOVA I justified that fast pre-cooling at –15 °C for 2.5h caused a significant decline in the number of microorganisms on both skinned and unskinned half carcasses, and this level could be maintained at a refrigerator temperature of +2 °C for 96 h.

3.2 The effect of processing (deboning) for the microbiological quality of the meat raw material (pork)

I justified with phase examinations that the processing (deboning) represents a crucial point in microbial quality, i.e. total plate count, coliform count. The other critical point is the transportation of different meat parts to other factories for secondary processing, which can increase total plate count and coliform count by 1-1.5 orders of magnitude. If the meat parts get frozen after processing the total plate count will not increase. Especially clipped meat cuttings (low heat capacity, large surface) increase the microbial content of the half dry sausage basic material. Improving the hygienic conditions of processing, and

eliminating certain meat parts, the microbial content of half dry sausage basic materials can be reduced from 10^6/g to 10^4/g

3.3 The choice of starter culture and the quantity and quality of the sugar source applied

I examined during preliminary laboratory tests the *Lactobacillus plantarum* strain *L1*, and firstly world-wide the *Leuconostoc mesenteroides spp. cremoris* sucrose-fermenting strain M1, and their mixed culture's effect on half dry sausage fermentation in the case of applying glucose, sucrose and lactose sugar sources. On the basis of microbiological and sensory qualifications, 9 combinations were included in the test production.

The result of the factory experiments suggested that the safest production could be realised using *Lactobacillus L1* and 29%sucrose content. The most excellent sensory properties of mixed culture were achieved by sucrose combined sugar source. The half dry sausage made with glucose combined sugar source also proved to be very good. According to the results of ANOVA the three combinations didn't differ from one other with respect of sensory properties.

3.4 Examination of the effect of the raw pig meat (pork) and white meat from different weight classes on the product's sensory properties

The heavy (over 140 kg live weight) swine are appropriate for producing excellent quality half dry sausage. The product's rheological features, cutting and chewing resistance, colour, flavour, and smell are largely affected by the quality of the basic material.

3.5 Evaluation of the results of production experiments carried out with different mixed cultures.

One of the aims of our experiments was to determine the effect of different starter culture combinations on the microbiological quality and the sensory properties of the half ready-, or ready made products manufactured using the same basic materials under identical production circumstances.

After accomplishing the analysis of the experimental samples produced at Győr, Ringa Ltd. Factory and that of the control product, we reached the following conclusions:

On the basis of the microbiological examinations of the samples containing starter cultures, these starter cultures dominated the product on the second day of the production, ensuring its microbiological stability. They ensured the conditions needed for necessary extent of reddening and water loss with their metabolic activity (lactic acid production). The features of the control product were improper during the critical period.

According to the results of the sensory evaluation, the No. II. sample proved to be the best among the applied starter culture combinations, considering the taste and aroma properties. The samples No. III. and IV. were placed in the category of good quality. Considering the other sensory properties, the control product was found to be of good quality. However, it was ranked last ("hardly acceptable" category) owing to its empty, sour flavour.

Reddening was in functional relationship with production time. At the start of production (day 0), the intersection of all products showed some dim, light greyish coloured spots. Looking at the intersections taken on the second day from the samples, it can be said that the sample No. III. showed nice red colour, even reddening. In the case of the control sample No. I., grey ring of approx. 5-6 mm in

diameter showed next to the cover. According to the examinations carried out on production day 7, every product showed even reddening, and that was appropriate also on day 14 and 21.

3.6 Examinations for recording the aroma profile of the half dry sausages

- Samples from the whole intersection of the experimental products contained a bigger quantity of aroma components than those from the inner core of 30 mm.
- It was found that either the whole intersection or the inner core of 30 mm showed similarity in the case of all three samples.
- On the basis of the aroma map,it seems probable that it is the composition of the basic material composition and the specific endogenous enzymes determine the formation of aroma profile in half dry sausages.
- The exact identification of the aroma components was not carried out because of the costs of the extremely large number of standard compounds.

4. NEW SCIENTIFIC RESULTS

- The total plate count of the pig carcasses used for the manufacture of half dry sausages, can be reduced by 1, possibly 2 log cycles using fast pre-cooling.

- The microbiological safety of the product can be guaranteed by using fresh basic material and reducing the application of handling procedures.

- The products made from heavy swine meat and white meat ensure the half dry products' sensory, aroma properties.

- The starter culture and sugar mixture combinations examined ensure the microbiological stability of products. Besides the generally used glucose, sucrose is also capable of contributing to the production of excellent quality products.

- The use of starter culture combinations also plays a key role in the microbiological stability of the product, and is responsible for the formation of aroma components.

5. PROPOSALS

- Fast pre cooling is proposed during the primary processing of both skinned and unskinned pig half carcasses because it results in a decrease of 2 log cycles in the total number of mesophilic and aerobic microorganisms.
- Optimal conditions should be ensured throughout the whole manufacturing process, i.e. deboning, cutting, meat grading etc.
- Factory production experiments justified that the products made from heavy swine meat and white meat basic material had optimal sensory properties, so their use is suggested in the production.
- The high microbial quality of the basic material used and the proper concentration of the starter culture (10^5 cell/g basic material) are the major conditions resulting in an excellent final product.
- The glucose or sucrose added at a concentration of 0,5% causes sour taste that meets the demand of Hungarian consumers.
- We carried out aroma profile analysis (under development in our Institute) on the final products in order to determine the properties of the half dry goods. The formation of aroma properties largely depended on the composition and the quality of basic material.
- Further experiments should be carried out for the identification and isolation of compounds determining the aroma profile. Moreover we will observe the aroma formation process during the various phases of production with further development of our method.

6. PUBLICATIONS OF THE TOPIC OF THE Ph.D. DISSERTATION

6.1.Publications

6.1.1. Foreign language publications

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