Effect of fixed factors on microbiological status of some meat products

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Abstract: This study was to assess the microbiological quality of commercial meat products of Institute for Animal Husbandry, Belgrade, Serbia. In the Institute various meat products produced in a small scale type of processing namely: Institute sausage, Barbecue sausage, Debrecen, Frankfurter and Extra sausage. Investigation was conducted during three years at the Microbiology laboratory. The products were submitted for testing twice monthly. The samples were placed in the polyethylene bag and then brought in the microbiology laboratory and immediately placed in the refrigerator. The next day, the sample product was removed in the refrigerator and placed in the laboratory bench for microbial analysis to begin. For all products there are variations in the total number of bacteria (CFU/g) depending on the year and season. However, during spring season, only the differences at Extra sausage and Barbecue sausage were significant (P<0.05). During the summer season was found very significant differences (P<0.01) in extra sausage. In the autumn, the effect of year was significant at the Institute sausage, Barbecue sausage, Debrecen and Extra sausage (P<0.05). During winter, the effect of year was significant (P<0.05) in Debrecen, and very significant (P<0.01) in Extra sausage. There are some variations depending of the season, which are statistically significant (P>0.05) and (P<0.01). Largest variations were found in Extra sausage.

1. Introduction

Due to rapid changes in dietary habits of most people generally and globally the demand for meat products is increasing. Over the years, the human requirements for food have been investigated and well known that meat is the main source of protein and also contain the important sources of vitamins and minerals. There are economic, dietary and sensory aspects that make meat processing one of the most valuable mechanisms for adequate supplying animal protein to human population. Processing of meat adds value to products and gave specific flavour, taste, colour or texture components [1,2]. Including the meat products in the diet...
sometimes associated risk of food poisoning from improperly processed products. This makes it vital that companies in the food chain maintain scrupulous standards of hygiene and are able to assure customers of the safety of their products [3]. In the Institute of Animal Industry various meat products produced in a small scale type of processing namely: Institute sausage, Barbecue sausage, Debrecen, Frankfurter and Extra sausage. The above mentioned products were classified as semi–durable meat products (raw-cooked meat products) based on official Gazette of Former Republic of Yugoslavia No. 26/93. The basic processing technologies of these meat products such as cutting and mixing are accompanied by various additional treatment that involve curing, seasoning, filling into casings, smoking, cooking. Microbiological analysis is an established tool in controlling the safety and quality of processed meat products. Recent advances have reinforced the role of microbiological testing of foods in food safety management [4]. Microbiological quality of such meat products is very important to be considered with regards to health significance of the consumer. Microbial food safety has emerged to be global concerns [5, 6, 7]. In this regard, some authors recommend the use of microbial testing to evaluate critical control points and good hygiene practices [8, 5, 9, 10]. The aim of the investigation is to evaluate the sanitary safety of the said products and to determine the effect of season and year as fixed factors on the microbiological status of semi-durable meat products.

2. Material and methods
Investigation was conducted during three years at the Microbiology laboratory of the Institute of Animal Husbandry Belgrade Serbia for the testing of the five (5) semi-durable meat products (raw-cooked meat products) namely: Institute sausage, Barbecue sausage, Debrecen, Frankfurter and Extra sausage.

2.1. Preparation of the raw cooked meat products (semi-durable products)
The basic processing technologies of these meat products such as cutting and mixing are accompanied by various additional treatment that involve curing, seasoning, filling into casings, smoking, cooking. The product component muscle meat, fat and non-meat ingredients are processed raw by comminuting and mixing resulting viscous mix then portioning in sausages, there after submitted to heat treatment smoking or cooking. These raw-cooked meat products are manufactured and marketed as sausages in small and large casings and their processing technology is different from all other processed product because of the utilization of comminuting equipment such as grinder and bowl cutter as essential in their manufacture.

2.2. Samples for the study
The five mentioned products were submitted for testing twice monthly. The samples were placed in the polyethylene bag and then brought in the microbiology laboratory walking distance about 50 meters away from the meat processing area and immediately placed in the refrigerator. The next day, the sample product was removed in the refrigerator and placed in the laboratory bench for microbial analysis to begin.

2.3. Sample preparation and analysis
Basic dilution was prepared at a ratio of (1:10). About 20 g of each product cut from the surface with the use of sterilized scalpel and scissors with forceps was mixed into a bottle with 180 ml of saline. For testing of Salmonella the 25 g of each product also cut into small pieces with forceps and was mixed to a bottle of 225 ml with selenit broth. Bottles were homogenised in homogenizer for 10 minutes. The bottle with selenit broth is left in the thermostat set to 37°C for 18-24 hours. Serial solutions were made up to 10⁴ using sterile universal bottles (test tubes) and pipettes. The selective media (table 1) were prepared according to the manufacturer’s instructions and used to culture for the presence of micro-organisms. Each tube with a selective substrate and 1 ml of the prescribed dilutions (based on Regulation on Microbiological Safety of food in trade-official Gazette of Former Republic of Yugoslavia No. 26/93).
2.4. Microbiological Analysis
For determination of the number of living organisms in a sample, cut a piece of 20g meat product using sterilized scalpel and scissors with forceps and transferred into a bottle with 180 ml of saline. The diluted sample is released from the pipette onto the solidified agar and spread on the surface by the use of sterile bent glass stick. In a test tube rack set 3-4 specimens per 9 ml of physiological solution. Per 1 ml micropipette shift in petri dish as the 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} dilutions using two petri dish to get the average so the sample have 3 petri plates. Then poured into each plate 10-15 ml of medium for the total number of bacteria thoroughly mixed by rotating the petri dish. Incubate for 72 hours at 30°C. After 72 hours of incubation at 30°C is the reading of the number of bacteria that valid anywhere in the Petri dish is between 30 to 300 bacterial colonies from the dilution and sow two Petri plates to draw an average.

**Table 1.** Microbiological culturing and examining of micro-organisms.

| Micro-organisms | Selective enrichment of the solid surface | Selective medium used | Method of culture | incubation temp.°C | Duration of incubation |
|-----------------|------------------------------------------|-----------------------|-------------------|-------------------|-----------------------|
| Total bacterial counts | NA | NA | Spread plate | 30°C | 72 hours |
| Escherichia coli | BZLŽB | VRBA | Pour plate | 44°C | 24 - 48 hours |
| Proteus | NB | BGA | Spread plate | 37°C | 24 - 48 hours |
| Staphylococcus aureus | SNB | BPA | Spread plate | 37°C | 24 - 48 hours |
| Clostridia | KTB | SFA | In tubes 14 cm. | 37°C | 24 - 48 hours |
| Salmonella | SB | XLD agar, SD agar | Spread plate | 37°C | 24 - 48 hours |

BZLŽB: Brilliant green lactose bile broth; NB: Nutrient broth ; SNB: Salty nutrient broth; KTB: Kitt Tarozzi broth; SB: Selenit broth; NA: Nutrient agar; VRBA: Violet red bile agar; BGA: Brilliant Green agar; BPA: Baird Parker agar; SFA: Sulfite agar; XLD: Xylose lysine deoxycholate agar; SD: Salmonella differential agar.

2.5. Statistical analysis
All obtained values of microorganisms counts were first transformed using base 10 logarithm for analysis and then used for GLM procedure processing. Statistical analysis was conducted using the software program SPSS [11] and following linear model.

\[ Y_{ijk} = \mu + G_i + S_j + e_{ijk} \]

where:
- \( Y_{ijk} \) - value of observation during jth season in ith year;
- \( \mu \) - Overall mean;
- \( G_i \) - Fixed effect of year;
- \( S_j \) - Fixed effect of season;
- \( e_{ijk} \) - Effect of random error.

3. Results and Discussion
Results of microbiological analysis of all examined final products are shown in table 2.
Table 2. Microbiological analysis of all examined final products.

| Microbiological Parameters | Frequency | Sampling Method | Interpretation of results |
|----------------------------|-----------|----------------|---------------------------|
| Salmonella                 | 3 (1 sample) | Destructive method on 25g/ml | Salmonella absent in 25g/ml |
| Coagulase-positive Staphylococci | 3 (1 sample) | Destructive method on 25g/ml | Coagulase-positive staphylococci absent in 0.01 g/ml (2) |
| Clostridia                 | 3 (1 sample) | Destructive method on 25g/ml | Clostridia were not present in 0.01 g/ml (2) |
| Proteus                    | 3 (1 sample) | Destructive method on 25g/ml | Proteus species not found in 0.001 g/ml (3) |
| E. coli                    | 3 (1 sample) | Destructive method on 25g/ml | E. Coli is not present in 0.001 g/ml (3) |

On the table can see that there were no Salmonella, Coagulase-positive Staphylococci, Clostridia, Proteus, and E. coli, detected from all tested products.

The total number of bacteria was expressed in CFU / g (table 3 and 4). In table 3, can see that for all products there are variations in the total number of bacteria depending on the year and season. However, during spring season, only the differences at Extra sausage (all differences) and Barbecue sausage (differences between the first and second year), were significant (P<0.05). During the summer season was found very significant differences (P<0.01) in extra sausage between the first and second and between second and third years of study.

In the autumn season (table 4), there are greater differences between the observed years. The effect of year (table 4) was significant at the Institute sausage, Barbecue sausage, Debrecen and Extra sausage (P<0.05). During winter, the effect of year (table 4) was significant (P<0.05 in Debrecen, and very significant (P<0.01) in Extra sausage.

As for the impact of the season, as shown on table 3 and 4, there are some variations, which are statistically significant (P>0.05) and (P<0.01). Largest variations were found in Extra sausage.

Therefore, could be concluded that a greater impact on the microbiological status of the tested products has on year, than season. It can be connected prior to hygienic requirements in production and not with the potentially greater presence of bacteria in a certain season.

Microbiological studies carried in several countries have reported high bacterial counts in foods [12]. Wei et al., 2006, state that in their research, the number of bacteria in meat products ranged from 1.55-5.81 CFU / g, indicating that the results of our research is more homogeneous and that are within the norms of our legislation [13-15].

Table 3. The effect of year during spring-summer seasons on microbiological status of meat products (log_{10} CFU/g).

| Season       | Product         | Year I | ±S.E. | Year II | ±S.E. | Year III | ±S.E. |
|--------------|-----------------|--------|-------|---------|-------|----------|-------|
| Institute sausage |                 | 3.946  | 0.413 | 3.482   | 0.096 | 3.453    | 0.091 |
Microbiological quality problems in donair depend largely on the following factors: low initial quality of raw meat and/or other ingredients, inefficient cooking process, improper sanitary practices for personnel, and for cooking/processing utensils [16,17].

The total plate count is a good indicator for the total bacterial load of the above mentioned varied products. Sometimes, raw or industrially prepared meat products are a favorable environment for developing microorganisms. Therefore, the microbiological analysis are important sanitary indicators, which emphasizes hygiene in processing and handling of products [18]. In this regard requires a constant examination by the producers themselves, so that their customers were protected [3,19].

Other studies of microbiological quality of meat products, carried out by many authors, stating that the spectrum of microbial susceptibility depends on a number of factors such as type of microorganism, molecular weight, deacetylation degree of chitosan; temperature and pH of medium [20,21,22,23].

Table 4. The effect of year during autumn-winter seasons on microbiological status of meat products (log\(_{10}\) CFU/g).

| Season | Product                      | Year I |          |               | Year II |          |               | Year III |          |               |
|--------|------------------------------|--------|----------|---------------|---------|----------|---------------|----------|----------|---------------|
|        |                              | Mean   | ±S.E.    | Mean          | ±S.E.   | Mean     | ±S.E.         | Mean     | ±S.E.    |               |
| Autumn | Institute sausage            | 3.190  | 0.243    | 3.910         | 0.045   | 3.345    | 0.045         |         |          |               |
|        | Barbecue sausage             | 4.390  | 0.088    | 4.425         | 0.114   | 3.624    | 0.114         |         |          |               |
|        | Debrecen                     | 2.650  | 0.650    | 3.300         | 0.045   | 2.645    | 0.045         |         |          |               |
|        | Frankfurter                  | 3.070  | 0.076    | 3.120         | 0.371   | 2.733    | 0.371         |         |          |               |
|        | Extra sausage                | 2.655  | 0.185    | 3.640         | 0.020   | 3.090    | 0.020         |         |          |               |
|                  | Winter          |          |          |          |          |          |
|------------------|-----------------|----------|----------|----------|----------|----------|
|                  | Institute sausage | 3.630    | 0.160    | 3.095    | 0.153    | 3.676    | 0.153    |
|                  | Barbecue sausage | 3.826    | 0.202    | 4.406    | 0.076    | 4.196    | 0.076    |
|                  | Debrecen        | 2.580    | 0.110    | 2.235    | 0.391    | 3.255    | 0.391    |
|                  | Frankfurter     | 2.530    | 0.271    | 2.645    | 0.179    | 3.111    | 0.179    |
|                  | Extra sausage   | 2.320    | 0.015    | 4.185    | 0.020    | 3.860    | 0.020    |

4. Conclusion

Our investigation showed that CFU/g in meat products are varied depending on the year and season. There are variations in the total number of microorganisms in all the products, but not all significant. However, some produce, such as Extra sausage and Barbecue sausage, were proved greater variation in the microorganisms. What is most important in concern of consumers, is that all the meat products we tested, are produced hygienically, because not detected any presence of Salmonella, Coagulase-positive staphylococci, Clostridia, Proteus species and E. coli.

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