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GROWTH AND ACTIVITY OF Str.faecalis ISOLATED FROM PASTEURIZED CANNED MEATS AND CHANGES IN THEM AFTER RADURIZATION WITH DIFFERENT DOSES OF GAMMA RAYS

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РЕЗЮМЕ

Исследованы консервы "Прест порк", инокулированные Стрептококус фекалис в экспоненциальной и стационарной фазах развития. Проведена пастеризация в течение 30 мин. при 65,5°С с последующим облучением гамма-лучами /0,05 и 0,2 Мрад/. Изучена динамика развития и дыхательная активность штаммов Стрептококус фекалис, изолированных из консервов во время их хранения при 4°С и при комнатной температуре в течение 6 месяцев. Установлены изменения в активности некоторых энзимных систем в изолированных штаммах и в продукте. Прослежены органолептические изменения во время хранения консервов.

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On a étudié des conserves "pressed porc", inoculées au streptococcus fecalis dans des phases de développement exponentielle et stationnaire. On a effectué une pasteurisation au cours de 30 min. à 65.5°C, suivie d'une irradiation aux rayons gamma aux doses de 0.05 et 0.2 Mrad. On a étudié l'intensité de développement, ainsi que l'activité respiratoire des souches streptococcus fecalis, isolées des conserves, au cours de leur stockage à 4°C et à une température d'ambiance pendant 6 mois. On a aperçu des modifications de l'activité de certains systèmes d'enzymes dans les souches isolées et dans le produit. Les modifications organoleptiques au cours du stockage des conserves sont aussi mentionnées.

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Investigations were carried out on canned pressed pork inocculated with Streptococcus faecalis in exponential and stationary growth phases. Pasteurization for 30 min. at 65.5°C was effected, followed by gamma-ray irradiation at levels of 0.05 and 0.2 Mrad. Growth dynamics and breathing

activity were studied for the strains of Streptococcus faecalis isolated from the cans during their storage at 4°C and at room temperature for 6 months. Changes in the activity of some enzymatic systems in the strains isolated and in the product were determined. Organoleptic alterations during the storage of cans were followed.

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Es wurden Konserven "Pressed porc" untersucht, die mit Streptococcus faecalis in der exponentiellen und stationären Entwicklungsphase beimpft waren. Nach einer 30 Minuten langen Pasteurisation bei 65.5°C wurde eine Bestrahlung mit Gamma-Strahlen in Dosen von 0.05 und 0.2 Mrad durchgeführt. Es wurden die Entwicklungsdynamik und die Atmungsaktivität der aus den Fleischkonserven isolierten Stämme von Streptococcus faecalis während ihrer Lagerung bei 4°C und bei einer Zimmertemperatur im Verlauf von 6 Monaten untersucht. Es sind die Veränderungen der Aktivität einiger Enzymsysteme in den isolierten Stämmen und im Produkt selbst festgestellt worden. Ausserdem wurden die sensorischen Veränderungen während der Konservenlagerung verfolgt.

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The established thermostability of the enterococci (11, 12, 14) and the different opinions about their role for storage and formation of the organoleptic quality of canned meat products, make it necessary to investigate the influence of a combined treatment on those microorganisms (2, 4, 9).

The scope of the present investigations is to establish the influence of the pasteurization temperature as well as the combined influence (thermal treatment and gamma irradiation° on the viability and activity of Str. faecalis in canned meat products and some changes in the biochemical indexes, organoleptic qualities and shelf life of the product, appearing under refrigeration and room temperature of storage.

Material and Methodics

As test material we used Pressed Pork innoculated with clean culture of resting cells of Str. faecalis in the exponential and stationary phase of development, obtained by a method described by us (6, 7) in quantity 10^5 in g/product, well mixed in the total mass of the product. The canned products in 300 g cans were pasteurized under a regime which guarantees 65,5°C for 30 minutes in the geometric center. About 8 hours after pasteurization, from the tested products one part were left unirradiated and the rest were irradiated with gamma rays with dozes of 0.05 and 0.2 Mrad at room temperature with a gamma source ^{60}Co and strength of the dose of 0.8 Mrad/hour. Test and controls of each variant were kept at refrigerated (2 - 4°C) and room temperatures. From each variant were obtained samples for microbiological abd biochemical investigations and organoleptic evaluation immediately after production, on the third, fourth, fifth and sixth month.

With the microbiological investigations we used MPB with 0,5% glucosis and 0,04% K_2HPO_4, nutritive media Chaina-Pery for the isolation of Str. faecalis. The strains of Str. faecalis from the canned products were cultivated and prepared for biochemical analyses after a method developed by us (6, 7). We followed the changes of the peroxydase and catalase activity of the product. From suspensions of isolated cells Str. faecalis in the exponential and stationary phase we investigated the respiratory and peroxydase activity.

The catalase activity was established immediately in a homogenate from the product obtained after the modified method of Krainev (1), and on the supernatant of homogenate the activity of peroxidase after the modified method of Popov (3). The respiratory activity of the Str. faecalis cells we established after the method of Warbourg (13).

The results from the biochemical investigations are statistically treated after the methods of variation analyses (5). The difference of the mean values of the separate indexes in the controls and test products and the isolated strains we

established to a degree of probability P = 0,05/= 95%). The organoleptic changes during the storage of the canned products were also followed.

Results and Discussion

With the microbiological investigations immediatly after the combined treatment we isolated Str. faecalis only from samples irradiated with 0,05 Mrad, independant from the phase of development of the introduced in the canned product strain. With storage under refrigeration (2 - 4°C) for 3, 4, 5, and 6 month, following irradiation with 0,05 and 0,2 Mrad of the canned products, we could not isolate Str. faecalis from the different variants. Str. faecalis we isloated only from unirradiated, innoculated in the stationary phase canned products after 4, 5, and 6 month of refrigeration storage. These results confirm our past investigations (6, 7) for the presence of a post-irradiation effect of mortality in storage of irradiated cells of Str. faecalis under refrigeration conditions. The isolation of Str. faecalis from unirradiated products, innoculated with culture in stationary phase, only after 4, 5 months of storage under refrigeration conditions, demonstrate the thermostability of the strain and elongation of the lag phase after thermal treatment (10).

After storage for 3 months at room temperature, we isolated Str. faecalis only from unirradiated products, innoculated with cells in the exponential phase and from irradiated, with 0,05 Mrad in the stationary phase. To the end of the sixth month we could not isolate Str. faecalis from products innoculated with cells in the exponential phase and irradiated with a dose of 0,2 Mrad.

The lack of Str. faecalis for six month from all variants of irradiated canned products, kept under refrigeration conditions and from the irradiated with 0,2 Mrad innoculated with cells in the exponential phase of development, kept at room temperature, we can explain by the increase in the bacteriostatic effect of the combined action - pasteurisation, gamma irradiation and refrigeration conditions in the

first case, and increased radio sensitivity of the young cells, in the second.

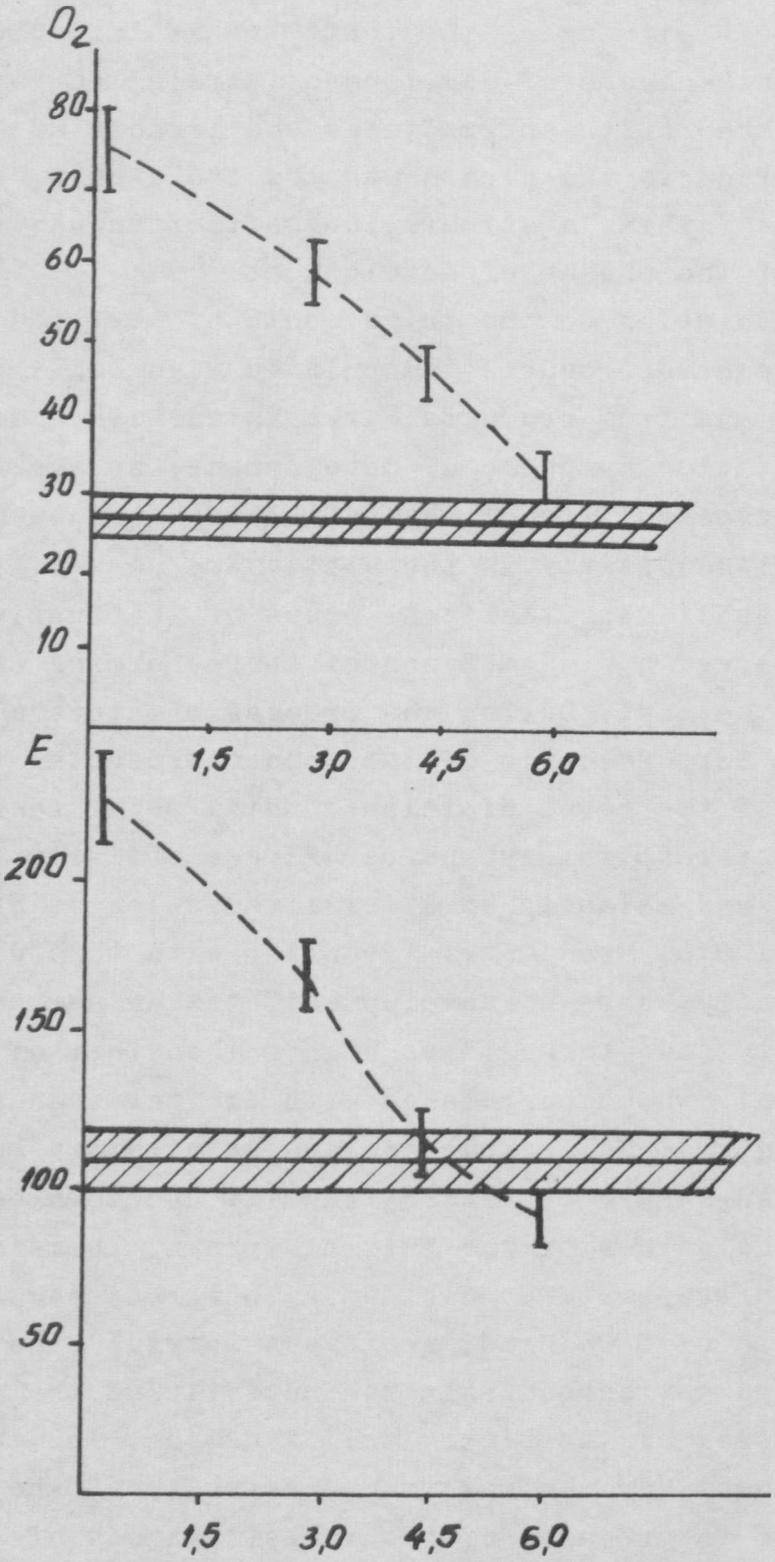
At the beginning of the test, the isolation of the introduced in both phases of development strain, only with the dose of 0,05 Mrad (with unirradiated and irradiated with a higher dose canned products the strain was not isolated), demonstrates that this dose exibits a stimulatinng effect upon Str. faecalis, independent of the phases of development.

The isolation on the third month of Str. faecalis, from unirradiated canned products innoculated with cells in the exponential phase and from products, irradiated with a dose of 0,05 Mrad in the stationary phase of development, speaks for a higher degree of thermostability of the cells in the exponential phase, and lower radiosensitivty in the stationary phase.

In establishing the total count of the survived microflora, we observed a predominance of spore forming cells at the beginning of the test. During the process of storage after innoculation with Str. faecalis of the canned products, their quantity in most of the cases diminished until total lack.

Highest respiratory and peroxidase activity of the isolated strains was established after irradiation of Str. faecalis with a dose of 0,05 Mrad. Str. faecalis, with this dose, isolated in stationary phase of development, has an augmanted respiratory activity (two-three times higher than that of the initial control strain), which correlated with its peroxydase activity ($P = 0,05$)(fig. 2). The peroxydase and respiratory activities of the isolated strain decrease gradually and towards the sixth month reach the values of the initial strain. Therefore, the pasteurization temperature combined with irradiation with gamma rays and a dose of 0,05 Mrad, increases manyfold the respiratory activity and correspondingly the activity of the peroxidase in the preocesses of the biological oxydation. The influence of the applied doses, on the peroxydase activity of the cells, is best seen from the results of the investigations after 4 and 5 months of storage, when the values of peroxydase activity of the irradiated with 0,05 Mrad and unirradiated cells are almost the same, while those of the irradiated with 0,2 Mrad are about twice higher.

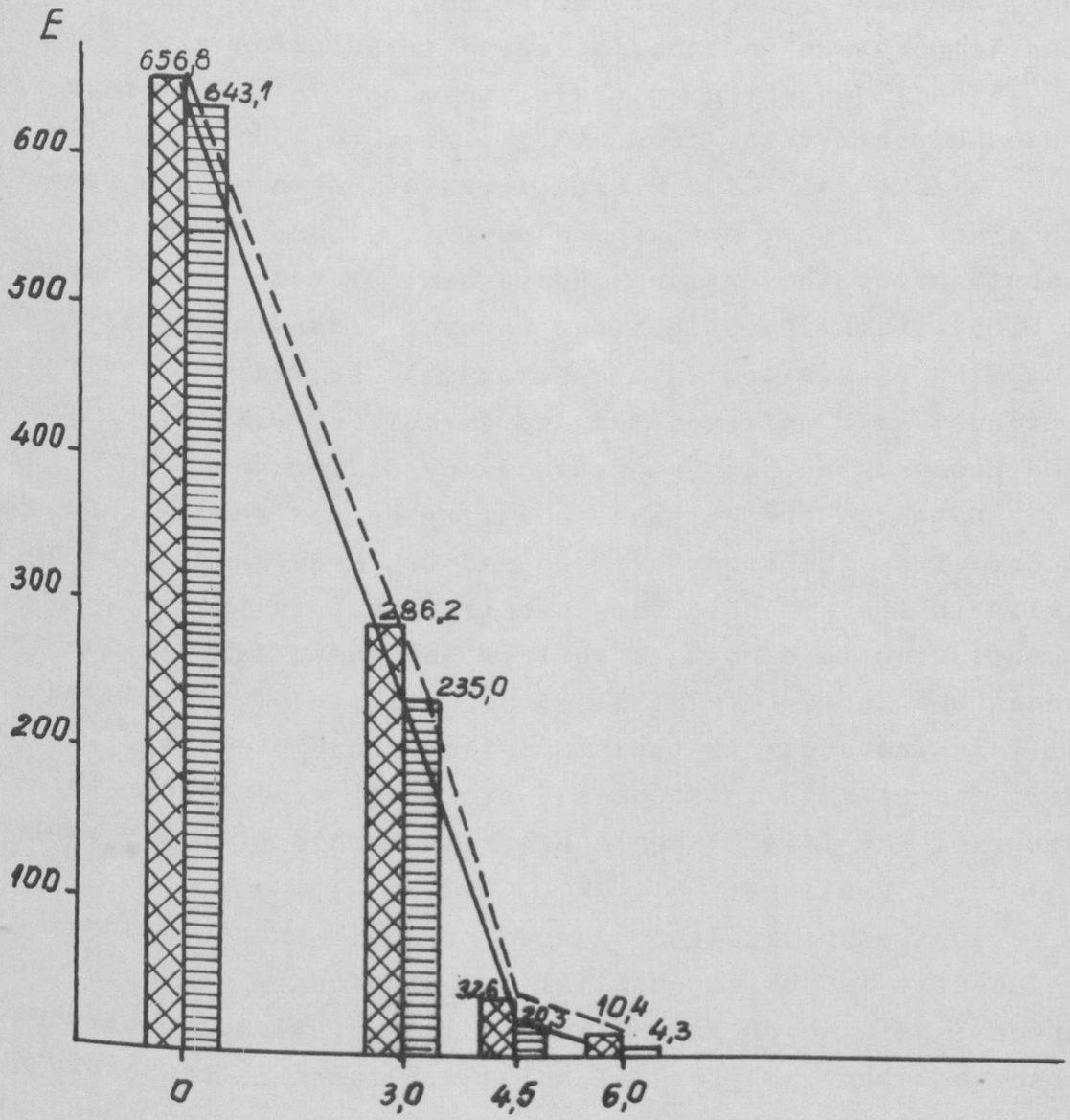
Respiratory (mm^3/mg/hr) and peroxydase activity of Str.faecalis innoculated in stationary phase and irradiated with 0,05 Mrad



mean values and reliable interval for the initial strain of Str.faecalis

mean values and reliable interval of isolated from the product strain of Str.faecalis

Mean values of peroxydase activity (E in 1 g product) from controls and product irradiated with doses of 0,05 and 0,2 Mrad in storage



mean values of peroxydase activity of irradiated product with a dose of 0,2 Mrad

mean values of peroxydase activity in controls and canned meat, irradiated with a dose of 0,05 Mrad

Parallel with the investigations on the development and activity of the isolated strains of Str. faecalis, we investigated also the changes with certain enzymes, developped in the product during its storage.

The peroxydase activity of the product demonstrates a gradual decrease immediately after the action of the pasteurization temperature and the following irradiation with gamma rays, which is observed on the sixth month in the storage of the canned meat (mean, from 643,1 $\pm$ 21,2 to 4,3 $\pm$0,29)(fig.3).

With a dose of 0,2 Mrad irradiation in canned products is observed a higher peroxydase activity, best expressed on the 3rf month after the action, independent of temperature impact (P = 0,05). With investigations however, immediately after the action, the escalation in the peroxydase activity is only of the product with unirradiated and irradiated with 0,05 Mrad canned products do not demonstrate any differences.

Data for the catalase activity do not demonstrate any influence from the doses of 0,05 and 0,2 Mrad gamma rays on its activity in the product. The investigations in this direction are continuing to elucidate whether the gamma irradiation influences the factors regulating the activity of the enzyme.

In the controls (unirradiated canned products, in which no Str. faecalis is introduces), stored at room temperature, even during the first 3 month are established strongly expressed signs of spoilage.

In the unirradiated canned products innoculated with Str. faecalis in the exponential and stationary phases of development, with which in the direct innoculations on hard media we pbserved abundant groth of only Str. faecalis and could not isolate other microorganisms including spore forming ones, after 6 months of storage at room temperature we could not establish signs of spoilage. This fact confirms the antagonistic action of Str. faecalis on the rest of the microflora and the significance of the enterococci for the durability of the canned meat products (8).

The controls stored under refrigeration, after the third mont have a taste of uncured salted pork meat, which on the 6th

month acquired an acid taste while the consistency is not changed which is characteristic for an uncured product.

Best organoleptic qualities (taste of a riped product and tender consistency - we established after 3 month of storage with unirradiated products, innoculated with Str. faecalis in the exponential phase. With the irradiated with 0,2 Mrad and stored for the same period of time, canned products with introduced cells of Str. faecalis in the exponential phase is felt a slightly different taste ofan irradiated product, without the characteristic taste of riped ham, while with the same, but with cells in the stationary phase, predominant is the taste of a riped product. The irradiated, but with a dose of 0,05 Mrad, products after three month of storage, their organoleptic indices are nearer to those of the unirradiated innoculated canned products.

After 4 to 5 month of storage, all test variants have a taste of riped product, expressed to a different degree, while the consistency is best expressed with the unirradiated canned products with innoculate in the exponential and stationary phases.

On the 6th month, best expressed organoleptic qualities of a riped product, have the unirradiated products innoculated with Str. faecalis in the exponential phase followed by those in the stationary phase and the irradiated with a dose of 0,05 Mrad on both phases of development.

The colour of all samples innoculated with Str. faecalis during the whole time of storage is better than that of the controls.

Conclusions

1. The pasteurization temperature retards the development of Str. faecalis in canned meat products, which fact is strongly expressed with cells introduced in the stationary phase of development.

2. With combined traetment of the canned products (thermal treatment and gamma irradiation) with a dose of 0,2 Mrad is prolongated the bacteriostatic action on Str. faecalis, while with a dose of 0,05 Mrad no bacteriostatic effect is observed.

3. Str. faecalis exibits higher thermal stability in the exponential phase of development and higher radiostability in the stationary phase of development.

4. The refrigeration storage after combinded treatment leads to a prolongation of the bacteriostatic activity.

5. The quantity of sporeforming forms decreases to a total lack during the process of storage of innoculated with Str. faecalis canned meat products.

6. The combined action with a dose of gamma irradiation of 0,05 Mrad increases manyfold the respiratory and peroxydase activity of Str. faecalis.

7. The peroxidase activity of the canned product is lowered during the storage period, while after irradiation with a dose of 0,2 Mrad it increases independant from the temperature of storage.

8. Best organoleptic qualities are established for refrigerated storage of unirradiated innoculated with Str. faecalis canned meat products, and worst - with the controls.

9. At room temperature the controls have well expressed signs of spoilage, while innoculated products are safe to the 6th month in the cases when abundant growth if Str. faecalis is observed without the development of other microorganisms.

10. After irradiation with a dose of 0,05 Mrad, the organoleptic qualities of the product are close to those of the unirradiated innoculated canned products, while with a dose of 0,2 Mrad is established a side-taste of an irradiated product.

L i t e r a t u r e

1. Kraynev, A., 1962, "Biochimia", 27, 5, 780
2. Klima, D., Petrichek, M., Houynyakova, M., 1970, XVI Congress na naouchnite rabotnizi po mesoto, Varna, Bulgaria, 413
3. Popov,T., Heykovska, L.,1971, Gigiena i sanitaria,10,89-92

4. Samoylenko, I., Ivanov, K., 1972, GMEI, 2, 113-116
5. Sepetmeev, D., 1968, Medyzinska statistika, Med. i fiskultura, Sofia
6. Stoychev et al., 1972, Sbornik dokladov, Konsultazia SEV, Sofia
7. Stoychev M., Brankova, R., Djejeva, G., 1972, II Nazionalna konferenzia po ispolsvane lachenyata v biologiata i selskoto stopanstvo, Sofia
8. Kafel, S. & Ayres, J.C., 1969, J. Appl.Bact. V. 32, 2, 217-232
9. Oberman, Helena, 1968, Zesz. neuk Politechn. lodzk,116,5-76
10. Stoychev, M., Djejeva, G., Ilieva, R., 1971, 17th Eur.Meet. of Meat Res. Work., Bristol
11. Stoychev, M., Djejeva, G., 1971, 17th Eur. Meet. of Meat Res. Work., Bristol, pp. 267-274
12. Shaumon, E,L., Reinbold, G.W., Clark, W.S., J. Milk and Food Technol., 33, 5, 192-196
13. Umbreit, W.W. and al., 1949, 2nd Edition, Burgess Publishing CO;, Minneapolis
14. Zivanovic, R., Oluski, A., 1965, Tadic Zivka, Technologia mesa, Beograd, 7-8, pp.198-204