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INFLUENCE OF SOME PASTEURIZATION TEMPERATURES ON THE DYNAMICS OF EVOLUTION AND BREATHING ACTIVITY OF STR.FAECALIS

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Summary,

Studied is the influence of different temperatures on phase growth prolongation of Str.faecalis. A colorimetric method is used for the estimation of biomass growth of Str.faecalis in the separate growth phases, based on cell carbon contents.

Established is the endogenic and exogenic breathing activity of the strain during the different phases of its growth, under the influence of different temperatures.

Demonstrated is a correlation between the acting temperatures, length of evolution phases, biomass growth, and breathing activity of Str.faecalis, based on which, valuable conclusions could be made, having direct connection with production, evaluation and storage of pasteurized canned meat.

ВЛИЯНИЕ НЕКОТОРЫХ ПАСТЕРИЗАЦИОННЫХ ТЕМПЕРАТУР НА ДИНАМИКУ РАЗВИТИЯ И ДЫХАТЕЛЬНУЮ АКТИВНОСТ КЛЕТОК STR.FAECALIS

Резюме

Исследовано влияние различных температур на продолжительность фаз развития Str.faecalis.

Применен колориметрический метод для определения прироста биомассы Str.faecalis в отдельные фазы его роста по содержанию клеточного углерода.

Установлена активность эндогенного и экзогенного дыхания штамма в отдельные фазы его развития при нормальных условиях и после воздействия различных температур.

Доказана зависимость между температурами воздействия, продолжительностью фаз развития, приростом биомассы и дыхательной активностью клеток Str.faecalis, на основе которой сделаны важные выводы, имеющие особенное значение для производства, оценки и хранения пастеризованных мясных консервов.

DER EINFLUSS EINIGER TEMPERATUREN DER PASTEURISATION AUF DIE DYNAMIK DER ATMUNGSAKTIVITÄT BEI STR.FAECALIS

Zusammenfassung

Untersucht wurde der Einfluss unterschiedlicher Temperaturen auf die Dauer der Entwicklungsphasen bei Str.faecalis.Nach dem Kohlenstoffgehalt der Zellen wurde kolorimetrisch die Zunahme der Biomasse bei Str.faecalis bestimmt.Ausserdem wurde die Einwirkung der unterschiedlichen Temperaturen auf die Aktivität der endogenen und exogenen Atmung des Stammes in den einzelnen Entwicklungsphasen erforscht.Dabei wurde Abhängigkeit der wirkenden Temperaturen bei den einzelnen Entwicklungsphasen von der Zunahme der Biomasse und der Atmungsaktivität bei Str.faecalis nachgewiesen,was von entscheidender Bedeutung für die Herstellung,Beurteilung und Aufbewahrung der pasteurisierten Konserven ist.

Investigation of the sensibility for the different kinds of microorganisms to temperature influence, presents, undoubtedly, a big interest from the stand point of science and practice.

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In the production of meat products, tha character of the remaining flora depends in lots of cases on the method and effectiveness of the thermal treatment (sterilisation and pasteurization). A significant problem in the production of canned products (ham, shoulder, pressed ham) submitted to thermal treatment under relatively low temperatures, is the isolation of faecal Streptococcus. Accoding to some authors (1,3,5,7,8,10), their presence in the microflora of the meat products is by no means a rare occurence. In literature exist data for the thermal resistance of faecal Streptococcus to higher temperatures of 70°C (6,10,12). However, rests unclear the thermal regime, which renders a total bactericidal action possible and the mechanism of augmenting the sensibility of these microorganisms to heating.

The scope of the present investigation is the study of some pasteurization temperatures and their influence on the dynamics of evolution and breathing activity of Str.faecalis.

Methodics.

The studies were made with culture of Str.faecalis var.liquefaciens, strain 775 having the following biochemical characteristics : grows in broths having a pH value of 9,6; NaCl 6,5% and metylenblau 1%; does not haemolyse blood agar; changes mannite, gelatine, glycerine, arginine, lactose and sorbite; does not change arabinose; grows at 45°C and after heating to 60°C for 30 minutes as well. Acid dggree after Törner - 97°.

The cultivation was made in Erlenmeyer flasks of 750 ml volume, on liquid broth with pH 7,2 containing : 10 g pepton (Merck); yeast extract (Oxoid) 10 g; 5 g NaCl; 10 g glucose; 400 g K_2HPO_4 all diluted in 1 l. water.

As solid nutritive media we used the same broth solidified by addition of 1,5 to 2% ag ar-agar.

For innoculation material we used 18 hours broth culture, received at 37°C. The microorganisms were separated from the media by centrigugation and double washing in physiological serum. The obtained bacterial mass was suspended in physiological serum and in this form was used for innoculation of the nutritive media. The suspension was heated for 30 minutes at 58°, 62°, 65.5°, 69° and 73°C. From the heated, and not heated suspensions, we innoculated with 5 ml, Erlenmeyer flasks contaning liquid nutritive media in the quantity of 250 ml. Cultivation of the innoculums was for 24 hours at 37°C under shaking.

Samples were taken each hour, beginning with the moment of the innoculation to the 24th hour, which samples, centrifugated and twice washed in physiological solution were used for following the dynamics of evolution and breathing activityof the investigated strain.

The growth of the biomass, during the separate phases of evolution, we determined colorimetrically, based on the quantitative determination of cell carbon, which method we used with eneterococci (9).

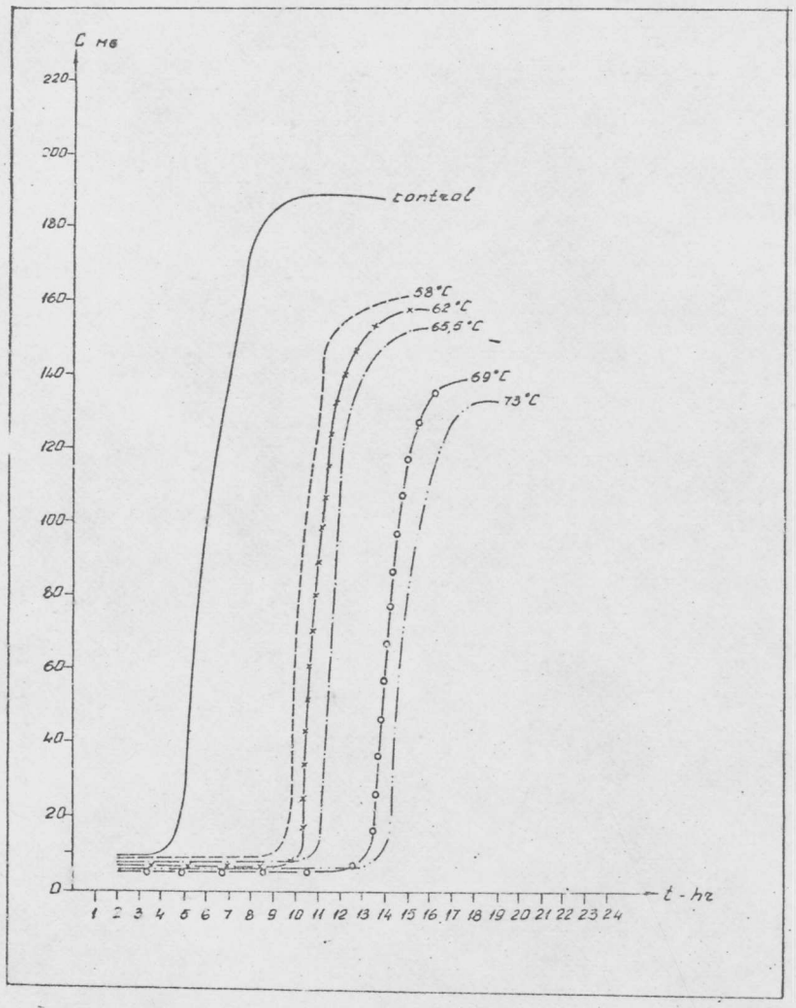
Oxygen consumption of the resting Atr.faecalis cells, was determined manometrically after the method of Warburg (11). In each Warburg vessel we put 1 ml suspension from the cells, 1.5 mlphosphate buffer (pH 7.4), in the inner vessel was poured 0,2 ml,20% KOH solution, and to this were added small paper cuts, which help retain the KOH during shaking of the vessels. When the endogenic breathing was determined in the side arm of the vessels 0,5 ml phosphate buffer was added; when studying the exogenic breathing, in the side arm was added 0,5 ml of the corresponding substrate. Incubation temperature was 28 ± 0.1°C. Cell breathing was determined in two parallel vessels, while each test was repeated four times.

Results and Discussion.

On graph 1 are determined the curves of growth and evolution of the culture of Str.faecalis,var.liquefaciens on not heated and heated to different temperatures of pasteurization culture.

Data from the investigations show, that non heated bacteria are in lag-phase during the first four hours of their cultivation and enter in logarithmic phase along the fifth hour,which lasts to the 10th hour from the time of the innoculation, from which moment begins the first stationary phase of evolution. During the logarithmic phase, the biomass with not heated control sample, increases to 20 fold in comparison with the starting culture.

In the trial samples heated to a temperature of 58°C for 30 minutes, the lag-phase was prolonged to the 9th hour,while the length of the logarithmic phase is shortened with about 2 hours.Parallel with these changes, is determined smaller growth of the biomass at the end of the logarithmic phase with about 13%, compared to that of the control.



Biomass growth in Str.faecalis cultures with no temperature influence and after different heat treatments.

The quantity of the biomass in the beginning of the stationary phase, starting on the 13th hour after the innoculation of the culture is equal to that, obtained on the 7th hour in the control, being still in the active logarithmic phase.

Same phenomenon is observed with the other temperatures of heating - 62°C and 65,5°C, while the lag-phase was prolonged averagely with 1 hour (with 62°C - to the 10th hour and with 65,5°C to the 11th hour).

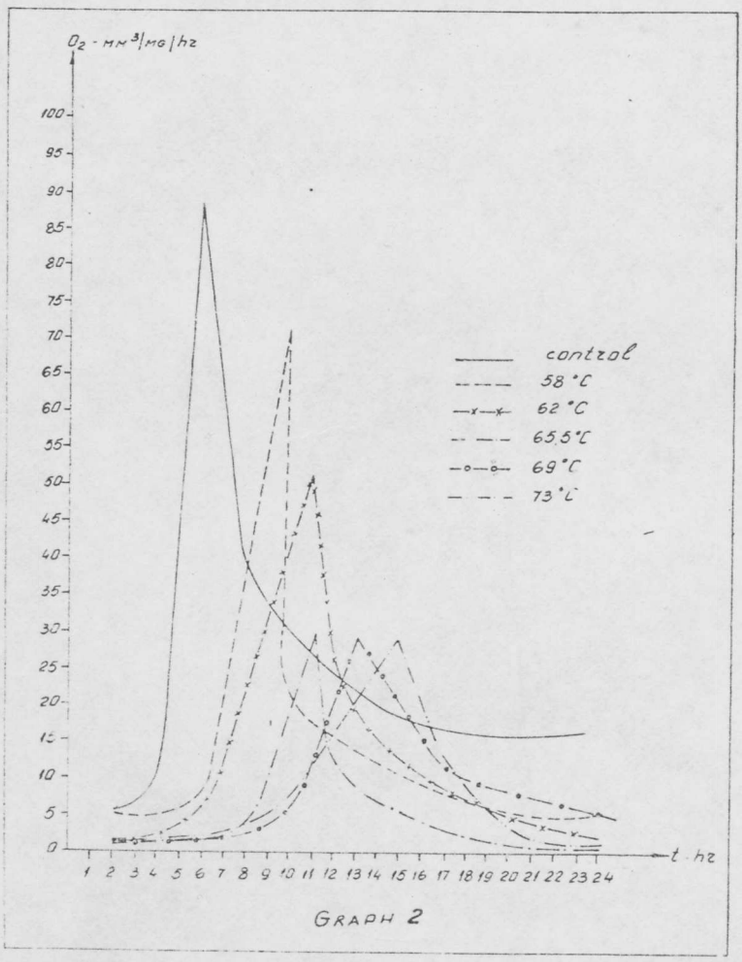
Same tendency of decreasing the total quantity of the bio mass is maintained with these temperatures along with the increase of the heating temperature, thus the higher temperatures of heating exibit lower evolution of biomass at the end of the logarithmic and the beginning of the stationary phases.

With the next temperature of 69°C, the logarithmic phase appears after the 13th hour and continues about 3 hours, while on the 16th hour it passes into the stationary phase.

– With 73°C the lag-phase continues to the 14th hour, after which begins the logarithmic phase which in turn continues to the 17th hour. Also with these two temperatures was determined decrease of the total quantity of biomass in the beginning of the stationary phase which corresponds to 25–28% less than the control.

These data show, that pasteurization temperatures to 73°C do not ensure total pasteurization effect with Str.faecalis var.liquefaciens, but increase from 2 to 3.5 times the length of the lag-phase. At the same time the length of the logarithmic phase is shortened and the total quantity of biomass at the end of the logarithmic phase decreases with the augmentation of the temperature influence.

On the bases of these results, could be derived important conclusions, having especially significant meaning in productiion, hygienic evaluation and storage of pasteurized meat canned products, in which the incidence of eneterococci is an important sanitary-hygienic and technological index.



GRAPH 2

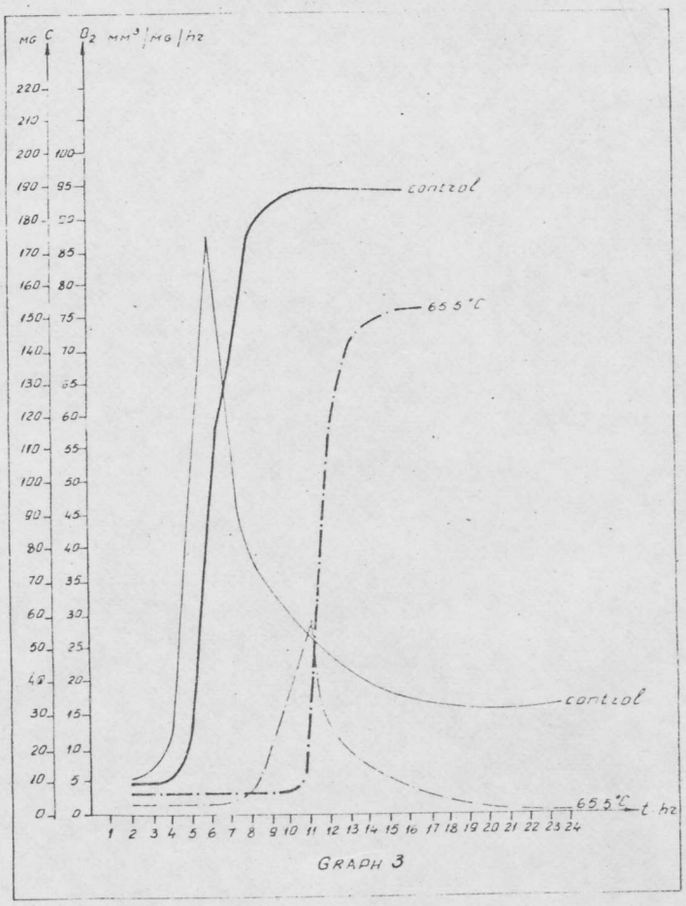
Respiration intensivity change of Str.faecalis cells during different phases of growth under normal conditions and after heat treatment under different temperatures.

On graph 2 are given the results in dynamics, showing the changes in the intensity of breathing of Str.faecalis var.liquefaciens, During the growth of the culture, in the tests without heating and after the influence of different pasteurization temperatures.

The oxygen used for one hour by Str.faecalis during the different phases of growth is calculated to 1 mg cell carbon.

The result exibits, that thepeak in oxygen need is found in different periods of the evolution of the culture and is in relation of the acting temperature. Highest intensity of breathing is observed in the control and fall in with the staft of the logarithmic phase of culture evolution. With increasing of the influencing temperature, the time for highest respiratory exibit is prolonged while with all temperatures it coincides with the appearance of the initial logarithmic phase of evolution.

Increasing of the temperature influence show a decrease of the used oxygen in the beginning of the logarithmic phase and from 85,25 $mm^3/mg/h\ O_2$ with the control, the maximum at 58°C heating of the culture attains 70.1 $mm^3/mg/h\ O_2$, and at 73°C to 29.15 $mm^3/mg/h\ O_2$.



Dependence between respiration activity and evolution phase of Str.faecalis.

This relation between the breathing activity and status of evolution of Str.faecalis is shown on graph 3.

Data show, that the intensity of exogenic breathing of Str. faecalis depends on the age and physiological status of the cells while its maximum coincides with the starting logarithmic phase, independantly of the length of the lag-phase.

Activity of endogenic breathing of Str.faecalis in the control and after being influenced by different pasteurisation temperatures is not significant and fluctuates between the limits from 2,0 - 8,4/O_2 mm^3/mg/h/ and is in relation to the age of the culture.

The observed correlation between the intensity of breathing of Str.faecalis and temperature influence, in which the intensity decreases with the augmentation of the temperature, has an important significance in the production and storage of the pasteurized meat products.

Conclusions.

1.Pasteurization temperatures of 58°C to 73°C, used in the production of pasteurized meat products do not ensure a total pasteurization effect with Str.faecalis var.liquefaciens,strain 755, but only prolongate the lag-phase about 3,5 from that of the non heated culture.

2.With the increase of the pasteurisation temperatures and equal length of influence (30 minutes) the lag-phase is lengthened, while the length of the duration of the logarithmic phase is shortened.

3.The total quantity of biomass at the end of the logarithmic phase decreases with the increase of the temperature influence.

4.The most active exogenic breathing of Str.faecalis coincides with the beginning of the logarithmic phase, independat of the length of the lag-phase and temperature influence.

5.The intensity of the exogenic breathing of Str.faecalis decreases with the augmentation of the temperature influence.

6.Activity of the endogenic breathing is negligeable and is in relation with the age of the culture.

7.The influence of pasteurisation temperatures to 73°C, used in meat processing, do not guarantee total pasterisation effect upon enterococci, and prolongate their lag-phase, decrease total count of the microorganisms and supress their breathing, which has a vital effect for production,hygienic evaluation and stora-

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ge of the pasteurized canned meat products.

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