THE TEMPERATURES INFLUENCE ON THE DEVELOPMENT OF STAPHYLOCOCCUS GENUS BACTERIA

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Abstract

The aim of this research is the influence of the physical, chemical and biological agents over the development of Staphylococcus genus bacteria that is achieved by relative study of these bacteria in both food and growth medium. Each of the agents listed above gives information about the bactericidal or bacteria static over the Staphylococcus genus bacteria. The behavior of these bacteria in the process of food preservation is different, depending on the preservation method (smoking, salting, microwave treatment, freezing and thawing, thermal treatment like pasteurization and sterilization). The behavior of the Staphylococcus genus bacteria in growth medium is influenced by the quality and quantity of the medium, the competitive character of the bacteria, the age of the cells, the pH of the medium and especially by the presence of those agents that can stimulate, inhibit or destroy these bacteria, agents like: NaCl, sugars (saccharine, glucose), acids (hydrochloric, tartaric, lactic, citric, acetic), potassium sorbet, egg yolk.
***Keywords**: the multiplication of Staphylococcus bacteria, staphylococcal enterotoxin (SE), temperature.*

Introduction

Of all physical, chemical and biological factors that influence the development of Staphylococcus bacteria and the production of SE, temperature is the main factor analyzed in this work.

Positive Gram cocci, Staphylococcus bacteria live many months in ordinary mediums, in the refrigerator or even at the chamber's temperature.

Experimental

The sterile nutritive basis for the study and the development of the bacteria outside the natural ecological niche consist in common used

mediums, and special mediums (elective and selective isolation medium, enrichment mediums, conservation mediums, identification mediums), following the requirements of the approved methods and the diagnosis kits.

The methods used are the following:

- The horizontal method for the enumeration and detection of positive-coagulant staphylococci, which uses the technique of the most probable number (MPN), according to SR EN ISO 6888-3.
- The horizontal method of aerobe cultivation done at 35-37°C for counting the positive-coagulant staphylococci on solid medium (Baird – Parker medium), according to SR EN ISO 6888-1.
- The horizontal method of aerobe cultivation done at 35-37°C for counting the positive-coagulant staphylococci on fibrinogen rabbit plasma medium, according to SR EN ISO 6888-2.
- The detection of staphylococcal enterotoxins (SE) in foodstuffs and growth medium through immunological tests such as:
  - The reaction of immune-precipitation through immune-diffusion in agar gel
  - The radio-immune test (RIA)
  - The immune-enzymatic test
  - Agglutination reactions

Results and Discussion

Staphylococcus aureus is a mesophyllum bacterium. It grows well in ordinary mediums, at temperatures of 35-40°C. It is a germ with a high thermal sensitivity: a population of 10^6 Staphylococcus aureus/ml can be completely inactive between 4-24 minutes at 54-60°C, in phosphate tampon, with neutral *ph*.

- The temperature's influence on the multiplication of Staphylococcus bacteria and on the toxicity factors

The temperature at which Staphylococcus aureus can multiply is between 10-45°C, with and optimum of 35-37°C, but they can also be produced at 25°C and 30°C in smaller quantities. It has been observed that the production of SEB also takes place in a small quantity at 16-20°C, (10-20mcg/ml), as compared to that produced at 37°C (340 mcg/ml), at equal microbial densities, as it appears in the graphic representation below (figure 1).

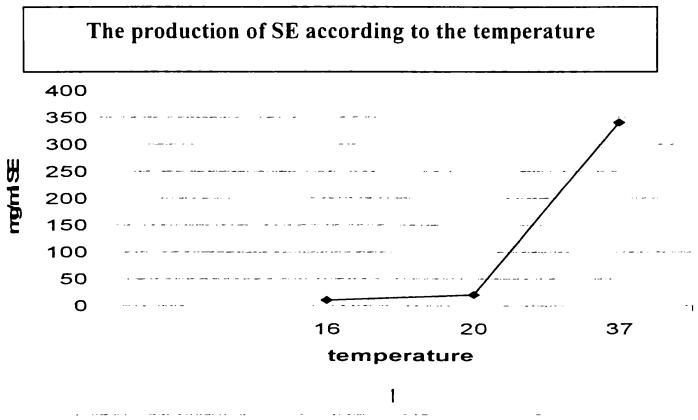

Fig. 1: The production of SE according to the temperature

At 15°C, *Staphylococcus aureus* does not produce enterotoxin, not even after 8 days of incubation.SEA, SEB, SEC and SED can be produced in BHI at temperatures of 19-39°C but SEB also at 45°C, as well as at 13°C.

PHYSICAL FACTOR	GROWTH	THE PRODUCTION OF ENTEROTOXINS
Temperature	6-46ºC	10-45ºC
Optimal temperature	37ºC	40ºC

The multiplication of Staphylococci and the production of enterotoxins vary with the temperature according to the graphic representation below, where white represents the SE production with an optimum of 40°C and black represents Staphylococci multiplication with an optimum of 37°C.

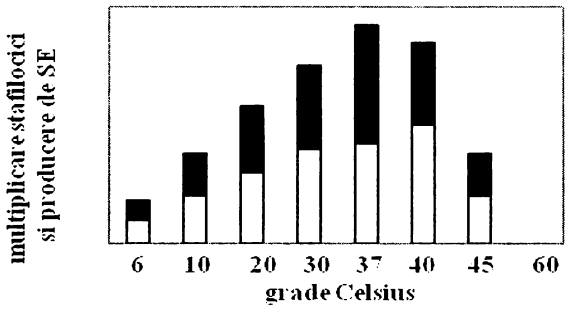

Fig. 2: Staphylococcus multiplication and SE production as influenced by the temperature

SE are very resistant to heat, the most heatproof bacteria of all bacterial toxins. The D_{100} value is of 1-2 hours and D_{120} is of 10-40 minutes as it can be seen in the graphic representation below.

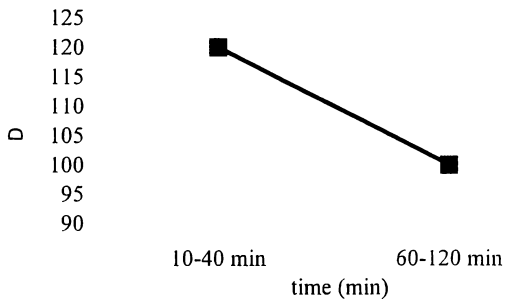

Fig. 3: Germicidal effect resulted from the correlation temperature-time

The heat becomes more active on SE in mediums with a *p*H < 5.3.

α haemolysin, the most toxic of all four types: α, β, γ, δ, is also influenced by the temperature. It is characterized by the production of a complete haemolysis zone, with unclear edges and becomes inactive at 40°C.

β haemolysin produces at 37°C extended zones of incomplete haemolysis, with clear edges. Its activity continues at 4°C when it transforms in a complete haemolysis the incomplete one obtained at 37°C. it is the most resistant of all four.

γ haemolysin has the highest thermal sensitivity and it is active only on human and rabbit red cells.

- The temperature's influence through technological treatments

The thermal treatment is the most efficient method of rendering the Staphylococcus inert in foodstuff. The thermal treatment applied to preserved food in hermetically closed tins is safe enough for the destruction of all staphylococci possibly present. Staphylococcic toxinfections produced by consuming preserved food are extremely rare and are caused by the contamination of the product after the thermal treatment. Fresh milk is frequently contaminated with Staphylococcus aureus, however, during the process of pasteurization applied correctly Staphylococcus aureus is surely eliminated. Staphylococcic toxinfections caused by the consumption of pasteurized milk are possible only if the milk has been contaminated through

the pasteurization process. Specialized literature comes with many proofs which show that high pasteurization (71.7°C-72°C), which is frequently applied to milk is sufficient for the elimination of staphylococci. Also, the milk's low pasteurization in tubes, 63°C for 30 minutes, a method that is still used in some countries, is efficient enough to eliminated all the staphylococci present in milk, although their number exceeds 10^6/ml (3, 11, 12).

Even though freezing and defrosting have a reduced effect on the viability of Staphylococci, storing contaminated meat at a temperature of -22°C determinates the elimination of 91% of staphylococci. In lean beef with a *p*H between 4.6 – 6.3, Staphylococcus aureus survives at 4 cycles of freezing-defrosting, but is reduced numerically in meat with a *p*H of 4.2. Staphylococcus aureus found in ox liver, experimentally inoculated and kept at a temperature of -29°C, for 29 days, has survived in a larger number than that found in liver kept at -29°C for 14 days and then 24 hours at a temperature of 21°C or 15 days at a temperature of -1°C. The development of flora of indigenous association of the liver has been found in Staphylococcus aureus when the liver was kept at 10°C or at 21°C. This explains why, generally, in meat kept at refrigerating temperatures staphylococci are found quite rare. In slices of bacon in vacuumized bags staphylococci develop together with the natural flora, but the development is improved if the number of saprophyte flora is lower and the storing temperature is higher. Adding potassium sorbat inhibits this development.

The thermal resistance of SE in foodstuff is far higher than the resistance of those from various tamponed solutions. This shows that thermal treatments that are usually applied to foodstuff during the treatment and before being served are not sufficient for rendering SE inactive, apart from those applied to preserved food in hermetically closed tins.

Staphylococcus aureus is more resistant to freezing temperatures than salmonellas and E. coli. Freezing and defrosting influence slightly the viability of this bacterium, but this processes make them a little more sensible at 7.5% NaCl, which shows that freezing can affect the cells but it does not kill them. The damages of the cellular membrane and the modifications of the metabolic activity suffered during the process of freezing influence the capacity of the cell to develop on highly nutritive mediums and less of not at all on simple mediums. Staphylococcus aureus cells kept at 5°C are less viable in acid mediums.

Microwave treatment of foodstuff is not safe enough for the annihilation of Staphylococcus aureus. In order to be efficient the level of this treatment must be in the deep layers of 100°C.

- The temperature's influence correlated with other factors on the multiplication of Staphylococcus bacteria and the production of enterotoxins.
- The temperature's influence correlated with the water's activity factor
- It seems that the reduction of the a_w value produced by adding NaCl plays a greater role in decreasing the SE production. The effect of a_w value is influenced by the *p*H and the incubation temperature (49.82). In a mixture of NaCl and KCl staphylococci grow and produce SE at a_w between 0.864-0.867 when incubated at 30°C and at values between 0.870-0.887 when incubated at 25° C.

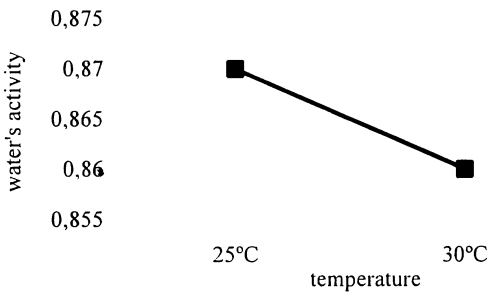

Fig. 4: Correlation between the water's activity factor and the temperature in the multiplication of Staphylococcus aureus

Studying the relation between a_w, *p*H and the temperature on the production of SEA, SEB and SEC in BHI with NaCl and saccharose it cannot be observed any difference in the production of enterotoxin in what concerns the influence of the two substances on the a_w, when the pH values were controlled. When the pH decreases because of the metabolisation of saccharose, the production of SE reduces.

- The temperature's influence correlated with the *p*H, chemical composition is shown in figure 5.

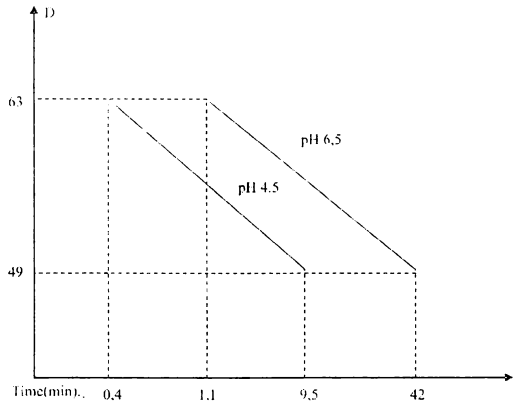

Fig. 5: The correlation of temperature/time/*p*h

Conclusion

Generally, in culture mediums Staphylococcus aureus is very sensitive to temperatures exceeding 46° C. At 54° C - 60°C, in neutral phosphate tampon and at a density of 6 x 10^6 -10^7 cells/ml it is killed in 4-24 minutes. The thermal resistance of this bacterium is influenced by the *p*H of the solution in which it is placed. Thus, in 0,1 M phosphate tampon it survives more in a *p*H of 6,5 than in *p*H 4,5 with a D_{49} value of 42 respectively 9.5 minutes and D_{63} 1,1 respectively 0,4. After a treatment at 52°C for 15 minutes 99% of the cells do not develop anymore in a medium with 7.5% NaCl, as they present micro damages. Thermal subtreatment produces to the bacteria a series of damages such as the damage of the cytoplasm membrane, the alteration of the metabolic capacity of cells and the degradation of ARN_R. Staphylococcus aureus cells submitted to sublethal thermal treatments regain tolerance to NaCl, bouillon with soy tryptone, nutritive bouillon with mannite. The recovery of stressed cells through heat can be improved by adding catalaze in the selective mediums. The omission of phosphates and of amined acids from the recovery mediums has a lethal effect, and the omission of Mg^{2+} slows down the reparation of the cells. It seems that the main reparation process which takes place during the recovery period of the stressed cells is the regeneration of ribosomes.

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