# Antimicrobial Effects of Linear Polyphosphates for Chosen Microorganisms

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# ZADÁNÍ DIPLOMOVÉ PRÁCE

(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

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| Jméno a příjmení: | **Bc. Adéla NOVÁKOVÁ** |
| Studijní program: | **N 2901 Chemie a technologie potravin** |
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| Téma práce: | **Antimikrobiální účinky lineárních polyfosforečnanů na vybrané mikroorganismy** |

Zásady pro vypracování:

1. **V teoretické části zpracujte literární rešerši týkající se charakteristiky, významu a využití lineárních polyfosforečnanů v potravinářství.**
2. **Charakterizujte tavené sýry a popište výskyt mikroorganizmů v tavených sýrech.**
3. **V praktické části proveďte sledování vlivu lineárních polyfosforečnanů na mikroorganizmy v koncentracích používaných v potravinářství.**
4. **Na základě teoretické části a výsledků praktické části formulujte návrhy a doporučení, týkající se využití lineárních polyfosforečnanů jako antimikrobních látek a zhodnoťte jejich využitelnost v potravinářství.**

Rozsah práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná**

Seznam odborné literatury:

**[1] GUNASEKARAN,Sundaram,MEHMET AK, M. Cheese Rheology and Texture. CRC Press 2003, pages 437, ISBN 1587160218**
**[2] FOX, P.F., Cheese: Chemistry, Physics, and Microbiology. Springer 1993, pages 577, ISBN 0412535009**
**[3] MOLINS, Ricardo A. Phosphates in Food. CRC Press 1991, pages 272, ISBN 084934588X**
**[4] DEMAN, John.M. Principles of Food Chemistry. Springer 1995, pages 530, ISBN 083421234X**

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ABSTRACT

Phosphates have been approved for use in dairy products primarily to protect flavour. It is also known that phosphates have antimicrobial properties. The objective of this study was to compare the effect of different phosphates in model system. This diploma work is aimed to studying of the antimicrobial effects of emulsifying salts JOHA HBS, JOHA S9 and SELF 690.

In addition, a panel of 6 gram-positive (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus*) and 4 gram-negative bacteria (*Escherichia coli, Serratia marcescens, Pseudomonas fluorescens, Salmonella* Typhimurium) were tested for their sensitivity against the polyphosphates. Gram-positive bacteria are generally more sensitive to phosphates then are gram-negative bacteria. Growth was totally inhibited by 0.4% and 0.5% concentration of phosphates for gram-positive bacteria.

Keywords

JOHA HBS, JOHA S9, SELF 690, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus, Escherichia coli, Serratia marcescens, Pseudomonas fluorescens, Salmonella* Typhimurium, emulsifying salts*;*

## ABSTRAKT

Fosforečnany byly schváleny pro mléčné výrobky k ochraně chuti a vůně. Je známo, že fosforečnany mají antimikrobiální vlastnosti. Tato práce se zabývá studiem antimikrobních vlastností tavících solí JOHA HBS, JOHA S9, SELF 690.

Předmětem studie bylo srovnat tento efekt na modelovém systému. Bylo srovnáváno 6 gram-pozitivních bakterií (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus*) a 4 gram negativní kmeny bakterií (*Escherichia coli, Serratia marcescens, Pseudomonas fluorescens, Salmonella typhi*). Gram-pozitivní bakterie byly citlivější než gram-negativní bakterie. U gram-pozitivních bakterií byl růst úplně zastaven koncentracemi fosforečnanů 0.4 až 0.5%.

Klíčová slova:

JOHA HBS, JOHA S9, SELF 690, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus, Escherichia coli, Serratia marcescens, Pseudomonas fluorescens, Salmonella* Typhimurium, tavicí soli;

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Motto

Timeo Danaos et dona ferentes.

Láokoón

# CONTENTS

# INTRODUCTION

Processed cheese is produced by blending shredded natural cheese of different types and degrees of maturity with emulsifying agents, and by heating the blend under a partial vacuum with constant agitation until a homogenous mass is obtained.

Processed cheese was initially manufactured without an emulsifying agent, but only after the introduction of citrate, and mainly phosphates, as emulsifying agents did the industrial production practicable. The other step in manufacture of processed cheese was combined numerous types of cheese, and inclusion of other dairy and non-dairy products.

Phosphates are additives widely used in the food industry, mainly meat and dairy processes, to protect flavour and increase bacteria and yields because of their water retention and emulsifying capacities. They are generally recognized as safe, and in the cheese industry they are used to solubilise calcium paracasinate, sequestering calcium and thus dispersing proteins. Calcium in the Ca-paracaseinate complex of natural cheese is removed by the ion.exchange properties of emulsifying agents, solubilising the paracaseinate, usually as Na-caseinate. [1]

Other beneficial effects provided by emulsifying agents are:

- hydrating and swelling of proteins;
- emulsifying fat and stabilizing the emulsion;
- controlling and stabilizing pH;
- forming an appropriate structure after cooling. [2]

Although phosphates have never been classified as antimicrobial agents, a number of investigations have reported that phosphates have antimicrobial activity. [3,4,5,6,7,8,9]

Because phosphates have a potential to be antimicrobial agents in foods, more information about the antimicrobial effects of phosphates is needed. [4]

The objective of the present study was to examine the antimicrobial effect of polyphosphates allowed for use in dairy and processed cheese productions.

There were applied there types of emulsifying agents:

1) JOHA HBS – mixture of polyphosphates (high amount of monomers in polymer chain) and phosphates (monomers),
2) JOHA S9 – mixture of polyphosphates (low amount of monomers in polymer chain) and phosphates,
3) SELF 690 – mixture of phosphates and orthophosphates.

The aim of this work was to obtain data on the inhibitory effect polyphosphates (emulsifying salts) on different microorganisms.

# THEORY

# 1 PHOSPHATES

A phosphate, in inorganic chemistry, is a salt of phosphoric acid. In organic chemistry, a phosphate, of organophosphate, is an ester of phosphoric acid. [10]

## 1.1 Chemical properties

The phosphate ion is a polyatomic ion with the empirical formula $PO_4^{3-}$ and a molar mass of 94.973g/mol; it consists of one central phosphorus atom surrounded by four identical oxygen atoms in a tetrahedral arrangement. The phosphate ion carries a negative three formal charge and is the conjugate base of the hydrogenphosphate ion, $HPO_4^{2-}$, which is the conjugate base of $H_2PO_4^-$, the dihydrogen phosphate ion, which in turn is the conjugate base of $H_3PO_4$, phosphoric acid. It is a hypervalent molecule. Phosphate is also an organophosphorus compound with the formula $OP(OR)_3$. [11, 12]

A phosphate salt forms when a positively-charged ion attaches to the negatively-charged oxygen atom of the ion, forming an ionic compound. Many phosphates are not soluble in water at standard temperature and pressure. [13]

In dilute aqueous solution, phosphate exists in four forms. In strongly-basic conditions, the phosphate ion ($PO_4^{3-}$) predominates, whereas in weakly-basic conditions, the hydrogen phosphate ion ($HPO_4^{2-}$) is prevalent. In weakly-acid conditions, the dihydrogen phosphate ion ($H_2PO_4^-$) is common. In strongly-acid conditions, aqueous phosphoric acid ($H_3PO_4$) is the main form. [12, 13]

## 1.2 Partition of Phosphates

It is now well established that three forms of phosphorus exist, white, black and red phosphorus. Behind this very simplified scheme, reality is much more complicated. In fact, each of these three forms has itself several crystalline modifications and in many cases the mechanisms of transformation are complex. [13]

### 1.2.1 White Phosphorus

White phosphorus ($P_4$) exists as individual molecules made up four atoms in a tetrahedral arrangement, resulting in very high ring strain and instability. This transformation, which is accelerated by light and heat, makes white phosphorus almost always contain some red phosphorus and appear yellow. [13]

This form appears as a waxy transparent solid matter burning spontaneously when in contact with the oxygen of air. Soluble in several classical solvents as benzene and carbon disulphide, white phosphorus does not react with water and can be easily stocked, melted or transported when protected by a layer of water. This form is very poisonous, the lethal dose being as low as 50 mg. [10]

White phosphorus is the most reactive of the three phosphorus forms and reacts with most of the elements. [14]

It oxidizes spontaneously and vigorously when in contact with oxygen, and also reacts with carbon dioxine. In both cases the final product is $P_4O_{10}$:

$$P_4 + 5O_2 \rightarrow P_4O_{10}$$

$$P_4 + 10CO_2 \rightarrow P_4O_{10} + 10CO$$

The process is highly exothermic and results in the formation of phosphoric acid of 80 to 90% purity. [3,15]

The reaction with oxygen has been extensively investigated for some conditions, reduced pressure of oxygen for instance; it is associated with a greenish glow. [10, 16]

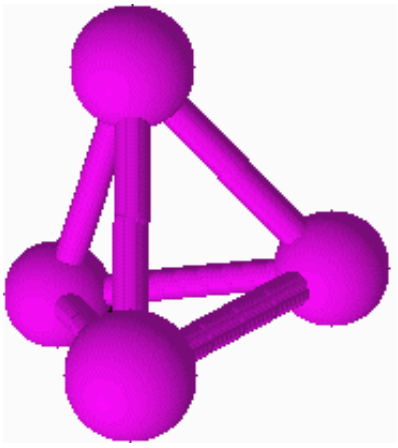


Figure 1 Structure of White Phosphorus [13]

### 1.2.2 Red Phosphorus

Several processes can be used to transform white phosphorus into red phosphorus. This transformation is accelerated if traces of iodine are added to white phosphorus. To the difference with the white form, red phosphorus does not ignite when in contact with oxygen and cannot be dissolved in the classical solvents of white phosphorus. [10, 12]

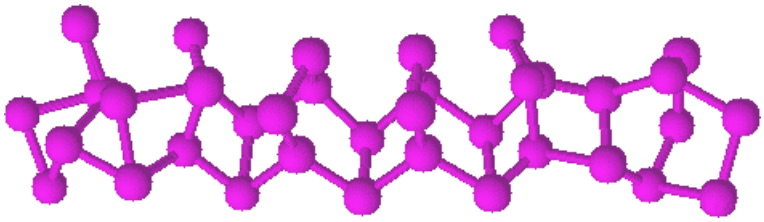


Figure 2 Structure of Red Phosphorus [13]

### 1.2.3 Black Phosphorus

The black form can be considered as a high-pressure form of the white phosphorus. Another way to produce black phosphorus is to heat a mixture of mercury and white phosphorus seeded with crystals of the black form. [14]

Black phosphorus in not soluble in the solvents of the white form suggesting this form is not molecular, but a polymeric arrangement. Is the most stable of the three forms of phosphorus and is chemically the least reactive. For instance, unlike white phosphorus, it does not ignite spontaneously. [10, 17]

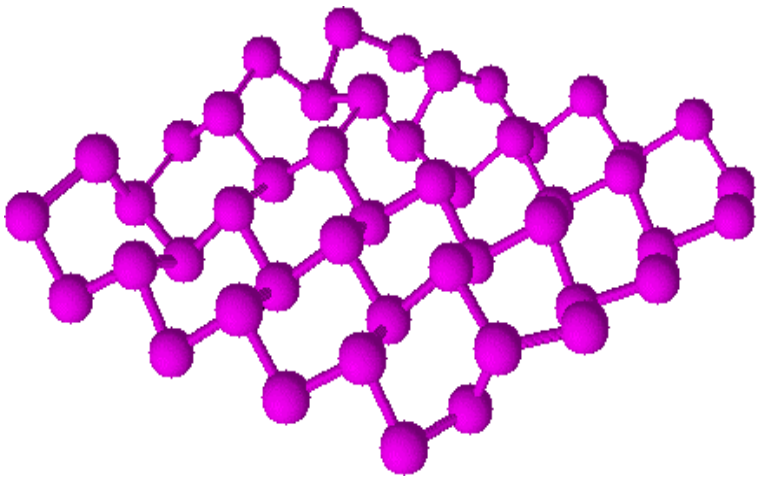


Figure 3 Structure of Black Phosphorus [13]

# 2 PHOSPATES IN FOOD

Phosphate not only plays a major role in human metabolic systems, especially in energy-transfer systems, and bone growth and health, it is also the main component unit in phosphate compounds that provide innumerable functions in the food industry. Phosphates can aid processing or improve quality of many foods, including meat, poultry and fish products; processed cheeses. The baking industry uses them in leavening systems, and soft-drink manufacturers add phosphate to cola drinks to enhance flavour. They work as emulsifiers in dairy products. Their toxicological properties are well known. Some of them, especially high-polymer phosphates, have a certain antimicrobial action which has been known since long ago. [11, 18]

The term “phosphate” refers to a salt of phosphoric acid. When many phosphate units are linked together into a more-complex structure, they go by the name of “polyphosphates”. [11]

## 2.1 Phosphates in Milk and Dairy Products

Some of the most important and oldest applications of food phosphates are found in the dairy industry, in which these flexible compounds are used for such diverse purposes as protecting condensed, sterilized milk against age gelation, increasing the solubility of dehydrated milk and whey powders, allowing production of novel dairy-based desserts, and improving texture, melting and spread ability of processed cheese. [3, 19, 20]

### 2.1.1 Phosphates in Fluid Milk Production

Type of milk the most commonly consumed in Czech Republic is cow milk. It is the reason why this discussion will emphasize the effect of phosphates on cow milk.

Bovine milks is a highly complex mixture of water (84 to 88%), proteins (3 to 4%), lipids (3 to 5%), carbohydrates (mostly lactose, 5%) and minerals (0.5 to 0.8%). [3]

In contrast to most proteins in food systems, milk caseins are especially depended on phosphates for structural integrity. Thus, $\alpha_{s1}$–casein, the principal protein in milk, is a phosphorylated protein that closely interacts with calcium ions. $\alpha_{s1}$-casein is also unique in that it exhibits bipolar clustering of polar and non-polar residues. Therefore, there is a hydrophilic, hydrated, charged, flexible zone in the molecule and a hydrophobic, globular

zone. The flexibility of the hydrophilic zone accounts for the response of αs1 – casein to changes in ionic environment, which are manifested through changes in molecular size, and allows for binding of positively charged ions, especially $Ca^{2+}$. In contrast, the hydrophobic zone gives origin to intermolecular association and allows for interaction with other casein molecules to bring about intermolecular aggregation. When the charge of the hydrophobic zone in $\alpha_{s1}$–casein in reduced through the binding of calcium ions phosphate groups, the size of the polar zone decreases and the resulting increase in molecular interactions favours precipitation or formation of micelles. Bipolar characteristics are also exhibited by $\alpha_{s2}$–casein, which contains several phosphoryl serine clusters that make it the most hydrophilic of caseins, and in β-casein. This contains only one phosphoryl serine residue and is the most hydrophobic among the caseins. Stabilization of milk casein micelles is primarily contributed by the amphiphilic κ-casein, a protein that contains no phosphoryl residues and, therefore, is unaffected by calcium ions. [3]

The hydrophobic zone in κ-casein interacts with similarly hydrophobic zones in other caseins. Research into the distribution of casein in the micelles has shown that part of the κ-casein was located between micelles. Progressive removal of intermicellar κ-casein indicated that 70% of the κ-casein was locate in the interior of the micelles, surrounded by α- and β- casein and calcium phosphate, and that the remaining 30% was between micelles.

Casein interacts with each other and with calcium phosphate to form spherical structures known as micelles, which contains $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-caseins in the general ration 3:1:3:1, for total of approximately 92% protein, the balance being mostly calcium phosphate, Micelles are composed of submicelles, which, in turn, are the result of interactions between the hydrophobic zones of the various casein molecules. [3]

Ester phosphate groups in $\alpha_{s2}$-casein are responsible for the high solubility of this protein at neutral pH values, so that neutralization of ionize phosphate groups by calcium ions leads to $\alpha_{s2}$-casein precipitation because of increased hydrophobic interaction between protein molecules.

The casein micelle phosphate component consists of two parts:

- A layer of ionic bonds (63% of total phosphorus)
- An absorption layer (37%)

bound weakly through van der Waals forces. The latter would constitute the colloidal calcium phosphate. [3, 21]

#### *2.1.1.1 Phosphates in Cheese Manufacture*

Some of the most important and oldest applications of food phosphates in the dairy industry are in the manufacture of cheese, particularly processed cheese, cheese spreads, and related products in which the basic role of phosphates in emulsification, and in manufacture of fresh cheese. [3]

Casein curd formation during cheese manufacture is a process that involves electrostatic forces among other factors. Changes in the charge of milk casein micelle surfaces may be brought about by alterations in the ionic environment and by lowering pH through direct-acidification, through acid production by selected bacterial starter cultures, or both, to give "acid curd". Among the acids approved for use in direct acidification (to pH 4.5 to 4.7) for cheese production is phosphoric acid, which has been found to increase water holding capacity (WHC) in such products as white brined and cottage cheeses and to improve flavour in mozzarella cheese of pH 5.4. On the other hand, precipitation of milk caseins may also be induced without appreciable pH changes by adding the enzyme rennet, hence the terms "sweet curd" or "rennet curd". [3]

Phosphates affect the rate of curd formation during cheese making with rennet in diverse ways depending on the type and concentration of phosphate used. The shorter chain ortho- and pyrophosphates have been shown to delay or inhibit milk curd formation by rennet; alterations in milk coagulum formation by the longer chain polyphosphates. [3]

Addition of food phosphates to achieve increased yields in cheese making has a two-fold economic impact because it may contribute to reduced waste treatment costs as well. These yield increases were not attributable to greater recovery of whey proteins (Table 1 and 2).

Table 1 Composition of Curd Resulting from Manufacture of Cottage Cheese With and Without SPG[a] [3]

| | **Level 1[b]** | | **Level 2[c]** | |
|---|---|---|---|---|
| **Component** | **Control** | **0.05% SPG** | **Control** | **0.20% SPG** |
| **Total solids (%)** | 26.14 | 25.36 | 25.59 | 24.88 |
| **Fat (%)** | 4.80 | 5.15 | 5.27 | 4.33 [c] |
| **Solids – not fat (%)** | 15.20 | 14.85 | 14.73 | 15.67 [c] |
| **Total protein (%)** | 11.99 | 11.02 | 12.82 | 10.76 [c] |
| **Casein protein (%)** | 11.72 | 10.69 | 12.48 | 10.39 [c] |
| **Whey protein (%)** | 0.28 | 0.33 | 0.34 | 0.36 [c] |
| **Lactose (%)** | 2.73 | 3.04 | 1.39 | 3.68 [c] |
| **Ash (%)** | 0.48 | 0.79 | 0.52 | 1.24 [c] |
| **Phosphorus (mg/100ml)** | 163.94 | 235.05 [c] | 150.86 | 381.01 [c] |
| **Calcium (mg/100ml)** | 70.16 | 136.71 [c] | 66.81 | 240.91 [c] |

[a] All curd components except total solids are calculated on the basis of 20% total curd solids.

[b] Total solids in skim milk was 10.33% for level 1 and 10.26% for level 2 because of variations in fat content.

[c] Significantly different from control ($p<0.01$)

Table 2 Composition of Whey Resulting from Manufacture of Cottage Cheese With and Without SPG[a] [3]

| | **Level 1[b]** | | **Level 2[b]** | |
|---|---|---|---|---|
| **Component** | **Control** | **0.05% SPG** | **Control** | **0.20% SPG** |
| **Total solids (%)** | 6.78 | 6.72 | 6.68 | 6.98 [c] |
| **Fat (%)** | 0.24 | 0.22 | 0.23 | 0.36 [d] |
| **Solids – not fat (%)** | 6.54 | 6.50 | 6.44 | 6.62 |
| **Total protein (%)** | 0.55 | 0.51 | 0.51 | 0.48 |
| **Casein protein (%)** | 0.08 | 0.06 | 0.08 | 0.09 |
| **Whey protein (%)** | 0.46 | 0.45 | 0.43 | 0.40 |
| **Lactose (%)** | 5.34 | 5.30 | 5.24 | 5.41 |
| **Ash (%)** | 0.66 | 0.69 | 0.70 | 0.73 |
| **Phosphorus (mg/100ml)** | 73.93 | 75.99 | 75.53 | 87.24 |
| **Calcium (mg/100ml)** | 109.37 | 100.41 | 104.20 | 80.75 |

[a] Means of 8 replications.

[b] Total solids in skim milk was 10.33% for level 1 and 10.26% for level 2 because of variations in fat content.

[c] Significantly different from control ($p<0.05$)

[d] Significantly different from control ($p<0.01$)

Processed cheese are blends of various cheeses that are heated under continuous agitation together with water, sodium chloride, and emulsifying salts, so as to melt the ingredients and form a plastic, homogeneous mix that can melted upon cooling. [3]

Chemically, the process consists of peptization and partial solubilisation of casein as a result of conversion from calcium to sodium caseinate. These effects are contributed by emulsifying salts, while a parallel physical phenomenon takes place whereby the casein gel is turned into a sol. The emulsion character of the final system is made possible increased availability of soluble proteins, which are essential for coating the largely increased fat globule surface area brought about in the system by simultaneous heating and agitation.

Because the capacity of an emulsifying salt to take casein soluble depends to a large extent on its ion exchange and calcium chelating properties, medium-, and long-chain polyphosphates are among the preferred agents in process cheese making. [3]

## 2.2 Antimicrobial Uses of Food Phosphates

The literature on microbial effects of food phosphates is divided in two groups:

1) effects of phosphates on bacteria in laboratory culture media;
2) effects of phosphate on microorganisms in food system.

In this work there were study the effects of phosphates on bacteria in laboratory culture media. [3]

### 2.2.1 Antimicrobial Uses of Phosphates in Dairy Products

The self-life of fresh, refrigerated pasteurized milk is limited by the eventual growth of psychrotrophic microorganisms, particularly by members of the genus Pseudomonas, most of which produce proteinases that cause detectable off-odors in milk long before bacterial numbers reach spoilage levels. [3]

Lactic acid starter cultures are essential in the dairy industry for production of fermented products and cheeses. An important factor in preparation and maintenance of dairy lactic acid started culture is inhibition of phages. These are cultures able to infect bacterial cultures and provoke lysis of bacterial cells, thus decreasing the activity of the cultures to such an extent that total batch failures may occur. Although very little is known about the effect of phosphates on viruses in foods, phosphates are used to induce resistance to phage

infection in lactic starter cultures and for phage inhibition or entrapment in milk and commercial phage inhibitory media. Phosphates have also been used to preserve bacterial cultures used in the dairy industry. [3]

Monosodium phosphate and sodium tripolyphosphate were the most effective for single cultures of *Lactobacillus bulgaricus*, whereas monosodium phosphate and disodium phosphate gave the best results with mixed cultures of *L. Bulgaricus* and *Streptococcus thermophilus*. In addition, trisodium phosphate was claimed to stabilize the viable cell count in fermented milk during storage. [3]

In opposition to the conclusions obtained in the studies are that these researchers described decreased acid production by culture of *Lactobacillus bulgaricus* grown in phosphate-containing milk or in phage inhibitory medium that contained phosphates. Moreover, morphological changes in lactobacilli from bacilloid rods to long chains were described that could not be reversed upon single transfer into phosphate-free skim milk medium. [3]

# 3 IMPORTANCE OF PHOSPHATES ON MANUFACTURING PROCESSING CHEESES

Processed cheese is manufactured from one or more of the natural cheeses with added emulsifying salts to give a prolonged shelf life. The basic premise is to stabilize the proteins that are normally affected already during one or more of the cheese-making steps. This is accomplished by rating and mixing cheeses with some emulsifying salts. The emulsifying salts serve to control the pH of the processed cheese and to bind calcium so that the protein can affectively stabilize the fat. The most common components of the commercial salt mixtures are phosphates, polyphosphates and citrates, although compound such as sodium potassium tartrate, complex sodium aluminium phosphate, or trihydroxyglutaric acid, could be used as well. The careful selection of cheeses, emulsifying salts and processing factors allows making process cheeses of varied textures suitable for many uses. [22, 23]

There are two major types of processed cheeses:

- Block cheese; this is a firm, slicing cheese with a relatively low moisture (40%) and high pH (5.7 – 6.3). This type of processed cheese can also be produced in the form slices.
- Cheese spread; this is a soft cheese with higher moisture content (50%) and a lower pH (5.4 – 5.8) [2]

The use of a blend cheese base, such as lactic or Cottage cheese, to act as a Carrar for herbs, vegetables or chopped cooked means is probably a spin-off from the use of processed cheese in the same manner. However, this worth nothing that processed cheese preparation are heat treated and this safe from the public health standpoint, while on spite of the low pH of the curds, the use of natural cheese curds could involve a public health risk unless the product is keep under correct refrigeration. [25]

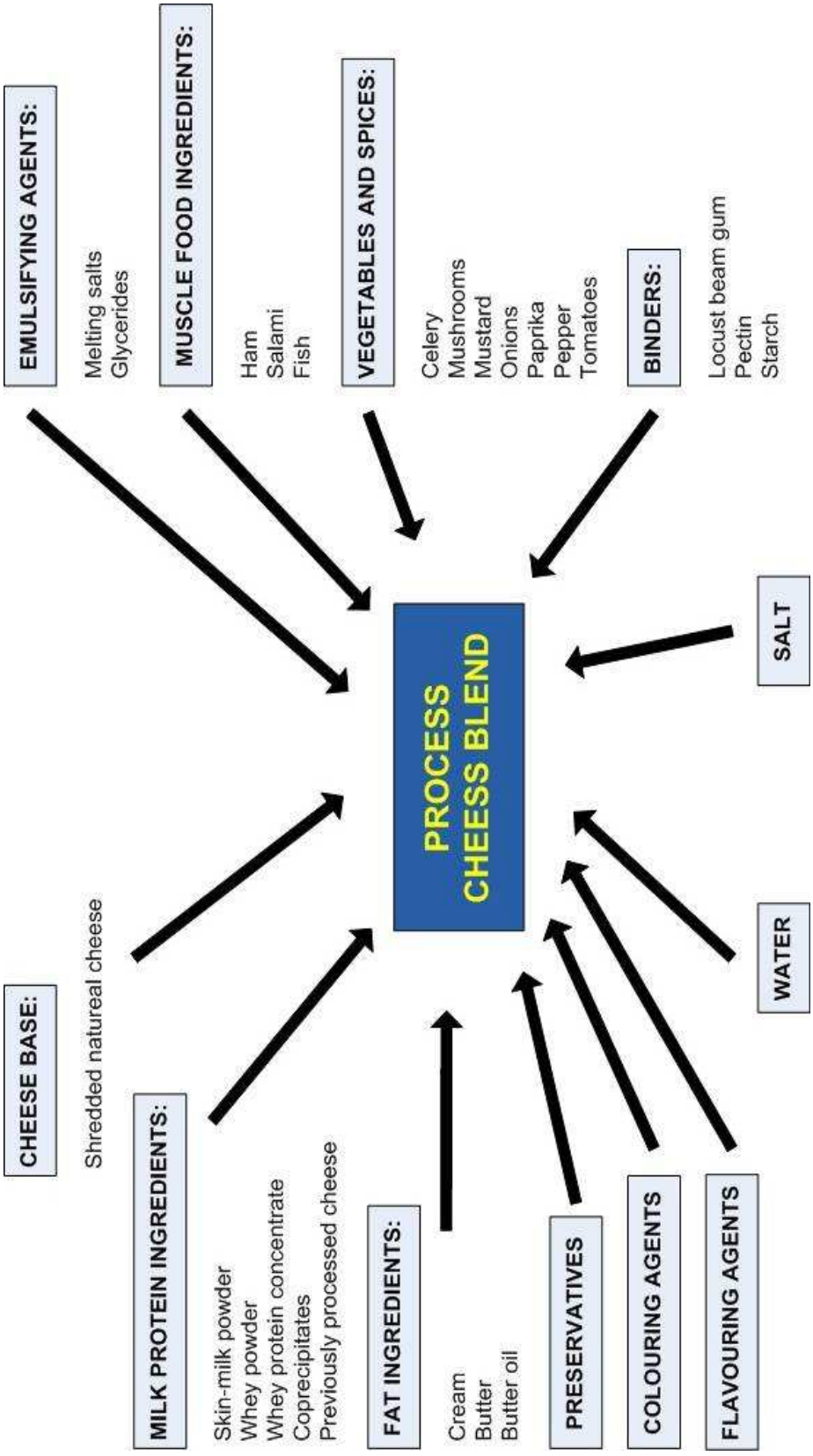
Processed cheese and cheese spread have traditionally been combined with other food such as chopped ham, pickles, lettuce, chips, onion, watercress, potatoes, carrots, coleslaw, etc. Some types are smoked after cooking, as we can see in Figure 4. [26]

Processed cheeses are further classified into different categories depending upon the ingredients used in their preparation. Although cheese is main ingredient, processed cheese in not a fermented food. [27, 28]

The principal advantages of processed cheese compared to natural cheeses are:

- Reduced refrigeration cost during storage and transport, which are very important in hot climates;
- They are more stable than natural cheeses during storage, which results in less wastage, a feature that may be especially important in remote areas and in households with a low level of cheese consumption;
- A certain amount of cheese which would otherwise be difficult or impossible to commercialize may be used, e.g. cheese with deformations;
- Better keeping quality, it means with less apparent changes during the prolonged storage;
- Another reason is great diversity of type and intensity of flavour, e.g. from mild to sharp, native cheese flavour or specific spices;
- They are attractive to children who generally do not like or appreciate the stronger flavour on natural cheeses;
- Adjustable packaging for various usage;
- Suitability for home use as well as for snack restaurants, for example in cheese burgers, hot sandwiches, spreads and dips for fast foods. [30,31]

Figure 4




*Ingredients used in the manufacture of processed cheese* [29]

## 3.1 Introduction – general characteristic

Natural cheese is one of the main ingredients used in processed cheese manufacture. The selection of the correct mixture of natural cheese ingredients greatly affects the texture and flavour of the final product. Natural cheese, a variety of other dairy ingredients can be used such as milk proteins, whey powders, cheese powders, skim milk powder and anhydrous milk. A further important ingredient group of the emulsifying salts, these are mixtures of calcium chelating salts, usually phosphates, polyphosphates or citrates. Although these salts are teemed “emulsifying salts”, they are not surface-active components in the strict sense. Various combinations of emulsifying salts are used to modify the texture and melting properties of the finished processed cheese product. [32]

Processed cheese was initially manufactured without an emulsifying agent: the first attempt was as early as 1895, but only after the introduction of citrate, and especially phosphates, as emulsifying agents did the industrial production of processed cheese become feasible. [30]

Processed cheeses are characterised essentially by composition, water content and consistency; according to these criteria, three main groups may be distinguished: processed cheese blocks, processed cheese foods and processed cheese spreads. More recently established sub-types of processed cheeses are: processed cheese slices and smoked processed cheese, the first sub-type belong to the category of processed cheese blocks, while the second could be either block or spread. [29]

Processing: Principles and techniques

The manufacturing procedure for processed cheese consists of operations performed in the following order:

- Selection of natural cheese;
- Calculation of the ingredients;
- Blending;
- Shredding;
- Addition of emulsifying agents;
- Thermal processing;
- Homogenization;

- Packaging;
- Cooling;
- Storage. [30]

### 3.1.1 Selection of natural cheese

Proper selection of natural cheese is of the most important for the successful production of processed cheese. Raw materials are ripened rennet cheese and sometimes other type of cheese which can be utilized for processed cheese. For melting basically all type of rennet cheeses are suitable and sour milk cheese can be added. The best melting characteristics are shown by hard and semisoft cheese types, among them especially Chester due to the specifically “fibrous” structure of the cheese mass. [30, 33]

Processed cheese quality, particularly taste and flavour, depends on proper natural cheese selection. The most important criteria for cheese selection are: type, maturity, flavour, consistency and structure, nature and character of the additives, legal requirements.

The selection and combination of natural cheeses of proper maturity is very important for processed cheese production. [29, 33]

However, some cheese with wrong physical characteristics can be used in processed cheese manufacture, as it is possible to correct them by smart blending. On the other hand natural cheeses with microbial defects they are not allowed to be selected for processing. [29, 33]

### 3.1.2 Calculation of the ingredients

Formulation of the ingredients is made such a way as to bring a desired finished product composition. Formulation is leaded to the basis of fat and dry matter contents of natural cheeses, including all mixed components, added water. [29, 33]

**Calculation of the batches**

When considering the type and degrees of ripening, analytical data for dry matter and fat in dry matter of the raw material must be known in order to calculate the production batches. The following sequence is possible:

a) Fixing the quantities in kg and % and addition of the amounts for dry matter and fat quantity.
b) Calculation of the theoretical fat content in dry matter according to

$$FiDM = \frac{m_f . 100\%}{m_{DM}}$$

c) Comparison of the theoretical FiDM with purposed one and eventual correction with cream, butter, low-fat or non-fat dry matter.
d) Calculation of yield Y according to

$$Y = \frac{m_{DM} . 100\%}{DM_T}$$

e) Calculation of the water to be added minus the water from the ingredients. Further, the amount of condensate must be deducted. This value must be determined by trials considering steam pressure, water content of the steam, temperature, time of heating and moisture content of product.

| | |
|---|---|
| FiDM | fat in dry matter |
| $m_f$ | fat quantity in kg |
| $m_{DM}$ | dry matter quantity in kg |
| $DM_T$ | time of heating dry matter in sec [29, 33] |

### 3.1.3 Blending

The basic step in the manufacture of process cheese is blending raw materials. The raw materials include the natural semi-mature cheese of shorter structure, emulsifying salts, and other ingredients. The recommended formulation for processed cheese block is 70-75% mild cheese and 25-30% semi-mature or mature cheese. On the other side, the blend

for processed cheese slices must contain 30-40% young cheese, 50-60 mild cheese, and only 10% mature cheese. Influences are shown in Table 3. [29, 33]

Table 3 Influence of ripening degree on the melting process. [29, 34]

| Factor | Advantages | Disadvantages |
|---|---|---|
| Young cheese (short ripening) | - Good dispersion, permitting a good emulsion of added butterfat;<br>- state of emulsion can be easily maintained during melting of the product. | - Long intensive treatment for protein digestion required;<br>- if melting time is too short, then a high-viscosity, rubber-like dough can form;<br>- tendency for a plain, sour taste;<br>- adheres easily to filling machine;<br>- pH value must be corrected;<br>- slow dispersion. |
| Old cheese (long ripening) | - Rapid dispersion, pronounced to spicy taste. | - Soft, sponge-like melting product with a mat appearance;<br>- has a tendency for “oiling”;<br>- low water binding capacity. |

Various other dairy and non-dairy ingredients are used in the production of different processed cheese types. Since the quality of the final product is influenced considerably by all the components present in the blend, the non-cheese components must also fulfil certain qualitative requirements. The most frequently used dairy ingredients are milk powder, casein, whey products, and milk-fat products. Colour and flavour may be added together with preservatives. [2, 29]

Milk powder is the dry form of skim milk. It contains not more than 1.5% fat and not more that 5% surface moisture. It has excellent flavour, nutritional value, and functional properties such as water binding, emulsification, and foam formation. It is also called dry milk, skim milk powder, and dried skim milk. Using skim milk powder for manufacturing

leads to changes of colours namely in consequence Maillard reaction. Especially, if total content of lactose is over 6% in the final product. [35]

Whey powder is the most common whey product. Whey may be regarded as skimmed milk less the casein. The casein protein is coagulated by acids or enzyme and is separated with the fat to form the cheese and this leaves the whey. Whey is rich in lactose and minerals but also includes the serum proteins – the albumins. Depending upon the type of cheese being formed, the whey may be "sweet" (from Cheddar and Swiss type cheeses) or acid (for example, from cream cheeses). [36]

### 3.1.4 Shredding

Shredding includes grinding of the components that are possible to make a better contact between emulsifying agents and blend ingredients. After that are all ingredients transferred directly into a processing cooker. This operation can be performed in many ways; shredded all ingredients alone, or in combination with other ingredients. [29]

### 3.1.5 Addition of Emulsifying Agent

The final phase in preparing the blend for processing is addition of emulsifying agents. Emulsifying agents are the most important in processed cheese production, where they are used to provide a uniform structure. Generally, polyphosphate application in the emulsifying agent blend result in processed cheese with superior structure and better keeping quality compared to other emulsifying agents. Phosphates and polyphosphates are used to add in quantity of 2-3% w/w. [34]

The affinity of phosphate to calcium ions is increased in following order: $NaH_2PO_4$ < $Na_2HPO_4$ < $Na_2H_2P_2O_7$ < $Na_3HP_2O_7$ < $Na_4P_20_7$ < $Na_5P_3O_{10}$ [29, 34]

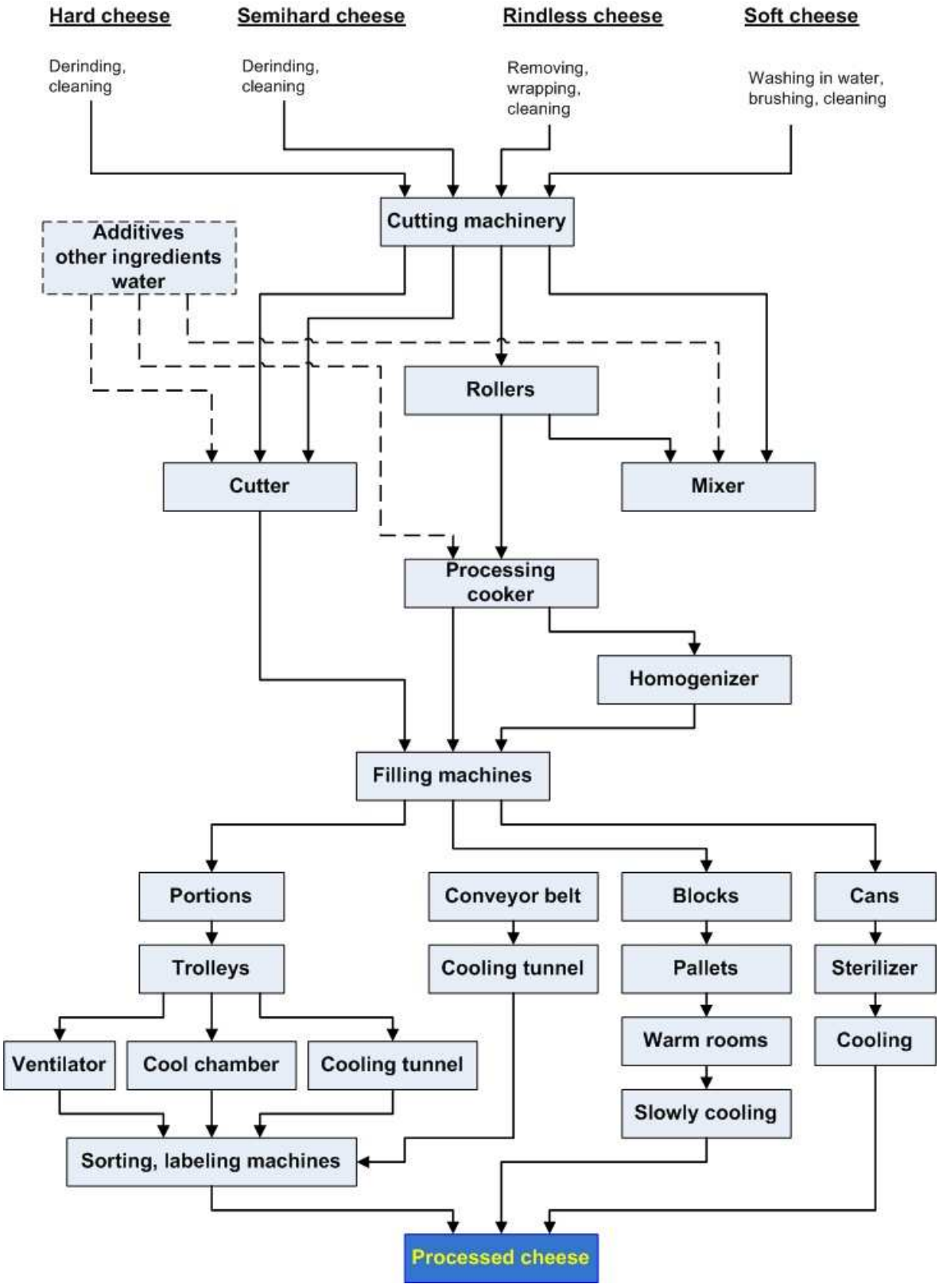



Figure 5 *Flow Chart of Processed Cheese Manufactureu* [29]

### 3.1.6 Thermal Processing

Processing means heat treatment of the blend. There is two possible way, how to made it. The first one is by direct steam and the second one is indirect steam, under a partial vacuum and with constant agitation. There are two types of cooking device:

1) Round (double-jacketed kettle, stainless-steel vessels of various size, fitted with corresponding lids and fitting for direct steam and vacuum);
2) Tube-shape (about 4 m long, fitted with one or two mixing worms).

If processing is carried out in a cooker, it means discontinuously, the temperature reached is 71 – 95°C for 4 to 15 minutes, depending on many various parameters. This heating also provides a pasteurization effect. In newly developed cooker, it is also possible to rich higher temperature up to 140°C.

After continuous processing, the blend is sterilized at temperature 130 – 145°C for 2 to 3 seconds in a battery of stainless steel tubes. [29, 30, 32]

Chemical, mechanical and thermal parameters used in cheese processing are listed in

Table 4.

Table 4 Chemical, Mechanical and Thermal Parameters as Regulating Factors in the Cheese Processing Procedures [29, 34]

| **Process conditions** | **Processed cheese block** | **Processed cheese slice** | **Processed cheese spread** |
|---|---|---|---|
| **Average of cheese** | Young to medium ripe, predominantly young | Predominantly young | Combination of young, medium ripe, overripe |
| **Water insoluble Nitrogen as a % of total Nitrogen** | 75 – 90% | 80 – 90% | 60 – 75% |
| **Structure of Emulsifying salt** | Predominantly long, high molecular weight polyphosphate, citrate | Long structure-building, not creaming, e.g. phosphate/citrate mixtures | Short to long, creaming, e.g. low and medium molecular weight polyphosphate |
| **Water addition** | 10–25% (all at once) | 5-15% (all at once) | 20-45% (in portions) |
| **Temperature** | 80-85°C | 78-85°C | 85-98°C (150°C) |
| **Duration of processing** | 4-8 min | 4-6 min | 8-15 min |
| **pH** | 5.4 – 5.7 | 5.6 – 5.9 | 5.6 – 6.0 |
| **Agitation** | Slow | Slow | Rapid |
| **Milk powder or whey powder** | 5 – 12 % | | |
| **Homogenization** | None | None | Advantageous |
| **Filling** | 5-15 min. | The quickest possible | 10 – 30 min. |
| **Cooling** | Slowly (10-12h) | Very rapid | Rapidly (15–30min) |

### 3.1.7 Homogenization

Homogenization improves the consistency, structure, appearance, and flavour of processed cheese. For a consumer is the main point of view, cheese products, processed cheese, are characterised by their flavour, texture and appearance. The texture of processed cheese is one of the most important factors for consumers. [32]

### 3.1.8 Packaging

Processed cheese is usually packed and wrapped in laminated foil in the form:

- tubes;
- cups;
- cans;
- cardboard;
- plastic containers;
- sausage form (usually smoking). [29]

Packaging materials having the ability to screen ultraviolet light have been developed for food products. Processed cheese in normal cellophane wax-coated wrappers becomes oxidized within 12 hours, and within 48 hours the top slice became inedible. This process can be stopped by addition of benzophenone. [37]

There are processed cheeses where the equilibrium relative humidity is above that of external climatic condition. The packaging material then serves the purpose of protecting from moisture loss. [38]

### 3.1.9 Cooling

The method and intensity of cooling depend on the type of processed cheese. These methods are shown in Table 4.

### 3.1.10 Storage

After packaging, the cheese can be stored in a cold storage until needed. Processed cheese should be stored at temperature between 5 to 10°C. [39]

# 4 APPEARANCE OF MICROORGANISMS IN PROCESSED CHEESE

Milk is extremely nutritious food. It is an aqueous solution of proteins, fats, and carbohydrates containing numerous vitamins and minerals. Milk has a pH of about 7.0 and is an excellent nutrient source for humans and animals as well as microorganisms. [40]

Microorganisms are important in milk and dairy products for free principal reasons:

- Pathogens or their toxins may constitute health hazards.
- Spoilage microorganisms or their metabolites may cause spoilage.
- Lactic acid bacteria and others may contribute in the preservative of milk and the production of desirable flavour and physical characteristics. [41]

Over the centuries, fermented milk products have assumed a key place in our diet. The sour milks are typical examples of fermented milk products. Buttermilk is made by adding starter cultures of *Lactococcus lactis* subsp. *cremoris* and *Leuconostoc citrovorum* to vats of skim milk. The *Lactococcus* ferments lactose to lactic and acetic acids, and the *Leuconostoc* continues the fermentation to yield various aldehydes and ketones, and the compound diacetyl. These substances, especially diacetyl, give buttermilk its flavour, aroma, and acidity. For sour cream, the same process is used, except that pasteurized light cream is the starting point. [40]

Cheese production begins when the casein curdles out of milk. Usually this accompanies a souring of the milk by *Lactococus*, but the process may be accelerated y adding rennin, an enzyme obtained from the stomach lining of a calf. The milk curd is essentially an unripened cheese. It may be marketed as cottage cheese, or pot cheese. Cream cheese also is unripened cheese with a butterfat content of up to 20 percent. [40]

To prepare ripened cheese, the milk curds are washed, pressed, sometime cooked, and cut to the desired shape. Often the curds are salted to add flavour, control moisture, and prevent contamination by moulds. If Swiss cheese is to be made, two bacterial species grow within the cheese; *Lactobacillus* species, which ferment the lactose to lactic acid; and *Propionibacterium* species, which produce organic compounds and carbon dioxine, which seeks out weak spots in the curd and accumulates as holes, or eyes. [37, 40]

## 4.1 Characteristics of bacteria

Gram-positive bacteria have cellular structure simpler than gram-negative bacteria. Gram-positive bacteria are those that are stained dark blue or violet by Gram staining, in contrast to Gram-negative bacteria, which cannot retain the stain, instead taking up the counterstained and appearing red or pink. The stain is retained by a high amount of peptidoglycan in the cell wall, which typically, but not always, lacks the secondary membrane and lipopolysaccharide layer found in gram-negative bacteria. The peptidoglycan can absorb the crystal violet, commonly used to stain bacteria.

The main purpose of gram staining is to visually differentiate groups of bacteria, primarily for identification. [42]

### *4.1.1 Staphylococcus aureus*

*Staphylococcus aureus* also known as golden staph is the most common cause of staph infections. It is a spherical bacterium, frequently living on the skin or in the nose of a person. Approximately 20–30% of the general population are "staph carriers". *Staphylococcus aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo (may also be caused by *Streptococcus pyogenes*), boils, cellulitis folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases, such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), and septicaemia. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the four most common causes of nosocomial infections, often causing postsurgical wound infections. [43,44]

Staphylococci are Gram-positive spherical bacteria. *Staphylococcus aureus* forms a fairly large yellow colony on rich medium. *Staphylococcus aureus* is a facultative anaerobe and opportunistic pathogen. *Staphylococcus aureus* is catalase positive (meaning that it can produce the enzyme "catalase"). [44, 45]

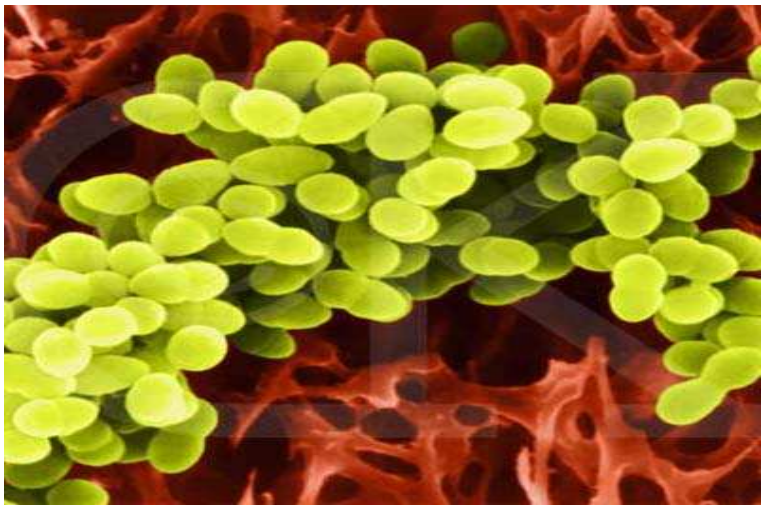


Figure 6 *Staphylococcus aureus* [46]

### *4.1.2 Bacillus subtilis*

*Bacillus subtilis* is a gram-positive, catalase-positive bacterium commonly found in soil. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme conditions. It has also been called “Hay bacillus” or “Grass bacillus”. It is bacillus because the bacterium is rod shaped or bacilli shaped. [47]

*Bacillus subtilis* is not considered a human pathogen; it may contaminate food but rarely causes food poisoning. *Bacillus subtilis* produces the proteolytic enzyme subtilisin. *Bacillus subtilis* spores can survive the extreme heating that is often used to cook food, and it is responsible for causing rapines — a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides — in spoiled bread dough. [41]

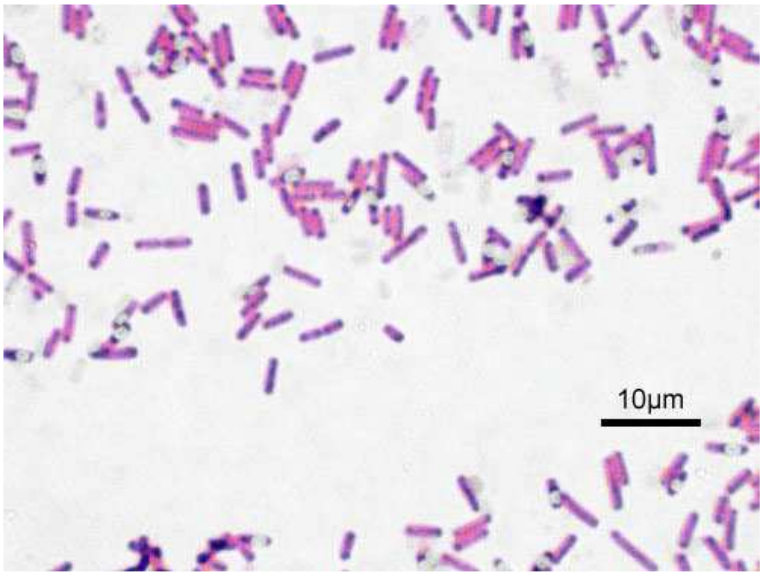



Figure 7 *Gram-stained Bacillus subtilis* [48]

### *4.1.3 Bacillus cereus*

*Bacillus cereus* bacteria are gram-positive, facultative aerobes, and like other members of the genus *Bacillus* can produce protective endospores. [49]

Bacillus cereus is responsible for a minority of food borne illnesses (2–5%). It is known to create heavy nausea, vomiting, and abdominal periods. Generally speaking, *Bacillus* food-borne illnesses occur due to survival of the bacterial spores when food is improperly cooked. This problem is compounded when food is then improperly refrigerated, allowing the spores to germinate. Bacterial growth results in production of enterotoxin, and ingestion leads to two types of illness, diarrheal and emetic syndrome. [49, 50]

A wide variety of foods including meats, milk, vegetables, and fish have been associated with the diarrheal type food poisoning. The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods such as potato, pasta and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks. [49, 50]

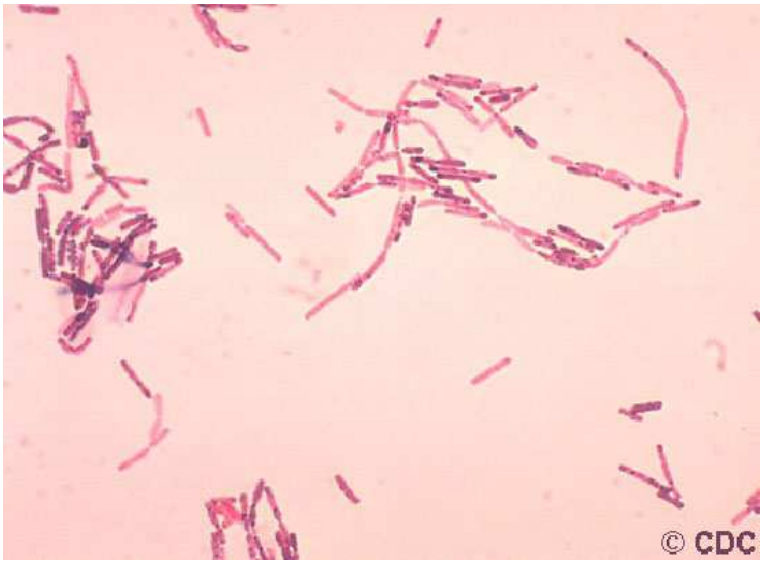



Figure 8 *Bacillus cereus* [51]

### *4.1.4 Bacillus brevis*

*Bacillus brevis* is a gram-positive aerobic spore-forming bacillus commonly found in soil, air, water, and decaying matter. It is rarely associated with infectious diseases. The antibiotics gramicidin and tyrocidine were first isolated from it. [52]

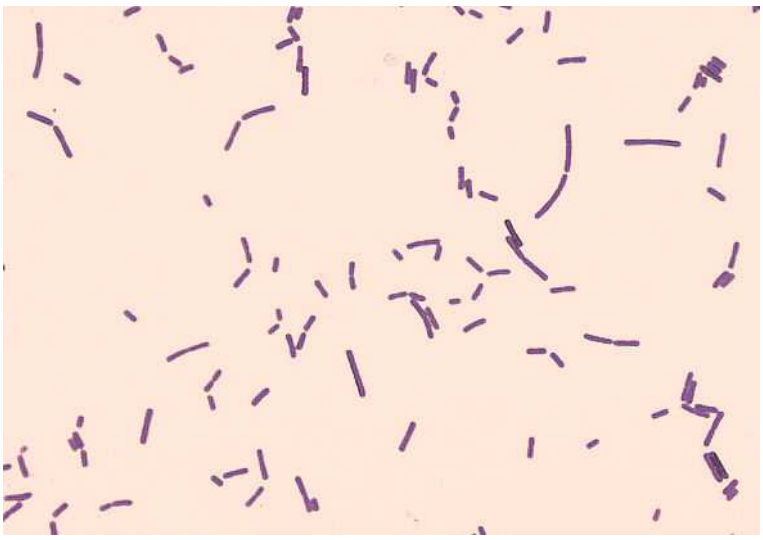


Figure 9 *Bacillus brevis* [52]

### *4.1.5 Bacillus sphaericus*

*Bacillus sphaericus* is a gram-positive aerobic spore-forming bacillus. *Bacillus sphaericus* is a naturally occurring soil bacterium that can effectively kill mosquito larvae present in water. B*acillus sphaericus* has the unique property of being able to control mosquito larvae in water that is rich in organic matter. *Bacillus sphaericus* is effective against *Culex* spp. but is less effective against some other mosquito species. [53, 54]

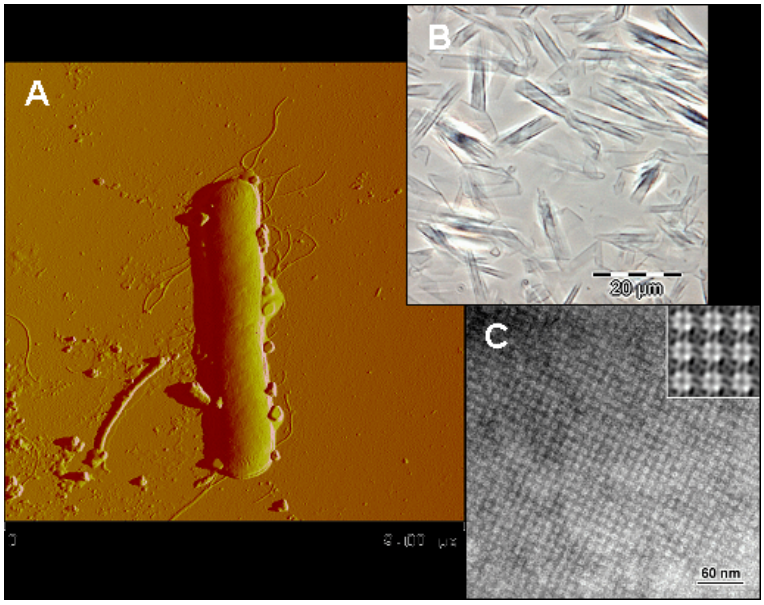



Figure 10 *Bacillus sphaericus* [55]

### *4.1.6 Micrococcus luteus*

*Micrococcus luteus* can be found in many places such as the human skin, water, dust, and soil. *Micrococcus* is generally harmless bacterium. *Micrococcus luteus* is gram-positive cocci that are 0.5 to 3.5 micrometers in diameter and usually arranged in tetrads or irregular clusters. They are generally strict aerobes and can generally reduce nitrate. *M. luteus* oxidises carbohydrates to $CO_2$ and water, and it does not produce acid from glucose as well as it does not make arginine dihydrolase or β-galactosidase. Some *Micrococcus* is pigmented bacteria; for example, *Micrococcus luteus* produces yellow colonies and *Kocuria rosea* (or more precisely *Micrococcus roseus*) produces redish colonies. *Micrococcus* species are oxidase-positive, which can be used to distinguish them from other bacteria like most *Staphylococcus* species, which are generally oxidase-negative. [56]

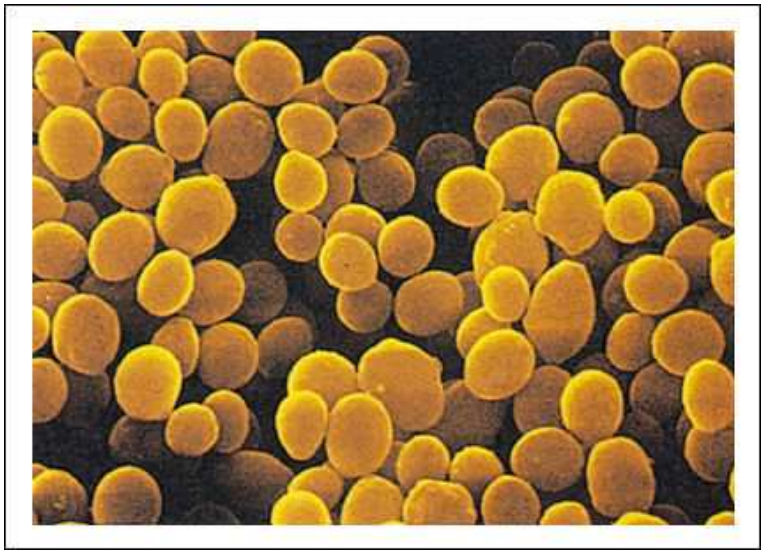


Figure 11 *Micrococcus luteus* [57]

### *4.1.7 Escherichia coli*

*Escherichia coli* are bacteria that are commonly found in the lower intestine of warm-blooded animals. Most *Escherichia coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls. [58]

*Escherichia coli* are a rod-shaped, and are typically 1-5 μm in length. *Escherichia coli* is gram-negative stains, and it is facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. *Escherichia coli*, better known as *E. coli*, is one of the most important model organisms and its genetics and biochemistry have been closely studied. [58, 59]

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In cases, virulent strains are also responsible for peritonitis, mastitis, septicemia and Gram-negative pneumonia. Recently it is thought that E. coli and certain other food borne illnesses can sometimes trigger serious health problems months or years after patients survived that initial bout. Food poisoning can be a long-term problem. [59]

Figure 12 *Escherichia coli* [60]

### *4.1.8 Serratia marcescens*

*Serratia marcescens* is a genus of Gram-negative, facultatively anaerobic, rod-shaped bacteria of the Enterobacteriaceae family. [59]

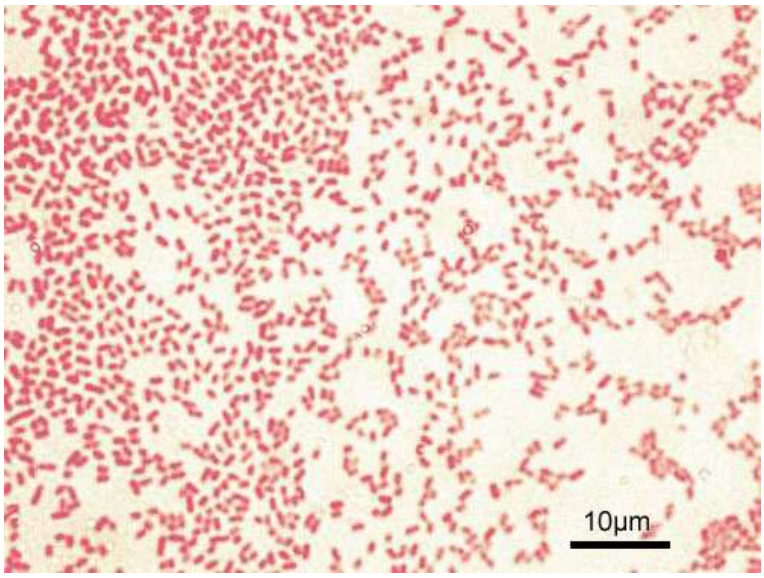



Figure 13 *Serratia marcescens* [61]

### *4.1.9 Pseudomonas fluorescens*

*Pseudomonas fluorescens* has multiple flagella. It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. [62]

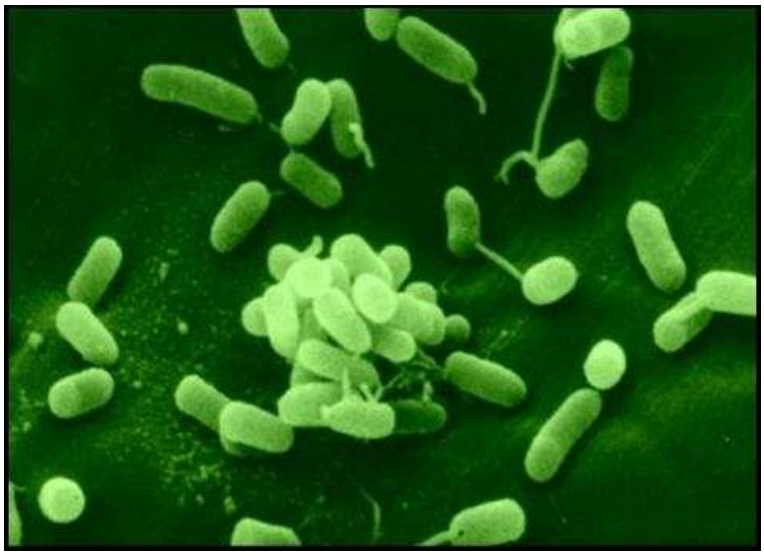


Figure 14 *Pseudomonas fluorescens* [63]

### 4.1.10 *Salmonella* Typhimurium

*Salmonella* Typhimurium is a rod-shaped, gram-negative bacterium which belongs to the *Enterobacteriacae* family. It is able to change its phenotype and alter its DNA to respond to changes or stimuli for survival purposes. This bacterium has gotten public interest since last year. [64]

It is a food born pathogen, spreading through food and water. It can also adapt to survive the cooking process and also has the ability to cross the gastric acid barrier (this is how they enter the human intestine). [65]

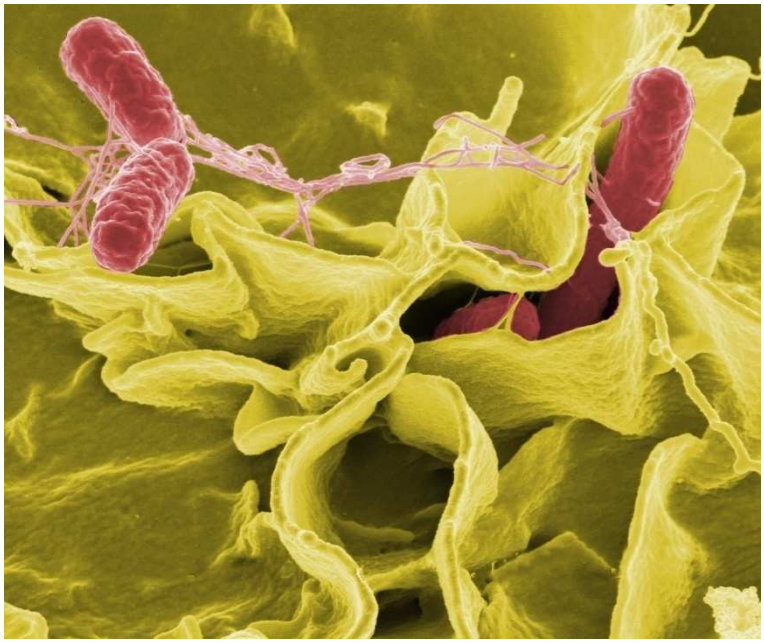


Figure 15 *Salmonella* Typhimurium [65]

# ANALYSIS

# 5 EXPERIMENTAL PART

## 5.1 Used chemicals

Beef extract (Himedia, Bombai, India)

Peptone (Himedia, Bombai, India)

Sodium chloride (Lach-Ner, Neratovice, CZ)

Agar (Himedia, Bombai, India)

JOHA S 9 (BK Giulini Chemie, Ladenburg, Germany)

SELF 690 (Chemische fabric BUDENHEIM, Germany)

JOHA HBS (BK Giulini Chemie, Ladenburg, Germany)

### 5.1.1 JOHA HBS

Emulsifying salt with bacteriostatic effect for the manufacture of processed cheese and processed cheese preparations.

**Composition:** E 452 sodium polyphosphate, E 339 sodium phosphate [66, 67]

Table 5 Specification of JOHA HBS

| | | |
|---|---|---|
| **Specification** | $P_2O_5$ content | 69.0% ± 1.0% |
| | pH - 1%, slurry | 6.0 ± 0.5 |

**Application:**

When used at an appropriate addition rate, JOHA HBS prevents the germination of bacterial spores. The recommended addition rate is between 0.3 – 1.0% calculated on the quantity of raw material to be processed. JOHA HBS is used together with other JOHA emulsifying salts. [66, 67]

### 5.1.2 JOHA S9

Emulsifying salt for the manufacture of processed cheese and processed cheese preparations. [37]

**Composition:** E 452 sodium polyphosphate, E 339 sodium phosphate [66]

Table 6 Specification of JOHA S9

| **Specification** | $P_2O_5$ content | 59.7 ± 1.0 |
|---|---|---|
| | pH - 1%, slurry | 9.0 ± 0.3 |

**Application:**

The recommended addition rate is between 2.8 – 3.2% calculated on the quantity of raw material to be processed.

### 5.1.3 SELF 690

Emulsifying salt for the manufacture of low-fat processed cheese.

**Composition:** E 450 c orthophosphate, E 339 sodium phosphate [66]

Table 7 Specification of SELF 690

| **Specification** | $P_2O_5$ content | 54.0 |
|---|---|---|
| | pH - 1%, slurry | 8.4 |

### 5.1.4 SELF 690

Emulsifying salt is for the manufacture of low-fat processed cheese.

**Composition:** E 450 c orthophosphate, E 339 sodium phosphate [66]

Table 8 Specification of SELF 690

| | | |
|---|---|---|
| **Specification** | $P_2O_5$ content | 54.0 |
| | pH - 1%, slurry | 8.4 |

**Application:**

SELF 690 has a very good influence for dissolving of proteins without creaming action.

## 5.2 Used equipments

Analytical balance (BA 110S, Kern)

Oast (STE 39/II, Chirana Brno)

Digester Varioklav H+P (Germany)

Sterilator (MEMMERT, model 100-800, Germany)

Biological oven TCH-54 (Laboratory instruments Praha)

Micropipettes (BIOHIT)

Biohazard Box Euroflow (Holand)

## 5.3 Microorganisms

For determination of the antibacterial activity spectrum, an additional selected set of 6 gram-positive organisms and 4 gram-negative organisms were used. The strains were taken from the ÚPI FT UTB.

**Gram-positive strains:**

*Staphylococcus aureus*

*Bacillus subtilis*

*Bacillus cereus*

*Bacillus brevis*

*Bacillus sphaericus*

*Micrococcus luteus*

**Gram-negative strains:**

*Escherichia coli*

*Serratia marcescens*

*Pseudomonas fluorescens*

*Salmonella* Typhimurium

## 5.4 Preparation of cultivating medium

- Preparation of Nutrient Broth

These strains of microorganisms were propagated on Nutrient Broth.

Ingredients:

- Beef extract..... 3.0g/l
- Peptone..... 5.0g/l
- NaCl..... 5.0g/l
- $H_2O$..... ..... 1000.0ml
- pH (25°C) 6.8

These strains of microorganisms were spread on liquid medium and incubated aerobically for 1-3 days at 30°C for Bacillus sp. and at 37°C for the others strains of organisms.

- Preparation of Plate Count Agar (PCA)

Characterization of PCA

Ingredients:

- Beef extract..... 3.0g/l
- Peptone..... 5.0g/l
- NaCl..... 5.0g/l
- Agar..... 15.0g/l
- $H_2O$..... ..... 1000.0ml
- pH (25°C) 6.8 – 7.2

- Preparation of saline

Saline is a general term referring to a sterile solution of sodium chloride in water. Saline was prepared from 8.5g sodium chloride who was into volumetric cupping-glass about capacity 1000mls filled up with distilled aqua.

## 5.5 Cultivation of microorganisms on agar plates

This method is used for determination minimum inhibitory concentration. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial inhibitors that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial agents that will prevent the growth of an organism after subculture on media.

Preparation of agar is described in chapter 5.4.

After sterilization of agar in infusion bottles the substance was poured by sterile method to Petri dishes. Saline was poured into eight test tubes 4.5 ml each and sterilized in digester. One larger colony was always removed by sterilized inoculating loop and mixed into saline. Than it was diluted by decimal system such way that into each test tube putted by micropipette 0.5ml of liquid from previous test tube with new sterilized pipette tip.

For solid agar which contained different concentration emulsifying salts, was putted by micropipette 0.1ml of inoculum of different concentration and spread with sterile hook to cover entire tray.

Dilution inoculum applied on trays was done such way that it was possible to deduct newly grown colonies microorganisms. In case of strong effect of salts there was used basic inoculum. Trays were incubating by temperature 30-37°C for 24 - 72 hrs. After that time there were grown colonies counted and it was established inhibition effect of particular salt. Used concentration of salts are given in table.8, concentration in 0% is given for the control.

Each test was repeated twice.

Table 9 Concentration of salts

| Concentration | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| **0.0** | 0 g/l | 0 g/l | 0 g/l |
| **0.1** | 1g/l | 1g/l | 1g/l |
| **0.2** | 2g/l | 2g/l | 2g/l |

| | | | |
|---|---|---|---|
| **0.3** | 3g/l | 3g/l | 3g/l |
| **0.4** | 4g/l | 4g/l | 4g/l |
| **0.5** | 5g/l | 5g/l | 5g/l |

From the number of colonies of microorganisms, after each dilution of inoculums it was calculated value of CFU/ml.

This value indicates number of bacteria able to create colonies.

Calculation of CFU/ml is expressed by a formula:

$$CFU = \frac{count\ of\ the\ colonies}{dilution} \cdot 10$$

# 6 MAIN PURPOSE OF THIS STUDY

The objective of the present study was to examine the antimicrobial effect of polyphosphates allowed for use in dairy and processed cheese productions.

There were applied there types of emulsifying agents:

1) JOHA HBS – mixture of polyphosphates (high amount of monomers in polymer chain) and phosphates (monomers),
2) JOHA S9 – mixture of polyphosphates (low amount of monomers in polymer chain) and phosphates,
3) SELF 690 – mixture of phosphates and orthophosphates.

Emulsifying agents JOHA HBS and JOHA S9 is made by BK Ladenburg GmbH, Germany. SELF 690 is made by Chemische Fabrik Budenheim, Germany. Ten model microorganisms were chosen: six gram-positive microorganisms *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus* and four gram-negative microorganisms *Escherichia coli, Serratia marcescens, Pseudomonas fluorescens, Salmonella* Typhimurium.

The aim of this work was to obtain data on the inhibitory effect polyphosphates (emulsifying salts) on different microorganisms.

In the first series were tested some microorganisms, because it was necessary to find the best model microorganisms, for this work.

# 7 RESULTS AND DISCUSION

## 7.1 Table part

This test was made for finding the best microorganisms in the next applications. On the agar plates were inoculated microorganisms in different dilution. In these tables there is shown the antimicrobial effect for chosen microorganisms. The results we can see in these tables and on the photos in the Appendices 1, 2, 3.

***Escherichia coli***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | 0 | 0 | 0 |
| 0.2 | 0 | 0 | 0 |
| 0.3 | 0 | 0 | 0 |
| 0.4 | 0 | 0 | 0 |
| 0.5 | 0 | 0 | 0 |

***Bacillus subtilis***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | xx | 0 | 0 |
| 0.2 | xx | 0 | 0 |
| 0.3 | xx | 0 | 0 |
| 0.4 | xxx | 0 | 0 |
| 0.5 | xxx | 0 | 0 |

***Micrococcus luteus***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | xx | 0 | 0 |
| 0.2 | xx | 0 | 0 |
| 0.3 | xxx | xxx | 0 |
| 0.4 | xxx | xxx | 0 |
| 0.5 | xxx | xxx | 0 |

***Bacillus sphaericus***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | 0 | 0 | 0 |
| 0.2 | x | 0 | 0 |
| 0.3 | xx | 0 | 0 |
| 0.4 | xxx | 0 | 0 |
| 0.5 | xxx | 0 | 0 |

***Serratia marcescens***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | 0 | 0 | 0 |
| 0.2 | 0 | 0 | 0 |
| 0.3 | 0 | 0 | 0 |
| 0.4 | 0 | 0 | 0 |
| 0.5 | 0 | 0 | 0 |

***Pseudomonas fluorescens***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | 0 | 0 | 0 |
| 0.2 | 0 | 0 | 0 |
| 0.3 | x | 0 | 0 |
| 0.4 | x | 0 | 0 |
| 0.5 | x | 0 | 0 |

***Staphylococcus aureus***

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| 0.1 | 0 | 0 | 0 |
| 0.2 | 0 | 0 | 0 |
| 0.3 | 0 | 0 | 0 |
| 0.4 | xxx | 0 | 0 |
| 0.5 | xxx | 0 | 0 |

***Salmonella* Typhimurium**

| Concentration of salt | JOHA HBS | JOHA S9 | SELF 690 |
|---|---|---|---|
| **0.1** | **0** | **0** | **0** |
| **0.2** | **0** | **0** | **0** |
| **0.3** | **0** | **0** | **0** |
| **0.4** | **0** | **0** | **0** |
| **0.5** | **0** | **0** | **0** |

***0*** ***none inhibition effect***
***x*** ***slight inhibition effect***
***xx*** ***medium strong inhibition effect***
***xxx*** ***very strong inhibition effect***

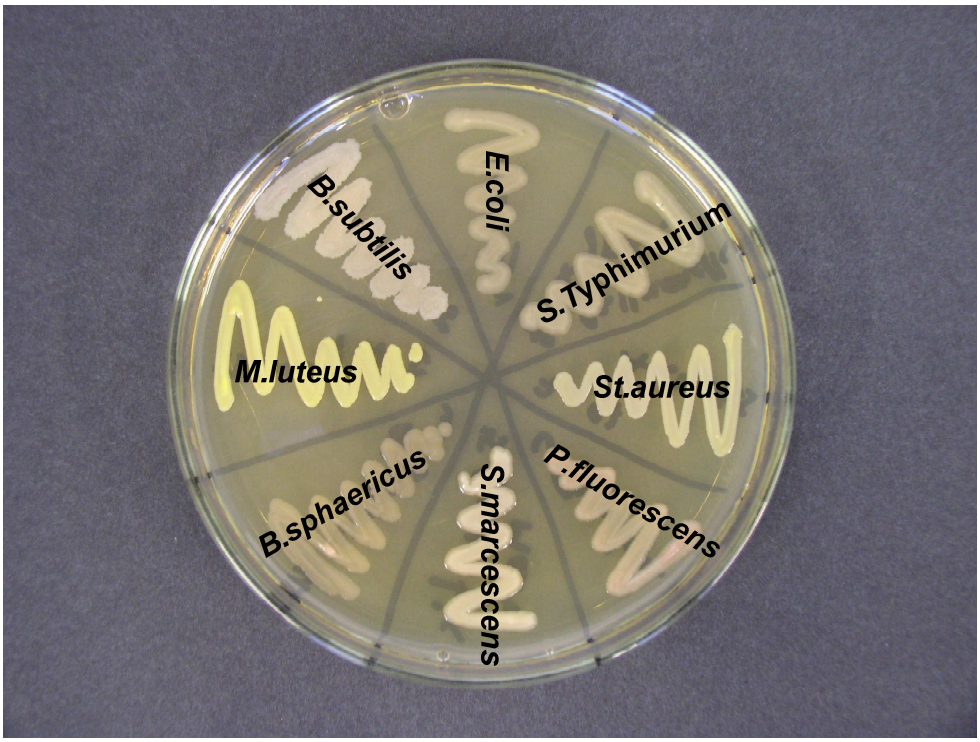



Figure 16 Control Petri dish

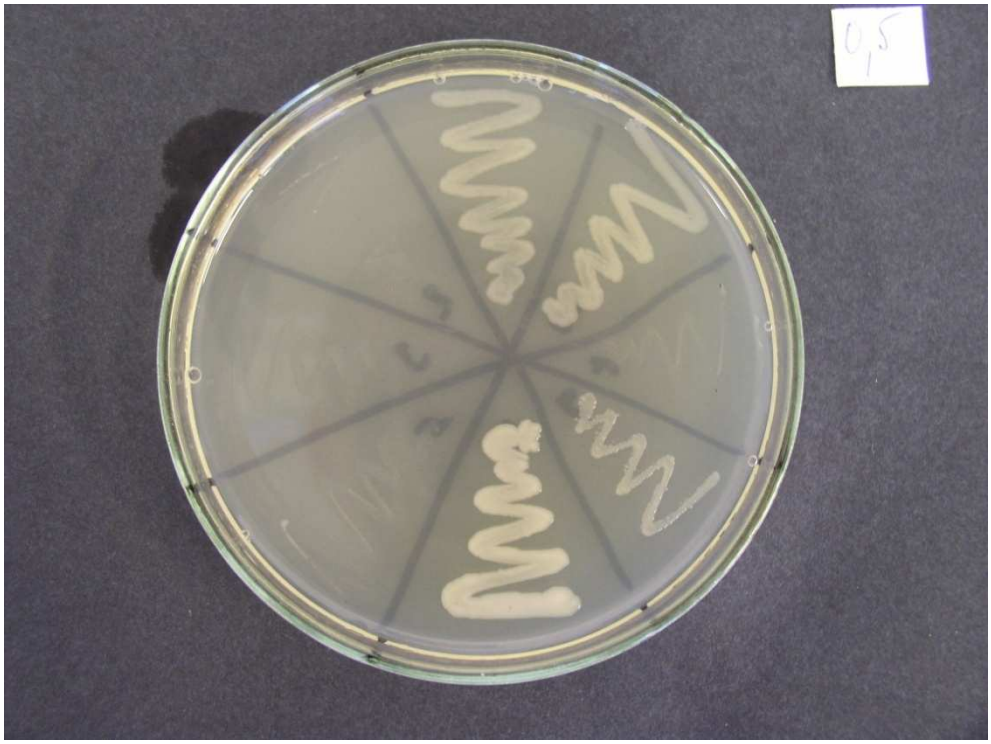



Figure 17 Petriho dish with JOHA HBS in concentration of 0.5%

The microorganisms were inoculated in the same sequence as a Figure 16.

## 7.2 Evaluation effect of emulsifying salts

The way how the bacteriostatic effects were tested is showed in section 5.5.

### *7.2.1* Evaluation effect of emulsifying salts for *Staphylococcus aureus*

On the table number 15 are shown the dependence of concentration of salts on CFU/ml.

Table 10 Growth inhibition of *Staphylococcus aureus* at 37°C by different emulsifying salt

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **10.775** | | |
| **0.1** | 10.725 | 9.136 | 10.008 |
| **0.2** | 10.625 | 9.049 | 9.901 |
| **0.3** | 10.641 | 9.021 | 9.371 |
| **0.4** | 0 | 0 | 0 |
| **0.5** | 0 | 0 | 0 |

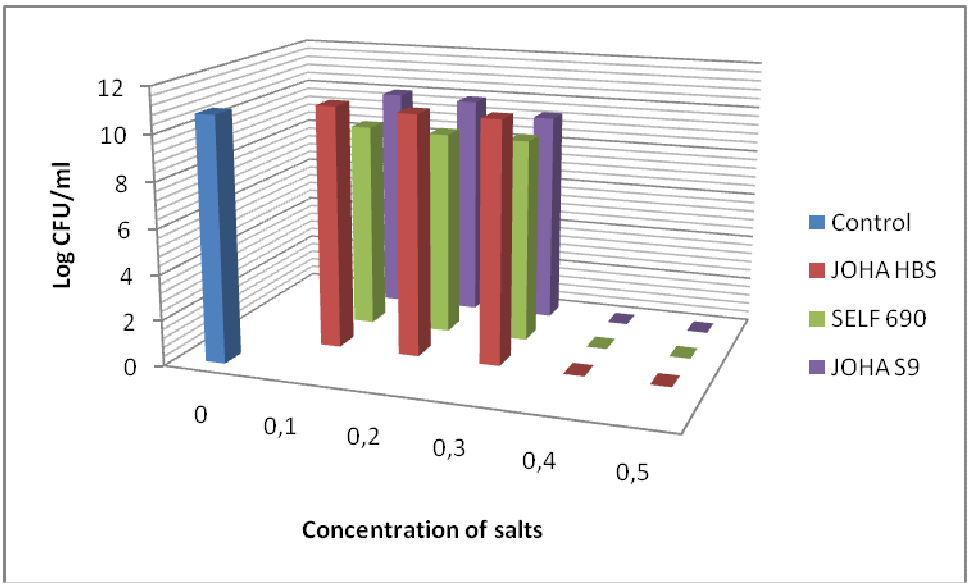


Figure 18 Growth inhibition of *Staphylococcus aureus* at 37°C by different emulsifying salts

The both, 0.4 and 0.5% polyphosphates were sufficient to inhibit vegetative growth of the organisms.

### *7.2.2* Evaluation effect of emulsifying salts for *Bacillus subtilis*

Dependence of concentration of salts on CFU/ml is shown on the table number 16.

Table 11 Growth inhibition of *Bacillus subtilis* at 30°C by different emulsifying salts

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **8.687** | | |
| **0.1** | 7.477 | 7.477 | 7.352 |
| **0.2** | 6.954 | 7.477 | 7.332 |
| **0.3** | 6.812 | 7.301 | 7.267 |
| **0.4** | 6.301 | 0 | 6.740 |
| **0.5** | 0 | 0 | 0 |

Lower concentrations of emulsifying salts are allowed to inhibit the growth *of Bacillus subtilis.* The both, 0.4 and 0.5% polyphosphates were sufficient to inhibit vegetative growth of the organisms.

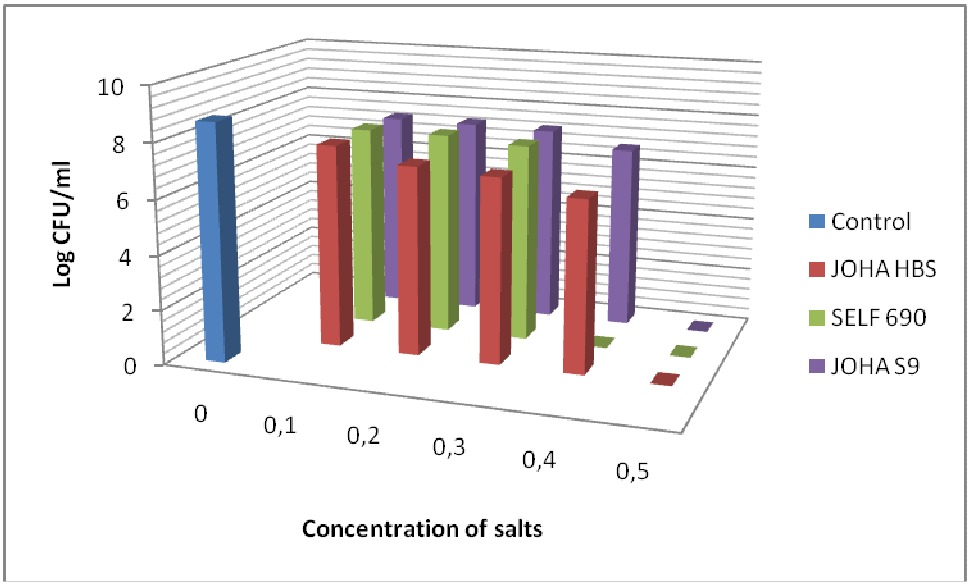



Figure 19 Growth inhibition of *Bacillus subtilis* at 30°C by different emulsifying salts

### *7.2.3* Evaluation effect of emulsifying salts for *Bacillus cereus*

Dependence of concentration of salts on CFU/ml is shown on the table number 17.

Table 12 Growth inhibition of *Bacillus cereus* at 30°C by different emulsifying salts

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **7.623** | | |
| **0.1** | 6.653 | 6.903 | 6.919 |
| **0.2** | 6.477 | 6.954 | 6.903 |
| **0.3** | 6.000 | 6.602 | 6.897 |
| **0.4** | 0 | 6.000 | 0 |
| **0.5** | 0 | 0 | 0 |

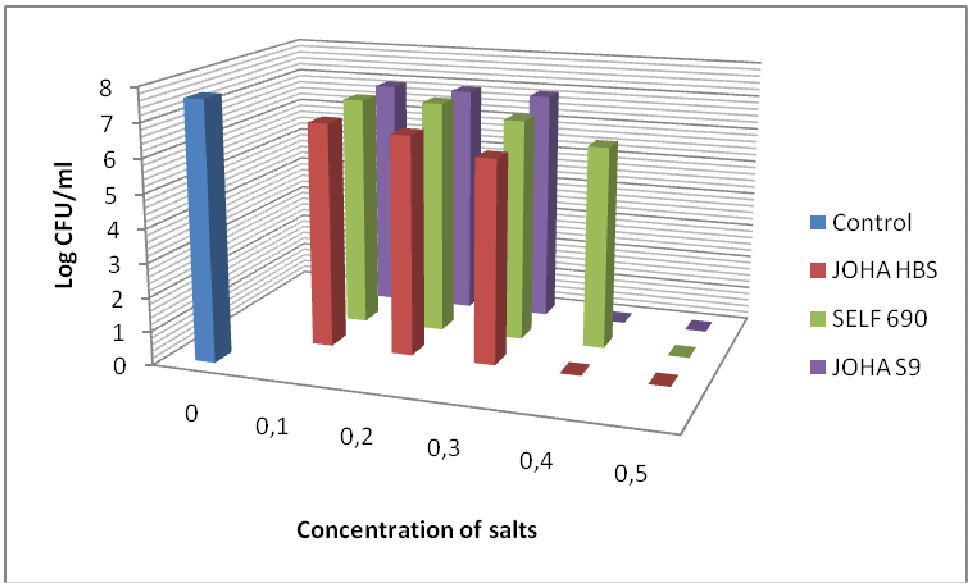



Figure 20 Growth inhibition of *Bacillus cereus* at 30°C by different emulsifying salts

Higher concentrations of polyphosphates were sufficient to inhibit vegetative growth of the organisms.

Evaluation effect of emulsifying salts for *Micrococcus luteus*

Dependence of concentration of salts on CFU/ml is shown on the table number 18.

Table 13 Growth inhibition of *Micrococcus luteus* at 30°C by different emulsifying salts

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **8.380** | | |
| **0.1** | 7.658 | 7.892 | 0 |
| **0.2** | 7.301 | 7.963 | 0 |
| **0.3** | 7.000 | 7.060 | 0 |
| **0.4** | 0 | 0 | 0 |
| **0.5** | 0 | 0 | 0 |

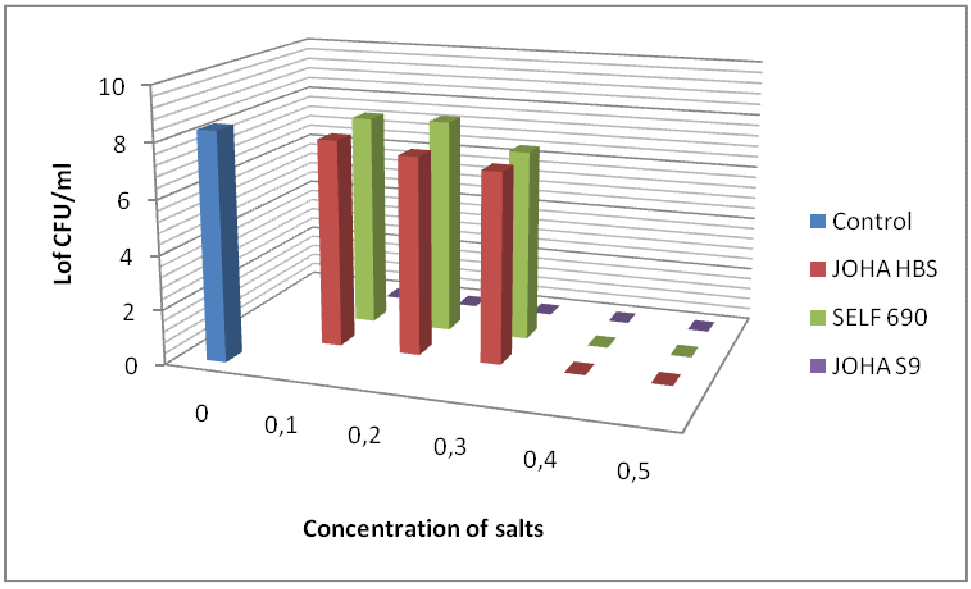



Figure 21 Growth inhibition of *Micrococcus luteus* at 30°C by different emulsifying salts

The both, 0.4 and 0.5% polyphosphates were sufficient to inhibit vegetative growth of the organisms. The emulsifying salt JOHA S9 totally inhibited the growth of *Micrococcus luteus.*

### 7.2.4 Evaluation effect of emulsifying salts for *Bacillus brevis*

On the table number 19 are shown the dependence of concentration of salts on CFU/ml.

Table 14 Growth inhibition of *Bacillus brevis* at 30°C by different emulsifying salts

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **8.7888** | | |
| **0.1** | 8.633 | 8.371 | 8.058 |
| **0.2** | 8.491 | 8.096 | 8.021 |
| **0.3** | 8.311 | 7.602 | 7.653 |
| **0.4** | 0 | 0 | 7.000 |
| **0.5** | 0 | 0 | 0 |

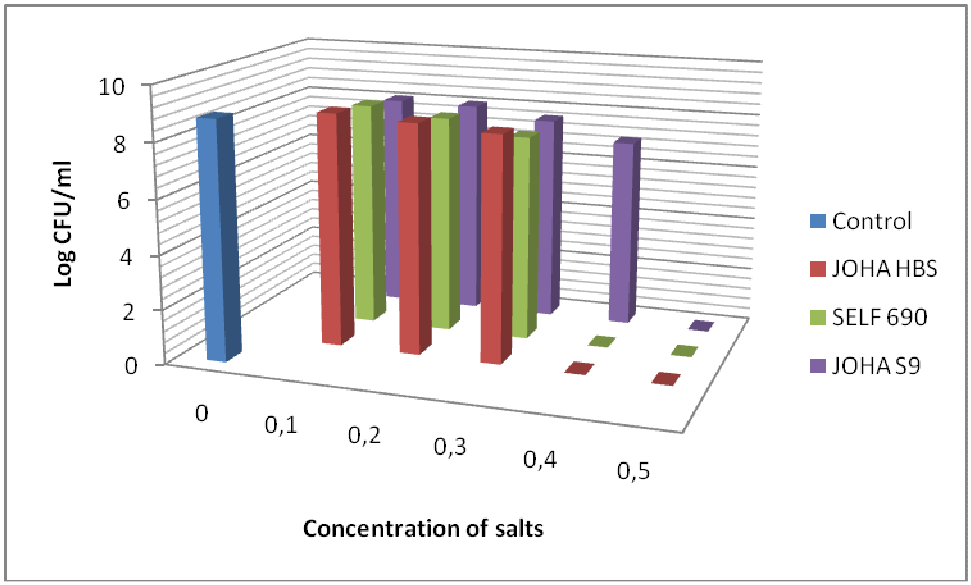



Figure 22 Growth inhibition of *Bacillus brevis* at 30°C by different emulsifying salts

The both, 0.4 and 0.5% polyphosphates were sufficient to inhibit vegetative growth of the organisms.

### 7.2.5 Evaluation effect of emulsifying salts for *Bacillus stearothermophilus*

On the table number 20 are shown the dependence of concentration of salts on CFU/ml.

Table 15 Growth inhibition of *Bacillus stearothermophilus* at 30°C by different emulsifying salts

| **Concentration of salt (%)** | **SALT** | | |
|---|---|---|---|
| | **JOHA HBS Log CFU/ml** | **SELF 690 Log CFU/ml** | **JOHA S9 Log CFU/ml** |
| **0** | **7.6232** | | |
| **0.1** | 0 | 7.448 | 7.049 |
| **0.2** | 0 | 7.423 | 7.238 |
| **0.3** | 0 | 7.243 | 6.301 |
| **0.4** | 0 | 0 | 0 |
| **0.5** | 0 | 0 | 0 |

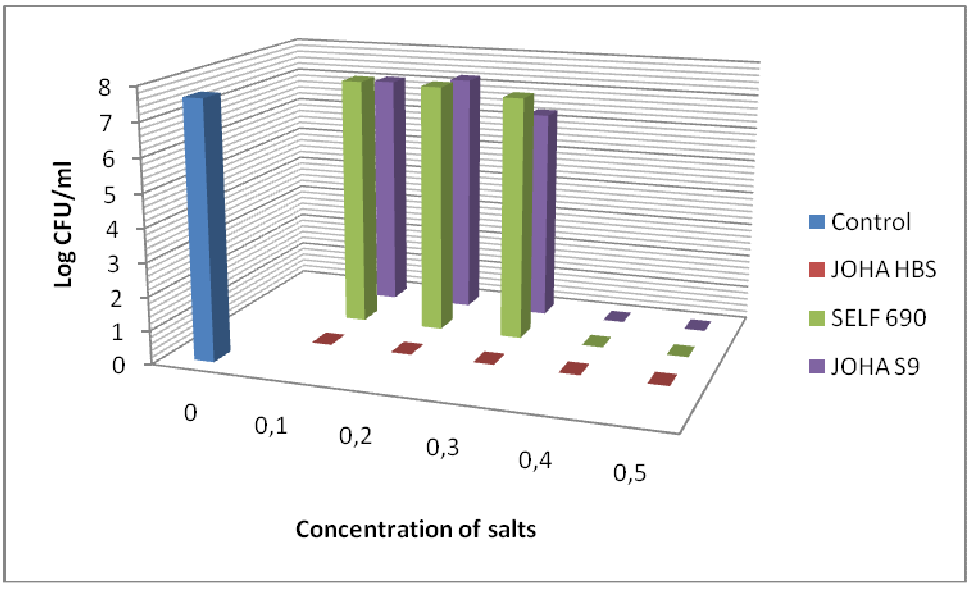



Figure 23 Growth inhibition of *Bacillus stearothermophilus* at 30°C by different emulsifying salts

JOHA HBS inhibited all growth of the organisms. The 0.4 and 0.5% polyphosphates were sufficient to inhibit growth of the organisms.

## 7.3 Evaluation effect of emulsifying salt JOHA HBS for microorganisms

On the figure 24 are shown tested microorganisms in all concentration of salt JOHA HBS in dependence on log CFU/ml.

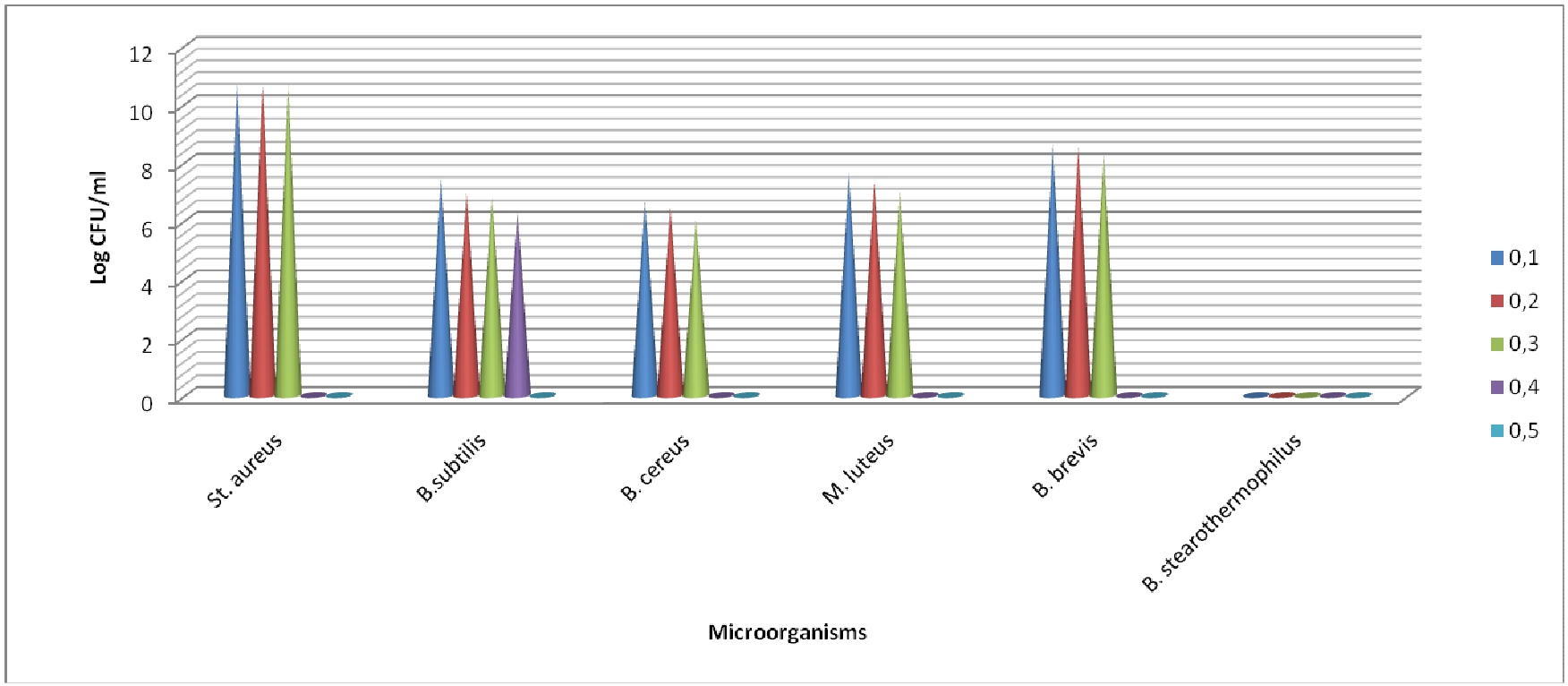



Figure 24 Emulsifying salt JOHA HBS

## 7.4 Evaluation effect of emulsifying salt SELF 690 for microorganisms

On the figure 25 are shown tested microorganisms in all concentration of salt SELF 690 in dependence on log CFU/ml.

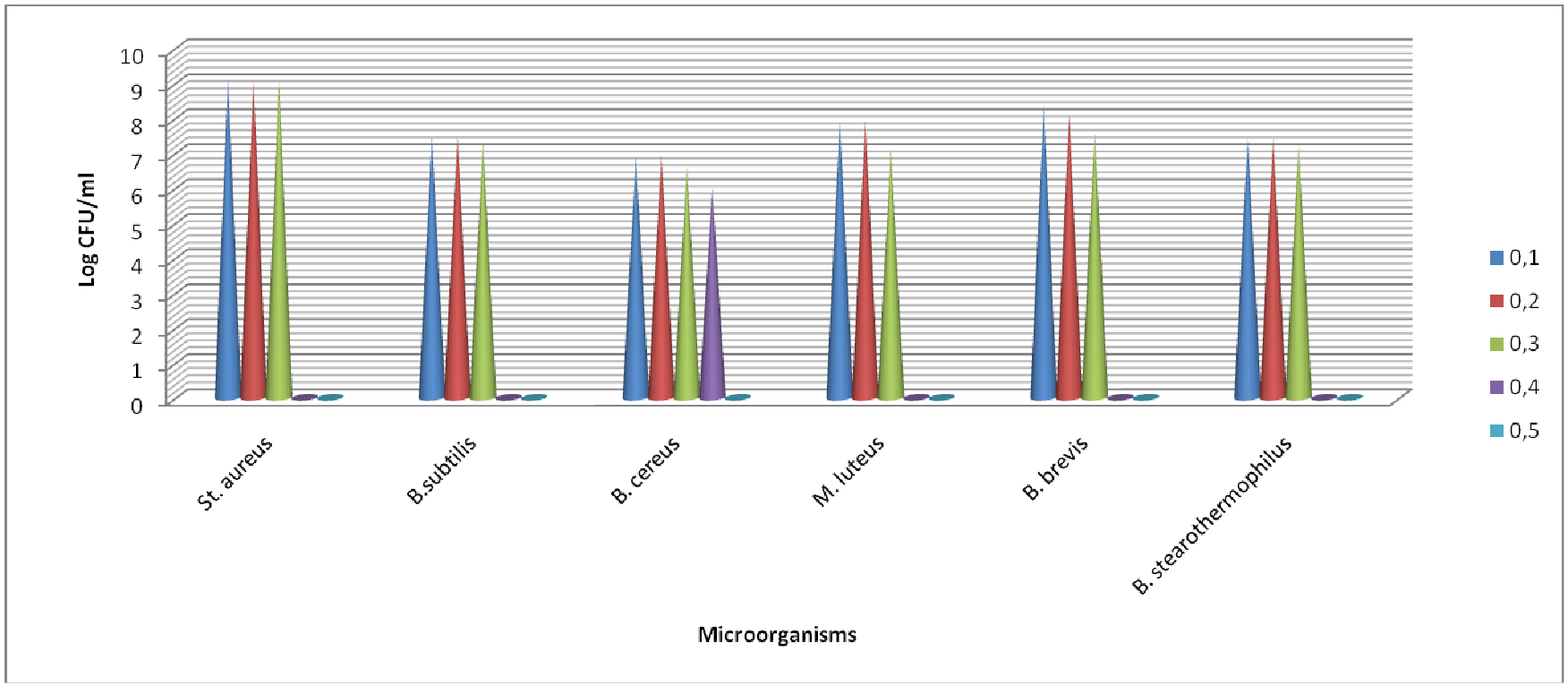



Figure 25 Emulsifying salts SELF 690

## 7.5 Evaluation effect of emulsifying salt JOHA S9 for microorganisms

On the figure 26 are shown tested microorganisms in all concentration of salt JOHA S9 in dependence on log CFU/ml.

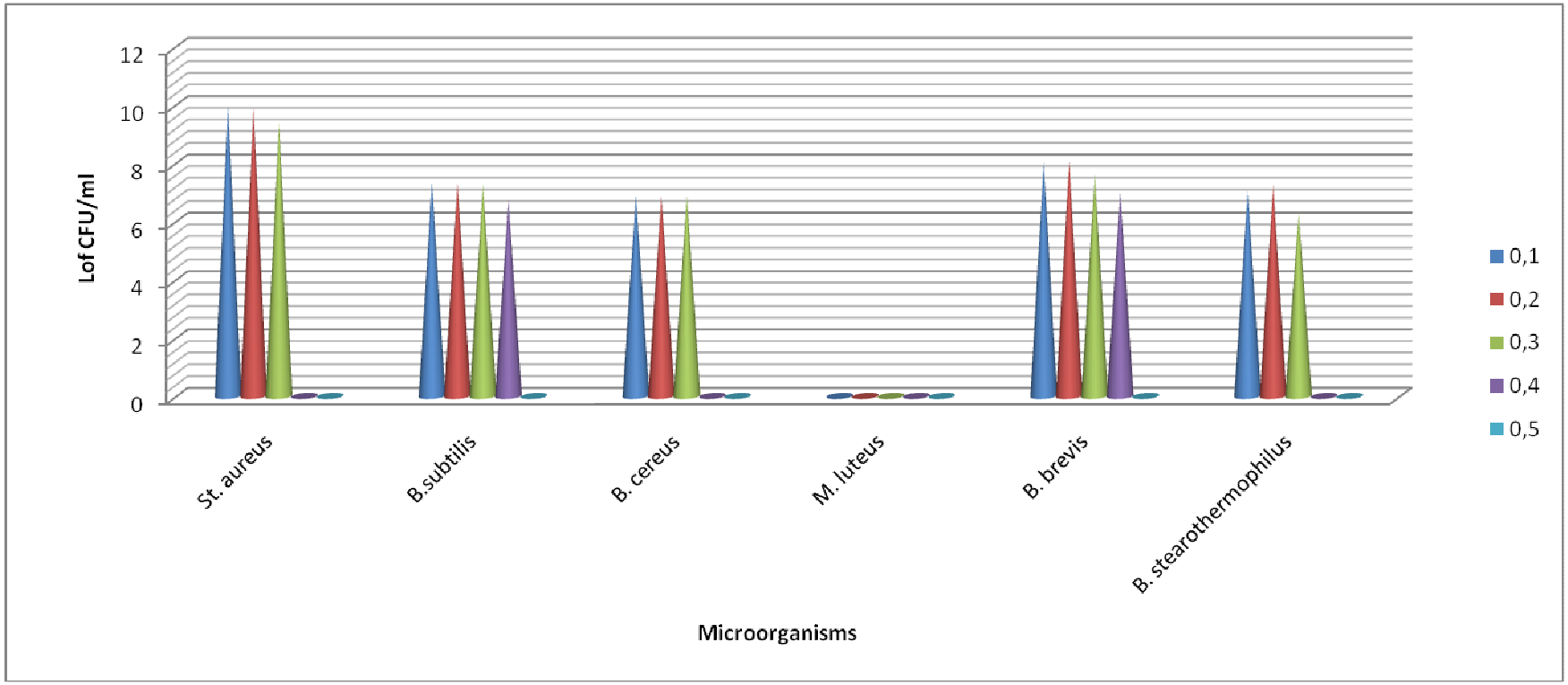



Figure 26 Emulsifying salt JOHA S9

## Discussion

Processed cheese is produced by blending shredded natural cheeses of different types and degrees of maturity with emulsifying salts, and by heating the blend under partial vacuum with constant agitation until a homogenous mass is obtained. Emulsifying agents play the major role in processed cheese production where there are used to provide a uniform structure during the melting process. Phosphates and polyphosphates are most common in quantity of 2-3% w/v. There were chosen three types of emulsifying salts: JOHA HBS, JOHA S9 and SELF 690. These salts were applied in the concentration 0.1 to 0.5% w/v in plate count agar.

Phosphates are used as additives, mainly in meat and dairy products, to improve quality of these foods. The antibacterial properties of phosphates are also well known. In this work, ten strains, six gram-positive and four gram-negative microorganisms were used.

The inhibition of ten microorganisms by three commercial phosphates (JOHA HBS, JOHA S9, SELF 690) was investigated. Ten model microorganisms were chosen: *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus sphaericus, Micrococcus luteus, Escherichia coli, Serratia marcescens, Pseudomonas fluorescens* and *Salmonella* Typhimurium. The microorganisms were inoculated in plate count broth with phosphates at concentration 0.1; 0.2; 0.3; 0.4; and 0.5% (w/v). Inoculated plates were incubated for 48 hours at 30 ± 1°C. The minimal inhibitory concentration (MIC) value was determined.

JOHA HBS (Table 24) showed MIC values of 0.1% (w/v) for *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Micrococcus luteus* and all strains were inhibited completely with 0.5% (w/v) phosphates. *Bacillus sphaericus* was inhibited completely with 0.1% (w/v).

JOHA S9 (Table 26) showed MIC values of 0.1% (w/v) for *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, and Bacillus brevis.* The concentration of 0.5% (w/v) was enough for S9 to suppress gram-positive microorganism's growth throughout the 48 hours incubation. *Micrococcus luteus* was inhibited completely with 0.1% (w/v).

SELF 690 (Table 25) showed MIC values of 0.1% (w/v) for *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus brevis, Micrococcus luteus* and all strains were

inhibited completely with 0.5% (w/v) phosphates. The best antimicrobial effect of this salt was shown on *Staphylococcus aureus*. These results we can see on Tables 15-20, and on Figures 16-24

For gram-negative bacteria *Escherichia coli, Serratia marcescenss, Pseudomonas fluorescens* and *Salmonella* Typhimurium, no significant microbial inhibition by phosphates was found. Appendices 1, 2, 3.

Other studies shown, that the polyphosphates have antimicrobial effects.

The growth of *Listera monocytogenes* in low mineral medium such as brain-heart infusion broth was inhibited by long chain sodium polyphosphates (SPP). Addition of polyvalent metal ions, such as $Mg^{2+}$ or $Ca^{2+}$, to the SPP-containing medium reversed the growth inhibition. Addition of SPP had little effect on growth of *L. monocytogenes*. [8]

The straight-chain polyphosphates with chain length of 3, 13, 15, and 21 were inhibitory to growth of *S. aureus* 196E, whereas the $PP_i$s (sodium acid $PP_i$ and tetrasodium $PP_i$) or the cyclic phosphate (sodium tetrametaphosphate) had no effect on the growth of the organisms. The binding sites of the condensed phosphates favour metal chelating and hence growth inhibition. In contrast, formation of a chelate ring may be hampered sterically in metaphosphates, and since the stability of complexes is generally influenced by the chain length of the polyphosphates, $PP_i$ metal complexes are less stable than longer-chain phosphate. [9]

The other tests showed that microbiological analyses (enumeration of viable cell counts, mesophilic sulphite-reducing clostridia, coliforms, yeasts and moulds) and sensory tests were performed at regular intervals. The results showed that polyphosphates had a beneficial effect on the shelf-life of the processed cheese spread tested in that thes significantly reduced ($P < 0.05$) the growth or survival rates of spoilage bacteria especially of mesophilic sulphite-reducing clostridia. Polyphosphates also beneficially influenced the sensory, including textural properties of the experimental processed cheese spread. All the samples containing less then 1% polyphosphates showed signs of butyric blowing significantly earlier ($P<0.05$), during the both accelerated shelf-life tests and regular refrigerated storage did the products fortified with 1% polyphosphate. In conclusion, the suitability of the long-chain polyphosphate formulation tested for shelf-life extension of processed cheese spreads was demonstrated. [69]

# CONCLUSION

In this study were found these results:

- Gram-positive bacteria are more susceptible then gram-negative bacteria to inhibition by various polyphosphates.
- *Bacillus sphaericus* was inhibited completely with 0.1% (w/v) JOHA HBS.
- *Micrococcus luteus* was inhibited completely with 0.1% (w/v) JOHA S9.
- The best antimicrobial effect of SELF 690 salt was shown on *Staphylococcus aureus*.
- JOHA HBS, JOHA S9, SELF 690 showed MIC values of 0.1% (w/v) for *Bacillus subtilis, Bacillus cereus, Bacillus brevis* and all strains were inhibited completely with 0.5% (w/v) phosphates.

Moreover, the microorganisms we completely inhibited by 0.5% polyphosphates, which clearly indicated the usefulness of these polyphosphates for prevention of contamination processed cheeses by these microorganisms.

In next studies is possible to apply this microorganisms into the processed cheese and try to find better concentration of the salts for the best antibacterial effect. It is necessary to find the lowest concentration of salts for our healthy life and especially for the life of our children.

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# LIST OF ABBREVIATIONS

WHC Water holding capacity

SPG Sodium polyphosphate glass

Y Yield

# LIST OF FIGURES

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# APPENDICES

Appendix 1 Evaluation effect of emulsifying salt JOHA HBS for chosen microorganisms

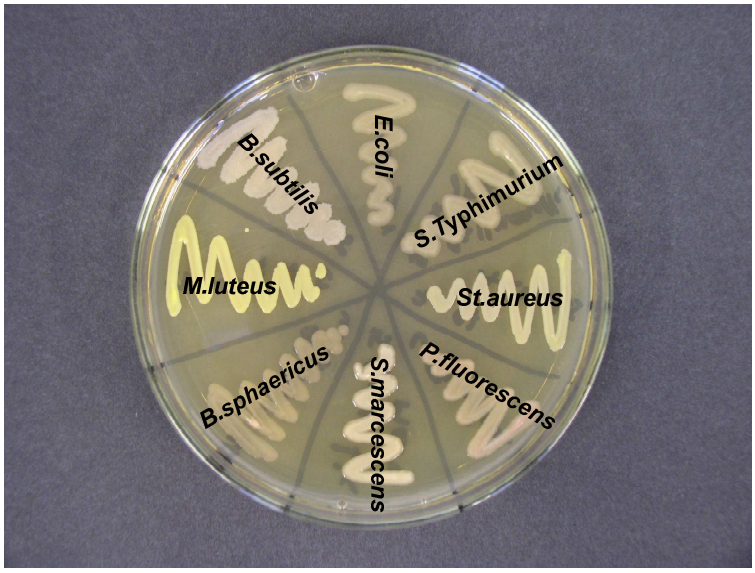



Fig. 1 Control Petri dish


Fig. 2 Petri dish with JOHA HBS in concentration of 0.1%

The microorganisms were inoculated in the same sequence as a Fig. 1.

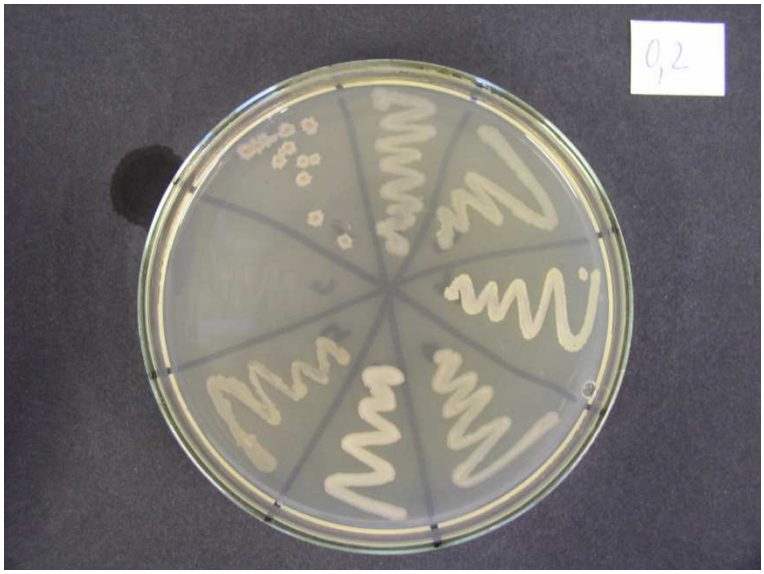



Fig. 3 Petri dish with JOHA HBS in concentration of 0.2%


Fig. 4 Petri dish with JOHA HBS in concentration of 0.3%

The microorganisms were inoculated in the same sequence as a Fig. 1.


Fig. 5 Petri dish with JOHA HBS in concentration of 0.4%


Fig. 6 Petri dish with JOHA HBS in concentration of 0.5%

The microorganisms were inoculated in the same sequence as a Fig. 1.

Appendix 2 Evaluation effect of emulsifying salt JOHA S9 for chosen microorganisms

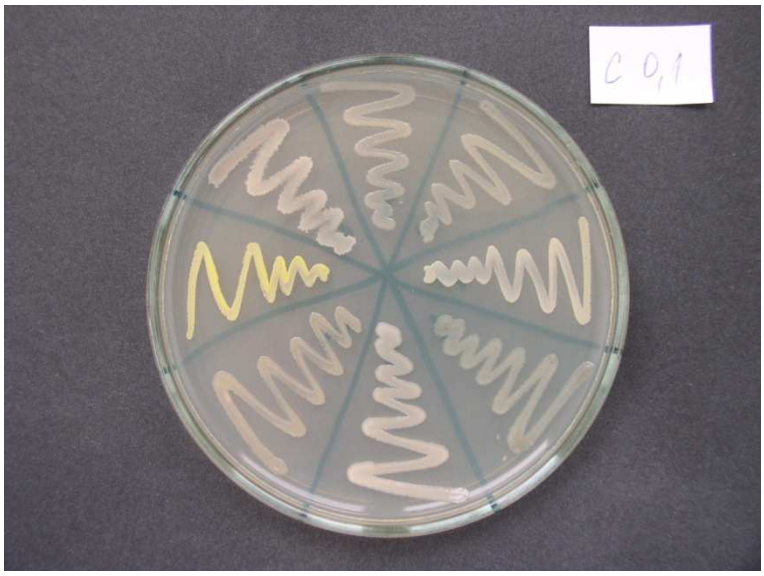



Fig. 7 Petri dish with JOHA S9 in concentration of 0.1%

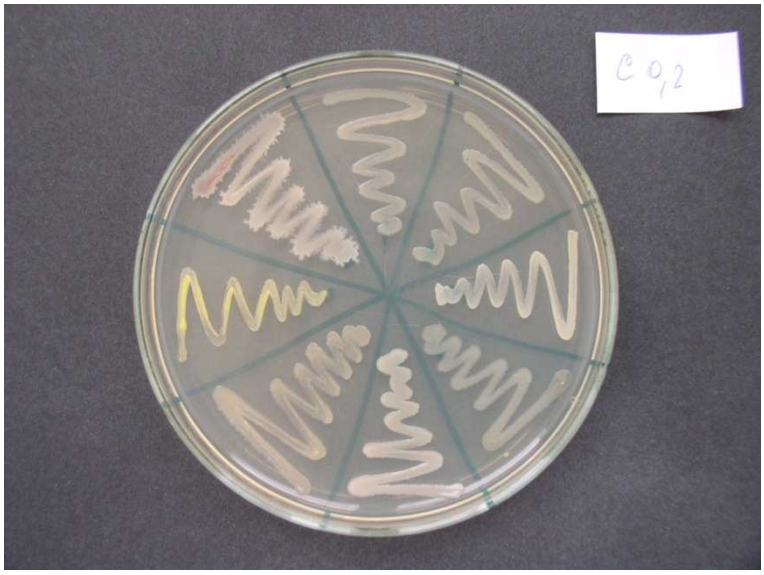



Fig. 8 Petri dish with JOHA S9 in concentration of 0.2%

The microorganisms were inoculated in the same sequence as a Fig. 1.


Fig. 9 Petri dish with JOHA S9 in concentration of 0.3%

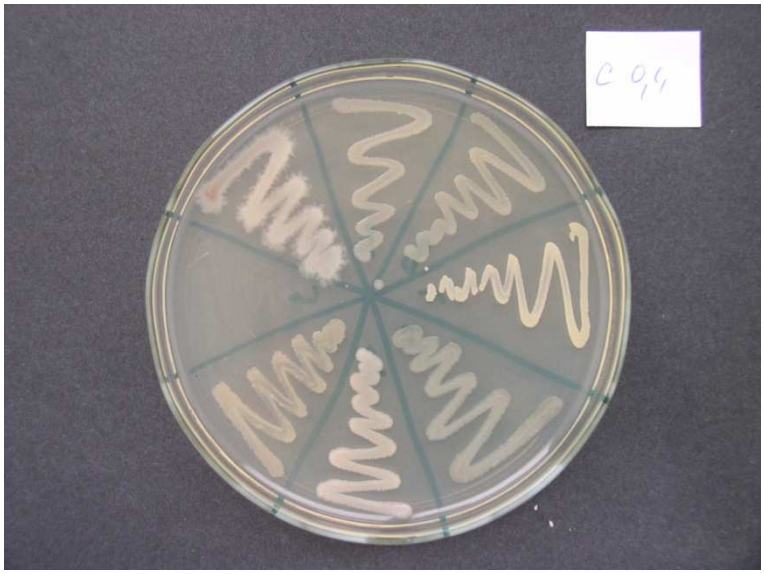



Fig. 10 Petri dish with JOHA S9 in concentration of 0.4%

The microorganisms were inoculated in the same sequence as a Fig. 1.


Fig. 11 Petri dish with JOHA S9 in concentration of 0.5%

The microorganisms were inoculated in the same sequence as a Fig. 1.

Appendix 3 Evaluation effect of emulsifying salt SELF 690 for chosen microorganisms

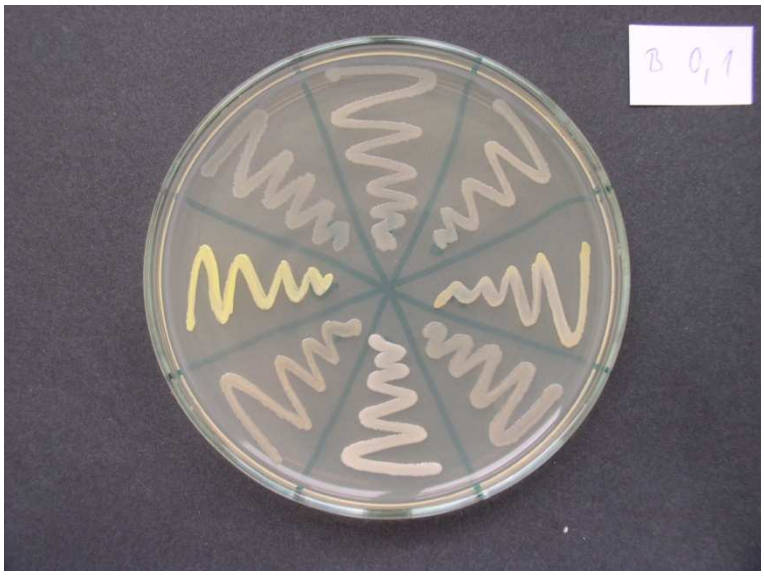



Fig. 12 Petri dish with SELF 690 in concentration of 0.1%

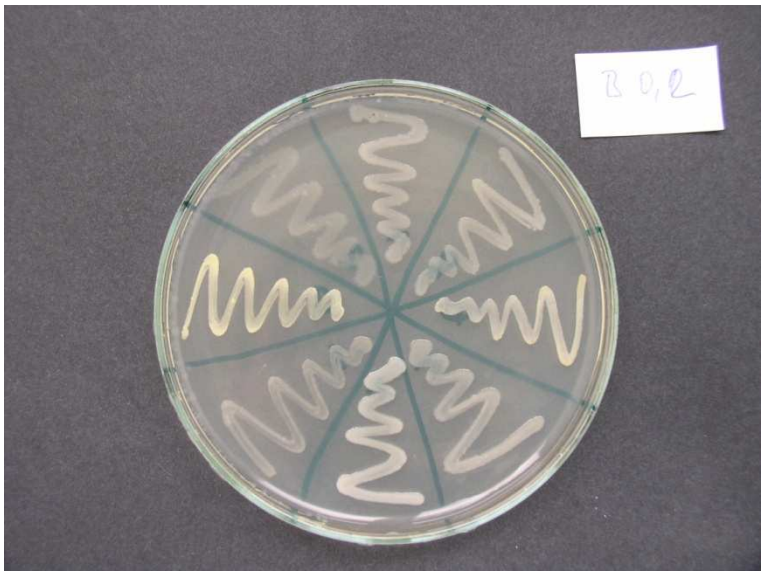



Fig. 13 Petri dish with SELF 690 in concentration of 0.2%

The microorganisms were inoculated in the same sequence as a Fig. 1.

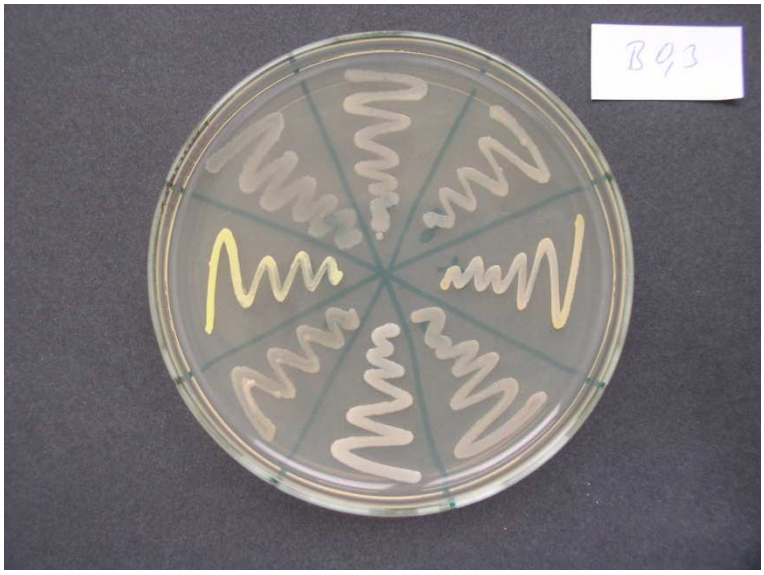



Fig. 14 Petri dish with SELF 690 in concentration of 0.3%


Fig. 15 Petri dish with SELF 690 in concentration of 0.4%

The microorganisms were inoculated in the same sequence as a Fig. 1.

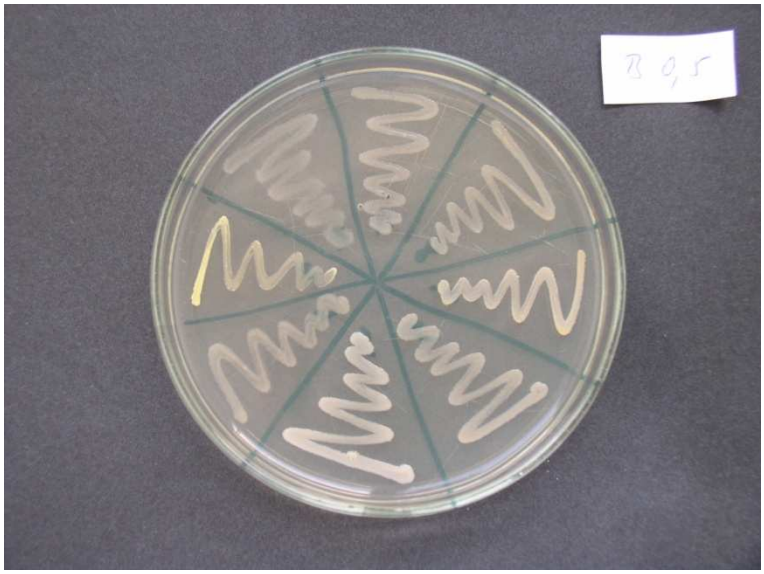



Fig. 16 Petri dish with SELF 690 in concentration of 0.5%

The microorganisms were inoculated in the same sequence as a Fig. 1.

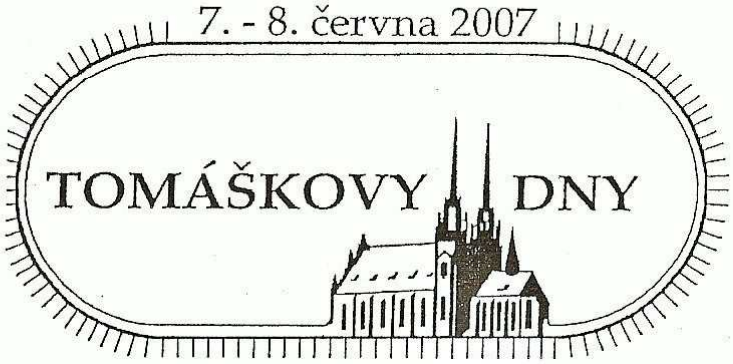
Appendix 4 PŘÍSPĚVEK KONFERENCE

**XVI. konference mladých mikrobiologů**

# TOMÁŠKOVY DNY 2007

sborník abstraktů

7. - 8. června 2007




Brno

7. – 8. 6. 2007

# Vliv přídavku různých druhů tavicích solí na růst vybraných mikroorganizmů

*Leona Čechová, Adéla Nováková, František Buňka*
*Ústav potravinářského inženýrství, Fakulta technologická, Univerzita Tomáše Bati ve Zlíně, Česká republika*

Pro dosažení jemné a homogenní struktury bez separace vody, tuku a proteinů je při výrobě tavených sýrů nutný přídavek tzv. tavicích solí (2-3 % w/w). V praxi se používají zejména sodné soli fosfátů a polyfosfátů. Klíčovou úlohou tavicích solí je upravit podmínky v tavené směsi tak, aby kaseinové frakce mohly uplatnit své emulgační vlastnosti. Odštěpení vápenatých iontů z kaseinových frakcí (fosfáty mají vyšší afinitu k vápenatým iontům) a jejich výměna za ionty sodné má za následek proces, kdy nerozpustný parakaseinan vápenatý je přeměněn na rozpustnější parakaseinan sodný. Afinita fosfátů k vápenatým iontům roste s jejich kondenzačním stupněm a s teplotou systému. Fosfátové tavicí soli jsou dodávány jako směsi orthofosfátů, difosfátů a polyfosfátů v různém kondenzačním stupni. Schopnost vazby vápenatých iontů může mít potenciál i z hlediska antimikrobiálních vlastností, neboť vápenaté ionty mohou stabilizovat buněčnou stěnu mikroorganizmů.

Byly vybrány tři druhy tavicích solí: (1) HBS – směs polyfosfátů s vysokým kondenzačním stupněm a orthofosfátů; (2) S9 – směs polyfosfátů (nižší stupeň polymerace než HBS) a orthofosfátů; (3) 690 – směs orthofosfátů a difosfátů. HBS a S9 vyrábí BK Ladenburg GmbH, Německo; 690 produkuje Chemische Fabrik Budenheim, Německo.

Vybrané druhy tavicích solí byly přidány do kultivačního média (Mueller-Hinton agar) v koncentracích 0,1 % (w/v) až 0,5 % (w/v). Na takto připravené půdy byly očkovány následující mikroorganizmy: *Escherichia coli, Salmonella* Typhimurium, *Serratina marcescens, Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus* a *Bacillus subtilis.* Výsledky byly odečteny po 48-hodinové inkubaci při 30 °C.

Z vybraných tavicích solí působila nejvíce antibakteriálně HBS, která účinkovala na grampozitivní mikroroganizmy při koncentraci 0,3% a vyšších, antibakteriální účinky na gramnegativní bakterie nebyly u této soli zaznamenány. U zbývajících dvou tavicích solí byly zaznamenány minimální inhibiční účinky na růst vybraných bakterií. Mírné antibakteriální účinky vykazovala sůl S9 při vyšších koncentracích pouze na růst *Micrococcus luteus.* Výsledky naznačují, že čím více obsahují tavicí soli polyfosfátů s vysokým kondenzačním stupněm, tím je větší antibakteriální účinek.

Poděkování: Tato práce vznikla za podpory projektu MŠMT: MSM 7088352101.

# Vliv přídavku různých druhů tavicích solí na růst vybraných mikroorganizmů

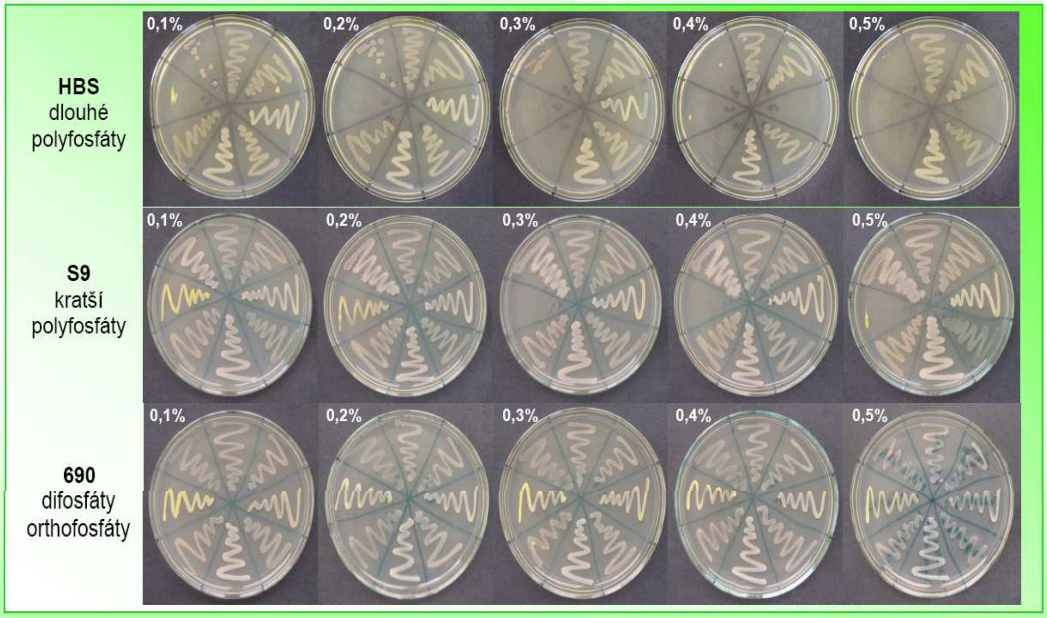
***Leona Čechová, Adéla Nováková, František Buňka***

Ústav potravinářského inženýrství, Fakulta technologická, Univerzita Tomáše Bati ve Zlíně, nám. T.G. Masaryka 275, 762 72 Zlín, Česká republika

## Úvod

- Pro dosažení jemné a homogenní struktury je při výrobě tavených sýrů nutný přídavek tzv. tavicích solí (2-3 % w/w). V praxi se používají zejména sodné soli fosfátů a polyfosfátů.
- Fosfáty a zejména polyfosfáty odštěpují vápenaté ionty z kaseinových frakcí (fosfáty mají vyšší afinitu k vápenatým iontům). Nerozpustný parakaseinan vápenatý je přeměněn na rozpustnější parakaseinan sodný.
- Afinita fosfátů k vápenatým iontům roste s jejich kondenzačním stupněm a s teplotou systému.
- Schopnost vazby vápenatých iontů může mít potenciál i z hlediska antimikrobiálních vlastností, neboť vápenaté ionty mohou stabilizovat buněčnou stěnu mikroorganizmů.
- Cílem práce bylo odzkoušet účinek tří tavicích solí obsahujících polyfosfáty v různém kondenzačním stupni na vybrané mikroorganizmy.

## Výsledky


## Materiál a metody

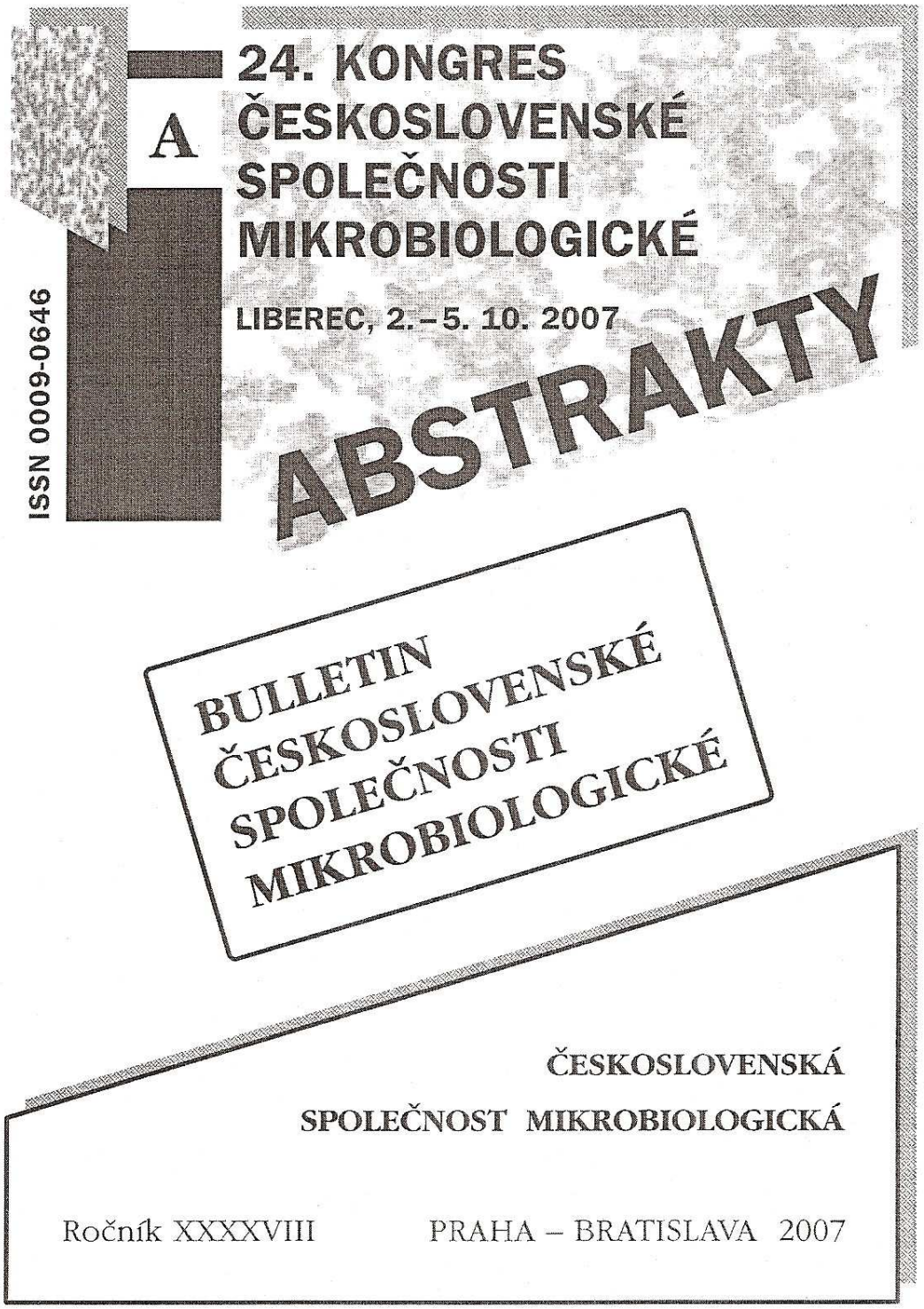
- Tři druhy komerčních tavicích solí: **HBS** (směs dlouhých polyfosfátů a orthofosfátů), **S9** (směs kratších polyfosfátů a orthofosfátů), **690** (směs difosfátů a orthofosfátů).
- Tavicí soli přidány do kultivačního média (Mueller-Hinton agar) v koncentracích 0,1 % až 0,5 % (w/v).
- Na půdy bylo očkováno 8 mikroorganizmů.
- Výsledky odečteny po 48 hodinách inkubace při 30 °C.

## Závěr

- Nejvyšší účinek na grampozitivní mikroroganizmy měla tavicí sůl HBS při koncentracích 0,3 % a vyšších. Antibakteriální účinky na gramnegativní bakterie nebyly u této soli zaznamenány.
- U zbývajících dvou tavicích solí byly zaznamenány prakticky zanedbatelné inhibiční účinky na růst testovaných bakterií.
- Mírné antibakteriální účinky vykazovala ještě sůl S9 při vyšších koncentracích, a to pouze na růst *Micrococcus luteus*.
- Výsledky naznačují, že čím více obsahují tavicí soli polyfosfátů s vysokým kondenzačním stupněm, tím je větší antibakteriální účinek.

**Tato práce vznikla za podpory projektu MŠMT: MSM 7088352101.**

Appendix 5 PŘÍSPĚVEK KONFERENCE



## Vliv vybraných fosfátových tavicích solí na růst bakterií druhu Staphylococcus aureus a rodu Bacillus

Buňková L., Nováková A., Buňka F.

*Ústav potravinářského inženýrství, Fakulta technologická, Univerzita Tomáše Bati ve Zlíně, nám. T.G.Masaryka 275, 762 72 Zlin*

Sodné soli fosfátů jsou tavicí soli používané v technologii výroby tavených sýrů. Jejich základní účinek spočívá ve schopnosti odštěpení vápenatých iontů z kaseinové matrice přírodních sýrů a jejich nahrazení ionty sodnými. Tavicí soli se dávkují v množství 2-3 % w/w. Schopnost fosfátů vázat vápenaté ionty roste s jejich kondenzačním stupněm a s teplotou systému. Byly vybrány tři tavicí soli: 690 – směs orthofosfátů a difosfátů; S9 – směs polyfosfátů a orthofosfátů; HBS – směs polyfosfátů s vysokým kondenzačním stupněm (vyšší stupeň polymerace než S9) a orthofosfátů. Vybrané druhy tavicích solí byly přidány do média (Mueller-Hinton agar) v koncentracích 0,1-0,5 % (w/v). Na takto připravené půdy byly očkovány mikroorganizmy: Staphylococcus aureus, Bacillus cereus a Bacillus subtilis. Výsledky byly odečteny po 48h inkubaci při 30°C. Z vybraných tavicích solí působila nejvíce HBS, která účinkovala na všechny 3 kmeny bakterií při koncentraci 0,3% a vyšší. Při těchto koncentracích nebyl zaznamenán růst bakterií. U zbývajících dvou tavicích solí nebyly zaznamenány významné inhibiční účinky na růst Staphylococcus i Bacillus. Mírné antibakteriální účinky vykazovala sůl S9 při vyšších koncentracích na růst S. aureus a B. cereus, kde došlo ke snížení počtu bakterií, avšak ke striktní inhibici růstu nedošlo. Výsledky naznačují, že čím více obsahují tavicí soli polyfosfátů s vysokým kondenzačním stupněm, tím je větší antibakteriální účinek.
Poděkování MŠMT: MSM 7088352101.

Univerzita Tomáše Bati ve Zlíně
Fakulta technologická

# Vliv vybraných fosfátových tavicích solí na růst bakterií *Bacillus* a *Staphylococcus aureus*

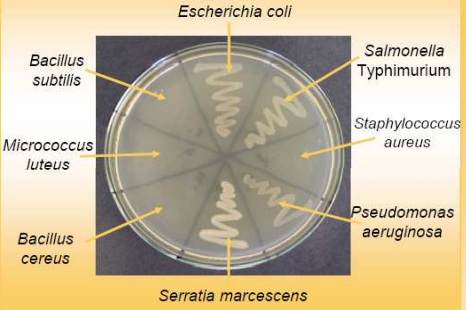
**Leona Buňková, Adéla Nováková, František Buňka**

**Ústav potravinářského inženýrství, Fakulta technologická, Univerzita Tomáše Bati ve Zlíně**

## ÚVOD

- K výrobě homogenních, jemných a roztíratelných tavených sýrů je nutný přídavek tzv. tavicích solí (2-3 % w/w). V praxi se používají zejména sodné soli fosfátů a polyfosfátů.
- Fosfáty a zejména polyfosfáty odštěpují vápenaté ionty z kaseinových frakcí (fosfáty mají vyšší afinitu k vápenatým iontům). Nerozpustný parakaseinan vápenatý je přeměněn na rozpustnější parakaseinan sodný.
- Afinita fosfátů k vápenatým iontům roste s jejich kondenzačním stupněm a s teplotou systému.
- Schopnost vazby vápenatých iontů může mít potenciál i z hlediska antimikrobiálních vlastností, neboť vápenaté ionty mohou stabilizovat buněčnou stěnu mikroorganizmů.
- V předchozí práci byly provedeny testy s 8 mikroorganizmy (*Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Micrococcus luteus*, *Bacillus cereus* a *Bacillus subtilis*).
- Bylo zjištěno, že polyfosfáty s vyšším kondenzačním stupněm účinkují zejména na grampozitivní bakterie (obrázek 1), přičemž účinné koncentrace se pohybovaly v intervalu 0,3 – 0,5 %.
- **Cílem práce bylo odzkoušet účinek tří tavicích solí obsahujících polyfosfáty v různém kondenzačním stupni na vybrané grampozitivní bakterie, a to na *Staphylococcus aureus*, *Bacillus cereus* a *Bacillus subtilis*.**

**Obrázek 1: Ukázka inhibičního účinku polyfosfátů s vysokým kondenzačním stupněm na vybrané mikroorganizmy**



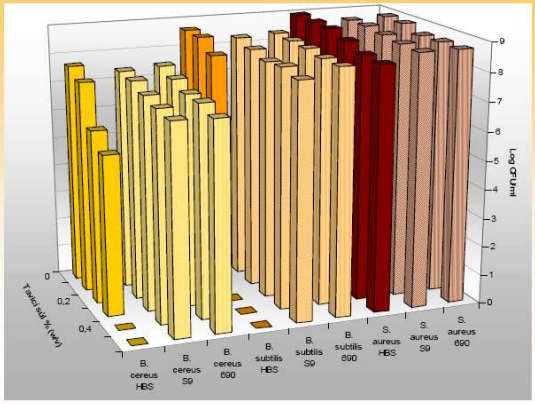

## MATERIÁL A METODY

- Použity tři druhy komerčních tavicích solí:
  - **HBS** – směs dlouhých polyfosfátů a orthofosfátů,
  - **S9** – směs kratších polyfosfátů a orthofosfátů,
  - **690** – směs difosfátů a orthofosfátů.
- Tavicí soli přidány v koncentracích 0,1 % až 0,5 % (w/v) do média (Mueller-Hinton agar).
- Na médium očkováno 100 µl přes noc narostené suspenze mikroorganizmů.
- Výsledky odečteny po 48 hodinách inkubace při 30 °C a následně přepočteny na CFU/ml.
- Testované mikroorganizmy:
  - *Staphylococcus aureus* subsp. *aureus* CCM 3953,
  - *Bacillus cereus* CCM 2010,
  - *Bacillus subtilis* subsp. *subtilis* CCM 1718.

## VÝSLEDKY A ZÁVĚR

- Ze studovaných tavicích solí měla nejvyšší účinek sůl HBS, a to na *Bacillus cereus* a *B. subtilis* v koncentracích 0,3 % (w/v) a vyšších. Na testovaný kmen *S. aureus* tato sůl nepůsobila (obrázek 2).
- Inhibiční účinky solí S9 a 690 v koncentracích 0,1 – 0,5 % (w/v) nebyly prokázány u žádného z testovaných kmenů mikroorganizmů.
- **Výsledky naznačují, že čím více obsahují tavicí soli polyfosfátů s vysokým kondenzačním stupněm, tím je větší jejich inhibiční účinek na testované mikroorganizmy rodu *Bacillus*.**

**Obrázek 2: Závislost počtu CFU studovaných grampozitivních bakterií na koncentraci tavicích solí.**




Tato práce vznikla za podpory projektu MŠMT: MSM 7088352101.

Appendix 6 Article in MLÉKAŘSKÉ LISTY – ZPRAVODAJ 105/2007

# VÝROBA SÝRŮ V PORTUGALSKU

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## Obecné charakteristika portugalského sýrařství

Pro Portugalsko je charakteristická tradiční výroba sýrů. Pod pojmem tradiční výroba sýrů je chápána výroba tradičních výrobků na farmách v menším objemu, což je determinováno především produkčními možnostmi a množstvím suroviny pro výrobu sýrů. Sýry jsou nejčastěji vyráběny z ovčího, kozího a v neposlední řadě též z kravského mléka, které ovšem v Portugalsku ve většině oblastí nemá vyhovující složení (odpovídající kvalitu) pro výrobu sýrů. Hlavním důvodem zhoršené kvality kravského mléka jsou klimatické podmínky (nedostatek vláhy, sucho), z čehož rezultuje nedostatek kvalitní pastvy a krmiva. Kravské mléko se proto používá pro výrobu sýrů jako směsné převážně s mlékem ovčím a kozím.

Sýry jsou v Portugalsku velmi oblíbené, spotřeba sýrů v Portugalsku je 3,2 kg na osobu a rok, což by odpovídalo spotřebě mléka ve výši cca 30 litrů. Vyšší spotřebě však brání přísné hygienické limity na průmyslovou produkci sýrů, nedostatek kravského mléka a vysoká cena tradičních maloprůmyslově na farmách vyráběných sýrů. Vysoká cena je dána vysokými režijními náklady, zejména vysokou pracností resp. nízkou produktivitou výroby.

Produkce tradičních sýrů v Portugalsku se pohybuje v rozmezí 15 000 až 20 000 t/rok. Z toho pouze 15 % této produkce je hygienicky kontrolováno, což je asi i důvod proč tyto výrobky nejsou v masovějším měřítku dodávány do supermarketů, na venkově slouží sýry pro vlastní spotřebu pro samozásobitele a pro prodej v maloobchodě. Sýry v Portugalsku jsou často konzumovány jako příloha k vínu, těstovinám, slouží k přípravě konvenience food, rychlému občerstvení snack-food apod.

Pro portugalské sýry platí, že ty nejkvalitnější sýry těžko najdete v obchodech, protože v Portugalsku převažuje stále tradiční výroba, kdy jsou sýry ručně vyráběny a jsou velmi

drahé. Produkce těchto sýrů je relativně vysoká, je však určena převážně pro domácí trh. Spotřeba některých druhů sýrů ve velkých městech je rovněž omezena jednak uplatňováním ochranné známky, nebo průmyslového vzoru na celou řadu druhů těchto sýrů a pak samozřejmě jejich vysokou cenou. Toto je jedna z příčin, proč průmyslový výrobci v rámci rozšíření sortimentu sýrů „kopírují“ řadu sýrů z jiných zemí, jako jsou: Camembert, Parmazán, Eidam, Čedar a současně je i tím omezován dovoz sýrů ze zahraničí.

Mezi nejvyhledávanější sýry jak již bylo dříve uvedeno patří tradiční portugalské sýry. Jednotlivé výroby tradičních sýrů jsou licencovány (mají povolení k výrobě od Ministerstva zemědělství) a to zákonem Regulamentar n°42/85 de 5/7. Ceny nejsou garantovány a odvíjejí se od tržních a konkurenčních vlivů.

**Charakteristika nejznámějších druhů sýrů**

Skupina čerstvých sýrů - sýr Rabacal

Sýr Rabacal se vyrábí z ovčího mléka, nebo směsného ovčího a kozího mléka. Jeho výroba je charakteristické pro oblast v okolí města Coimbra.

Hlavní část produkce je deklarována jako čerstvý sýr, v opačném případě sýr zraje kratší dobu a lze jej pak označit jako čerstvý měkký sýr. Jeho váha je 500 g. Tvar je kruhový, o průměru 12 cm a výšce 3 až 5 cm. Sušina tohoto sýra je cca 45 % (w/w), hlavní podíl sušina je tvořen tukem, dále bílkovinou a v malém množství je obsažen zbytkový cukr.

Je to tedy čerstvý sýr, měkký, bílé barvy. Sýr má zrát v průměru 21 dní, protože však poptávka převyšuje nabídku, uvádějí se tyto sýry na trh jako čerstvé.

Používaná technologie je klasická tak jako u nás při výrobě obdobných druhů sýrů. Koagulace probíhá asi hodinu při 25 – 30 °C. Zajímavé je že pro srážení mléka se u většiny sýrů používá extrakt z Artyčoku. Následuje zpracování sýřeniny, odpouštění syrovátky, lisování, solení a příp.zrání sýrů. V Portugalsku jsou často používány k výrobě sýrů separační techniky.

**Fermentované ovčí sýry**

**Sýr Serra da Estrela**

Tento sýr je jedním z nejznámějších a nejoblíbenějších sýrů. Často je nazýván Serra sýr a je z pohledu ekonomického a organoleptického nejvýznamnější z tradičních sýrů. Jeho výroba je přísně kontrolována. Považuje se totiž za národní sýr a „gurmánskou specialitu“.

Jeho váha je 1,2kg nebo 2kg. Tvar je kruhový, o průměru 18cm a výšce 8cm. Těsto je kompaktní, zřídka s oky, je měkký (čerstvý) nebo polotvrdý (zralý). Sušina sýra je cca 67,5% (w/w), z čehož je patrno, že jde o sýr lisovaný a následně zrající. Sušina je tvořena především bílkovinami (25,5g ) a tuky 31,5g , což odpovídá tuku v sušině cca 47%.

Serra jen sýr pojmenovaný dle nejvyšší portugalské hory (Serra da Estrela). V okolí této hory je velmi příznivé mikroklima a vhodné pastviny pro pasení velkých stád ovcí. Typická stáda ovcí složená jak z černých rovněž tak bílých plemen ovcí Bordaleira da Serra da Estrela jsou nejlepší na produkci mléka. Průměrná roční produkce ovčího mléka v Portugalsku je cca 12 milionů litrů mléka. Sýr může být vyráběn pouze na farmách, které jsou lokalizovány v oblasti Serra da Estrela a používají na srážení mléka výtažek z artyčoku.

Dle portugalských standardů má sýr Serra cylindrický tvar bez ostrého ohraničení. Typický sýr má základnu 15 – 20cm a výšku 4 – 6cm.V sýru jsou pouze malá oka, slonovité až bílé barvy, sýr má máslovou texturu, což vede k velké deformaci sýra při krájení (rovněž v důsledku vysokého obsahu tuku – pozn. autorů). Má nakyslou mléčnou příchuť a čisté typické aroma po ovčím mléce. Kůra sýra je tenká, hladká a má slámově žlutou barvu. Sýr zraje po dobu 30 – 45 dní při teplotě 19 - 30°C, relativní vlhkosti 75 - 90%, starší sýry zrají až 6 měsíců.

Sýr Serpa

Nejznámější produkční oblastí tohoto typu sýru je oblast Alentejo. Jedná se o ovčí sýr, vyrobený hlavně z ovčího mléka, ale i s přídavkem mléka kozího. Tento sýr je vyráběn z ovčího mléka získávaného od plemen Merino a Campanica, kde je průměrná velikost stáda 300 - 500 ovcí. Chemické složení ovčího mléka – sušina 19% (w/w) je tvořena celkovými

proteiny 6,5 %, tuk 7,5 %, hustota 1,04g/$cm^3$, kyselost 26,3ml 0,1 N NaOH na 100g mléka, pH 6,6.

Hmotnost sýru Serpa je 1,5 až 2kg, zřídka 3kg. Tvar je kruhový ve tvaru bochníku o průměru 15 až 25cm, a výšce 5 – 7cm. Serpa je tvarohový sýr, bílé barvy. Textura je kompaktní s oky, pikantní až nakyslé chuti. Ve 100g sýru je 42,0 g vlhkosti (sušina 58% w/w), 24,8g bílkovin, 25,7g tuků, 0,3g cukru.

*Detailnější popis technologie výroby sýrů uvedou autoři v následujícím článku.*

Appendix 7 Article in MLÉKAŘSKÉ LISTY – ZPRAVODAJ 106/2008

# Technologie výroby ovčích sýrů v Portugalsku

Pokračování článku „Výroba sýrů v Portugalsku“.

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V předcházejícím článku byly charakterizovány dva nejznámější sýry a sice sýr Serra Estrela, který je vyráběn většinou manufakturním způsobem na chovatelských farmách a sýr Serpa vyráběný z ovčího, příp. přídavkem kozího mléka. Tento sýr je vyráběn průmyslovou technologií za použití separačních technik.

V dalším textu je popsána technologie výroby sýru Serra da Estrella tak jak je praktikována v současnosti.

## Sýr Serra da Estrela

Tento sýr je jedním z nejznámějších a nejoblíbenějších sýrů. Považuje se totiž za národní sýr a „gurmánskou specialitu“. Je vyráběn ze syrového ovčího syrového mléka, jak je však uvedeno v předcházejícím článku může být aplikován přídavek kravského mléka.

Serra jen sýr pojmenovaný dle nejvyšší portugalské hory (Serra da Estrela). V okolí této hory je velmi příznivé mikroklima a vhodné pastviny pro pasení velkých stád ovcí. Typická stáda ovcí složená jak z černých rovněž tak bílých plemen ovcí Bordaleira da Serra da Estrela jsou nejlepší na produkci mléka. Mléko je získáváno během laktace, která je v období říjen až květen a trvá cca 210 -240 dnů. Sýr může být vyráběn pouze na farmách, které jsou lokalizovány v oblasti Serra da Estrela a používají na srážení mléka výtažek z artyčoku. Má status AOC (Appelation d´ Origine Controleé).

Tabulka I. Chemické složení ovčího mléka ovcí plemene Bordaleira da Serra da Estrela

| Voda | Tuk | Bílkoviny | Laktoza | Popeloviny | |
|---|---|---|---|---|---|
| 80,01 | ±2,7 | 7,8 ±1,6 | 6,0± 1,3 | 4,4 ± 0,5 | NA2 |
| 80,9 | 1,6 | 7,4 ±1,5 | 6,0 ± 0,9 | 4,6 ± 0,5 | 0,9 ± 0,1 |
| 81,2 | 6,8 | 6,8 | 5,1 | 0,9 | |

1 ± znamená 95 % (koeficient spolehlivosti)

2 neuvedeno

Hlavními technologickými operacemi při výrobě jsou:

1. Koagulace (srážení) mléka
2. Zpracování koagulátu (krájení, drobení, vytužování zrna, oddělování syrovátky
3. Lisování sýřeniny
4. Solení
5. Zrání sýrů

Dojení ovcí se provádí dvakrát denně, a sice před východem slunce (vypouštění na pastvu) a při západu slunce, kdy se ovce vrací zpět z pastvy. Dojení se provádí do malých nádob. Riziko mikrobiální kontaminace z vemen, vlny a rukou pastýřů je značné, proto se věnuje hygieně získávání mléka zvýšená pozornost.

Před samotným zpracováním se mléko udržuje v teplém stavu max. po dobu 30 – 60 minut, následuje filtrace mléka, zbavení pevných částic, nečistot apod. srážení mléka se provádí v malých nádobách o objemu 20 litrů. Důvodem je snaha co nejrychleji zpracovat mléko, aby nedocházelo k nežádoucím změnám.

Srážení mléka probíhá, buď v teplé vodní lázni, nebo u jiného přirozeného zdroje tepla např. u krbu. Ke srážení se používají syřidlové enzymy rostlinného původu, získané jako výtažek z usušené rostliny Artyčoku (Cynara cardunculus L). Biochemická studie této rostliny je velmi zajímavá, vzhledem k rozsahu a zaměření článku bude uvedena v dalších

publikacích. Studie popisuje sýřící a koagulační sílu, dále proteolytické a lipolytické působení získaných enzymů. Způsob praktického využití a aplikace přípravy syřidla je uváděn v následujících modifikacích:

- sušená rostlina se přidává do teplého mléka, provede se promíchání po několik minut, následuje filtrace přes jemný tkaninový filtr (plachetku),
- nebo se provede macerace usušené rostliny v malém množství solného roztoku, získáme hmotu pastovité konzistence, kterou přefiltrujeme a dále přidáváme do sýřeného mléka,
- macerace se provede stejným způsobem, získaná pasta je v plátěném obalu ponořena do mléka, následuje míchání a vymačkání z obalu do sýřeného mléka.

Doba srážení mléka je velmi variabilní dle jednotlivých farem a je závislá na teplotě a ročním období. Udává se např. v rozmezí 30 do 45 minut při teplotě 20 až 30°C, 28 až 240 minut při teplotním rozmezí 17 – 40°C, příp. jiné kombinace. Množství surového rostlinného extraktu přidaného k mléku se udává od 11 do 22g/l, resp. 32 až 40g/l při kratší době srážení (37 až 90 min).

Následně se koagulát (sražené mléko) krájí ručně (sýrařské nože, lžíce) v celé hmotě na čtverce, po krátkém odpočinku se rozkrájí a drobí na menší kousky a vytužuje na pevnější zrno. Současně probíhá oddělování syrovátky. Celý tento proces trvá až 45 minut, ve zkrácené formě pouze 10 minut. Mimo uvedený způsob jsou používány již různé mechanizační pomůcky, jako jsou cylindrické odlučovače syrovátky, nebo jsou požívány tvarožníkové pytle, kdy však získáváme po vylisování homogenní pastu, která pak může být formována do plachetkového tkaninového obalu.

Solení sýrů je prováděno jednak z důvodů zlepšení senzorické jakosti, konzervačního efektu a současně i zvýšení sušiny sýry. Solení je prováděno:

a) přídavkem soli do mléka před sýřením, kdy se přidává cca 30g nerafinované kuchyňské soli na l litr mléka, nebo sůl jako součást přídavku pastovitého syřidla (macerát v solném roztoku),

b) nebo se provádí solení až po ukončení lisování posypem kuchyňské soli (NaCl) na povrch a spodní část sýra v množství 5-9g/$cm^2$, sýry jsou dále obraceny a sůl je na povrchu roztírána.

Lisování sýrů se provádí po oddělení syrovátky většinou ve tkaninových pytlích (tvarožnících), které jsou vkládány do odkapních nádob, přikryty víkem a zatíženy kameny. Po vylisování jsou tvarovány do tvořítek.

Zrání sýrů probíhá v samostatných zracích prostorách, kde je důležité zajistit požadované parametry zrání,a sice teplotu a vlhkost. Vzhledem k tomu, že výroba je manufakturního charakteru není v častých případech zajištěna účinná regulace těchto parametrů. Např. od října do května byly registrovány v různých farmách teploty v rozmezí 19 – 30°C a průměrná vlhkost 75 – 95%. V zimních měsících jsou však teploty zrání nižší a pohybují se kolem 10°C, což má pozitivní vliv na průběh zrání. Uváděné údaje z dalších farem jsou však vzhledem k dříve uváděným skutečnostem velmi variabilní. Během zrání se sýry každodenně obrací z důvodu zachování tvaru, rovnoměrného zrání v celé hmotě a prosolení. Po 8 až 15 dnech se na povrchu sýra objeví načervenalá mazlavá plocha. Dle výrobců je výskyt této skvrny indikátorem dobrého finálního výrobku a souvisí s rozvojem požadované zrací mikroflóry.

Dále se sýry umývají v horké vodě, balí se zracího plátna (plachetky) tak, aby byl dosažen požadovaný cylindrický tvar finálního výrobku. Sýr se omývá v teplé vodě. Zrání sýrů probíhá 30 až 45 dnů, starší sýry zrají až 6 měsíců.

Výtěžnost sýrů tj. spotřeba mléka na výrobu 1kg sýru po ukončeném zrání (45 – 50 dnů) se udává 5,5 až 6,0 litru, tj. cca 17% výtěžnost z 1kg mléka. Výtěžnost je odlišná dle ročního období, kdy složení mléka kolísá. Obecně se udává podstatně vyšší výtěžnost v zimním období.

Průběh zrání byl sledován ve třech zónách sýra, a sice centrální, střední a povrchové. Během zrání sýrů jsou proteiny silně rozpouštěny (solubilizovány). Proteolýza v centrální a povrchové zóně je značně rozdílná. Vysoký stupeň proteolýzy na povrchu sýra je i v korelaci s odlišnou texturou a barvou mezi vnitřní částí a povrchovou částí, což odpovídá hypothese o dostředivém způsobu zrání. Dochází rovněž k lipolýze tuku, což však není významné. Vyšší obsah těkavých mastných kyselin (máselná, isovalerové) je dle některých autorů zárukou vysoké senzorické jakosti sýra. Obsah těkavých mastných kyselin je však ve srovnání s plísňovým sýrem Roquefort poloviční.

Tabulka II. Procenta celkového dusíku, ve vodě rozpustného dusíku a dusíku aminokyselin v sýrech a obsah – poměr korespondenčního zracího koeficientu (dle různých autorů)

| Celkový dusík | Rozpustný dusík (%) | Dusík aminokyselin | Zrací koeficient |
|---|---|---|---|
| 3,1 – 4,2 | 1,0 – 2,0 | 0,1 – 0,3 | 32 – 56 |
| 2,8 – 4,0 | 1,1 – 1,7 | 0,1 -0,4 | 35 – 48 |
| 3,62± 0,7 | 1,2± 0,4 | NA3 | 32± 13 |

1 Definován v procentech jako rozpustný dusík k celkovému dusíku

2 ± 95 % konfidenčního intervalu (koeficient spolehlivosti)

3 nedostupný, nevyužitelný

Vzhledem k tomu, že sýr je vyráběn ze syrového mléka, je z hlediska zdravotní nezávadnosti důležitý obsah a složení mikroflóry v sýrech. Vzhledem ke skutečnosti, že sýr zraje při nižší teplotě a po dobu 45 dnů (resp. až 60 dnů) není uváděn výskyt přežívajících zdraví nebezpečných patogenních mikroorganismů, mimo menšího výskytu *Escherichia coli*. Spektrum mikroflóry obsažené v sýru je velmi široké a základem je mikroflóra syrového mléka. Z technologicky významné mikroflóry, která se nachází ve zralém sýru je uváděn výskyt proteolytické mikroflóry 8 x 105/g, lipolytické mikroflóry 11-424 x 106/g, kvasinky méně než 200 x 106/g, plísně méně než 143 x 106/g. Z jednotlivých druhů mikrobů jsou uváděny *Lacotococcus lactis* subs. *lactis* a *cremoris*, *Leuconostoc mesenteroides* subsp. *dextranicum* a *cremoris*, *Lactobacillus helveticus*, *Lacobacillus acidophillus*, *Lactobacillus casei* a *plantarum*. Jako dominantní jsou však udávány rod *Lactococcus*, *Lactobacterium plantarum* a *Lacotabacillus casei*. Přítomny byly rovněž plísně *Geotrichum candidum* a *Penicilium rogueforti* a červeně pigmentující kvasinky *Rhodotorulaceae* a *Torulopsoideae*.

Dle portugalských standardů má sýr Serra cylindrický tvar bez ostrého ohraničení. Typický sýr má základnu 15–20cm a výšku 4–6cm. V sýru jsou pouze malá oka, slonovité až bílé

barvy, sýr má máslovou texturu, což vede k velké deformaci sýra při krájení (rovněž v důsledku vysokého obsahu tuku). Má nakyslou mléčnou příchuť a čisté typické aroma po ovčím mléce. Kůra sýra je tenká, hladká a má slámově žlutou barvu. Sýr je deklarován ve dvou jakostních třídách, a sice jako máslový (pravý, ryzí) a starý (prozrálý) zrající až 6 měsíců.