# EFFECTIVENESS OF DISINFECTING APPLIANCE DUSEPT ON G- AND G+ BACTERIA OF HEAT-TREATED MILK

# ÚČINNOSŤ DEZINFEKČNÉHO PRÍPRAVKU DUSEPT NA G- A G+ BAKTÉRIE TEPELNE OŠETRENÉHO MLIEKA

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## SÚHRN

V experimente sme hodnotili účinnosť dezinfekčného prípravku na báze chlóru tromi metódami na zmes $G^+$ a $G^-$ baktérií a na mikroorganizmy obsiahnuté v pasterizovanom mlieku. Pri použití kvalitatívnej platňovej metódy bolo účinné pôsobenie 2 %-ného roztoku na zmes $G^+$ a $G^-$ baktérií. Pri kvalitatívnej metóde s použitím skleneného nosiča bol 2 %-ný roztok taktiež účinný na zmes $G^+$ a $G^-$ , 1 %-ný a 0,1 %-ný roztok nebol účinný. Pre mikroorganizmy mlieka na nosiči 2 %-ný roztok bol neúčinný. Pri kvantitatívnej metóde bol 2 %-ný roztok po 20 minútovom pôsobení na baktérie obsiahnuté v mlieku účinný.

**KĽÚČOVÉ SLOVÁ: účinnosť dezinfekcie, dezinfekčný prostriedok, Dusept, mlieko, baktérie, kvalitatívna metóda**

## ABSTRACT

We were evaluating the effectiveness of disinfecting appliance Dusept on mixture of $G^+$ and $G^-$ bacteria and on the micro-organisms of heat-treated milk by three methods. By using the qualitative method effective action of 2% reagent was found on mixture of $G^+$ and $G^-$ bacteria. By the qualitative method with carrier the 2% reagent was also effective on the mixture of $G^+$ and $G^-$ bacteria, 1% and 0,1% reagents were not effective. The 2% reagent was not effective on the micro-organisms of milk on a carrier. The 2% solution was effective when applying the quantitative method by 20 minutes actuation on bacteria contained in milk.

**KEY WORDS: effectiveness of disinfection, disinfecting appliance, Dusept, milk, bacteria, qualitative method**

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# HEAT-TREATED MILK DISINFECTION BY DUSEPT APPLIANCE

## DETAILED ABSTRACT

In this work we were testing cleaning and disinfecting appliance Dusept containing the effective substance sodium hipochlorite made by Inc. Duslo Šaľa.

Effectiveness of disinfecting appliance Dusept was tested on mixture of $G^+$ (*Enterococcus faecalis)* and $G^-$ (*Escherichia coli*) bacteria in sample of milk. Three methods were used:

1. Qualitative method on stiff soil where the disinfecting appliance was added [11].
2. Qualitative method with glass carrier [12].
3. Qualitative method with fixed glass carrier [7].

There was a twenty-four-hour cultivation of $G^+$ and $G^-$ on a slanted agar prepared before the testing of the disinfecting appliance. The $G^+$ and $G^-$ bacteria were mixed 1:1 right before the experiment by which the tested mixture of $G^+$ and $G^-$ was created. After sterilizing and partial cooling there was the disinfecting appliance added into the prepared nutrient medium GTKA. Then there was an exact amount of Dusept added, so as the resulting arose effective concentration was 2%, 1%, and 0,1%.

Concurrently check tests were carried out, which differed from the previous, in that there was no disinfectant operative solution applied on the plates with some dried micro-organisms on them. Before application of the suspension of $G^+$ and $G^-$ bacteria and milk, the total amount of micro-organisms in 1 ml was determined.

By using the quantitative method we carried out statistical evaluation of the experiments. Then the basic variation-statistical characteristic x, s, v (%) were calculated.

As shown in Table 1, when using of 2% concentration of operative solution of disinfectant appliance Dusept nor the bacteria of milk neither the mixture of $G^+$ and $G^-$ bacteria have grown in nutrient medium. We can state, that the 2% concentration was effective on full scale on all micro-organisms used for the testing and thus on the micro-organisms contained in milk.

Qualitative method with glass carrier enabled us to evaluate the disinfecting effectiveness of Dusept on the micro-organisms fixed on the surface of the carrier (Table 2).

It was also found using the quantitative method with carrier, that 2% concentration of Dusept is effective on mixture of $G^+$ and $G^-$ micro-organisms. No vital (living) forms of bacteria were found fixed on the surface of the carrier in any of the 5 repetitions of the experiment.

Regarding the findings acquired both of the qualitative methods, in case of a long-term disinfecting at low concentrations (1% and 0,1%) resistance of micro-organisms could be created on the disinfectant appliance Dusept. Then the resistant organisms could also survive the application of increased concentrations. It can be assumed that this is the reason why the producer recommends using 5% concentration with minimum 30 min operation time.

We examined the effectiveness of the disinfectant appliance on surfaces with dried microbial contamination. The quantitative method was used.

Table 4 provides the results of evaluation of effect of the solution of Dusept on $G^+$ and bacteria. We used Dusept with 0,5 concentration which was left to react for 4 min. After the application we found out that the amount of the micro-organisms entrapped on a glass carrier were decreased by the order of $10^2$.

We determined effectiveness of disinfecting appliance Dusept on mixture of $G^+$ and $G^-$ bacteria and micro-organisms contained in milk, using 3 methods. We determined different effective concentrations of Dusept concerning the used method as well as the examined micro-organisms.

## INTRODUCTION

One of the basic assumptions in each branch of food industry is the hygienic cleanliness of both the production area and non-production area, as well as the inhibition of contamination of the production materials before they are used in the process of production and at the moment of storage of the final product. The clearing and consecutive decontamination of these areas is one of the crucial preventive factors, which avoid the expansion of micro-organisms. The default of sanitary regime brings vast after-effect, which can lead to health-violation of a consumer and to concern's economic casualties. The aim of the sanitary processes are arrangements which disable the expansion of the primary contamination of the micro-organisms and also inhibit the secondary contamination of the micro-organisms and ineligible allothigene substances [1].

If there are no high-standard abstergent and decontaminating preparations, they need appropriate application techniques are needed for the producer to supply the required effectiveness. [2,3,4]. There are different characteristic systems for mechanized cleaning decontamination in the course of sanitation of volume units, tubes or areas. The most frequently used are different mobile pressure appliances, circuit cleaning (CIP-Cleaning in Place), irreversible cleaning (Central Cleaning System), cleaning with partial dismantling (COP-Cleaning out Place), hose-stations [5].

The most frequently used system is CIP-cleaning, the advantages of which are decreasing costs, optimalization of water and detergents usage, decreasing of decontaminating facilities and water vapour. This system also enables the increased exploitation of technological facility because tanks and tubes can be cleaned immediately after their emptying and they can be refilled again after they are cleaned. The CIP-system minimizes manual labour [6].

The CIP system in dairies, breweries and beverages industry raises the level of security, because the operating personnel do not have to enter the tanks and thus avoid the possibility of injuries which are often caused by having a fall on a slippery surface. At the same time the CIP-system improves the sanitation of the final product by abidance of the cleaning programme, whereby there are good results achieved and the quality of the product also improves.

Chemical disinfection is connected with application of organic reagents or inorganic chemicals as disinfection appliances [8]. Disinfecting appliances as well as abstergent appliances must carry out a number of requests like: microbicidal properties, resistance of the appliance when applied, gaining the optimal efficiency from 20°C to 40°C, non-toxic and non-irritant action in required concentrations, material tolerance, easy solubility in alcohol in all required concentrations and an easy rinsing, minimal redeposition, acceptable or no odour, a good cleaning effect of combined appliances, simple application and stability in the course of storage and application concentration, simple analytical analyses, price availability and economic capacity [9]. In the terms of microbial contamination it should have deadly effect on the entire spectrum of micro-organisms, even at very low temperature and at the presence of organic impurities. The optimum choice must be effective at the presence of impurities, residues of cleaning specimen and different pH of medium [10].

## MATERIAL AND METHODS

In this work we were testing cleaning and disinfecting appliance Dusept containing the effective substance sodium hipochlorite made by Inc. Duslo Šaľa. The producer recommends the application of operative solution with 5% concentration for the standard disinfection of areas and facilities in food manufactories. They declare that the product Dusept has been tested on gram-positive ($G^+$) and gram-negative ($G^-$) bacteria. According to the tests, Dusept is effective on the gram-positive bacteria ($G^+$) in form of 0,03% solution with two minute-action duration, as regards the gram-negative bacteria ($G^-$) it is effective in form of 0,8% solution with four minute-action duration.

Effectiveness of disinfecting appliance Dusept was tested on mixture of $G^+$ (*Enterococcus faecalis)* and $G^-$ (*Escherichia coli*) bacteria in sample of milk. Three methods were used:

1. Qualitative method on stiff soil where the disinfecting appliance was added [11].
2. Qualitative method with glass carrier [12].
3. Qualitative method with fixed glass carrier [7].

There was a twenty-four-hour cultivation of $G^+$ and $G^-$ on a slanted agar prepared before the testing of the disinfecting appliance. The $G^+$ and $G^-$ bacteria were mixed 1:1 right before the experiment by which the tested mixture of $G^+$ and $G^-$ was created. After sterilizing and partial cooling there was the disinfecting appliance added into the prepared nutrient medium GTKA. Then an exact amount of Dusept was added, so as the resulting effective concentration was 2%, 1%, and 0,1%.

When the glass carrier was used, there were sterile glass plates of 25x10x1mm format put into the sterile Petri basins and sealed with 10ml of 24-hour suspension of the $G^+$ and $G^-$ mixture and 10ml of milk. The plates were moved out in 1 ″ hour and then they were put into 3 sterile Petri basins and sealed by 5 ml of disinfectant appliance Dusept with 2%, 1%, and 0,1% operative solutions.

The glass plates were transferred by sterile pincers from the disinfecting solutions into the sterile Petri basins, where they were sealed with 5 ml sterile solution of saline with peptone. The rinse of the Petri basins lasted for 5 min. The glass plates were aseptically transferred into the test tubes which were containing nutritive medium (10ml) and then they were put into the thermostat, where they were cultivated by 30°C for 24-48 hours. After the cultivation the growth of the colonies were being monitored.

Using the quantitative method with fixed glass carrier, there were 0,1 ml of 24-hour suspension of $G^+$ and $G^-$ mixture (0,05 $G^+$ and 0,05 $G^-$), or 0,1ml of milk, aseptically applied on the glass plates in Petri basins of 50x50x5 format. The specimen was spreaded onto the whole surface of the plate and left to dry in room temperature. When it was dry, the glass plates were put into the thermostat and the next day there was 20 ml of operative solution of disinfectant appliance was used. The plates with milk were sealed with 2% concentration of Dusept for 4 minutes.

Concurrently check tests were carried out, which differed from the previous tests in that the disinfectant operative solution was not applied on the plates with some dried micro-organisms on them. Before application of the suspension of $G^+$ and $G^-$ bacteria and milk, the total amount of micro-organisms in 1 ml was determined.

By using the quantitative method we carried out statistical evaluation of the experiments. Then the basic variation-statistical characteristic x, s, v (%) were calculated.

## RESULTS AND DISCUSSION

The disinfectant appliance Dusept made on the sodium hipochlorite basis was being evaluated by three methods. As shown in Table 1 with the application of 2% concentration of operative solution of disinfectant appliance Dusept in nutrient medium nor the bacteria of milk neither the mixture of $G^+$ and $G^-$ bacteria did grow. We can state, that the 2% concentration was effective on full scale on all micro-organisms used for the testing and so on the micro-organisms contained in milk.

At 1% and 0,1 % concentration of the operative solution of the disinfecting appliance Dusept we observed a positive growth of $G^+$ and $G^-$ bacteria in all repetitions of the experiments. Resulting from this, these concentrations are not effective on the mixture of the tested micro-organisms $G^-$ (*E. coli*) and $G^+$ (*Enterococcus faecalis*) and they do not inhibit their growth. In the check-test, where there was no disinfectant appliance applied, the positive growth of bacteria was manifested, as well as the growth of bacteria in milk. Disinfectant appliance Dusept was not effective on the micro-organisms contained in milk at 1% and 0,1% concentration.

Qualitative method with glass carrier enabled us to evaluate the disinfecting effectiveness of Dusept on the micro-organisms fixed on the surface of the carrier (Table 2).

It was also found using the quantitative method with carrier, that 2% concentration of Dusept is effective on mixture of $G^+$ and $G^-$ micro-organisms. There were no vital (living) forms of bacteria found fixed on the surface of the carrier in any of the 5 repetitions of the experiment.

1% concentration of Dusept liquidated micro-organisms only partially. At 0,1%concentration positive growth of the mixture of micro-organisms was recorded.

The action of the Dusept-solution on the micro-organisms of milk spreaded on the carrier is shown in Table 3.

Table 1: The results of testing the effectiveness of disinfecting appliance Dusept by qualitative method

| Number of experiment | Mixture of E. Coli and Enterococus faecalis | | | Control | Milk | | | Control |
|---|---|---|---|---|---|---|---|---|
| | Concentration of disinfectant solution in the nutritive medium (%) | | | | Control of disinfectant solution in the nutritive medium (%) | | | |
| | 2 | 1 | 0,1 | | 2 | 1 | 0,1 | |
| 1 | - | + | + | + | - | +/- | +/- | + |
| 2 | - | + | + | + | - | +/- | +/- | + |
| 3 | - | + | + | + | - | +/- | +/- | + |
| 4 | - | + | + | + | - | +/- | +/- | + |
| 5 | - | + | + | + | - | +/- | +/- | + |

Legend: + fog, ring, sediment, positive growth; - negative growth; +/- faint, odd growth of bacteria

Table 2: The results of testing the effectiveness of disinfecting appliance Dusept on micro-organisms by qualitative method with carrier

| Experiment No. | Mixture $G^+$ (Enterococcus faecalis) and $G^-$ (E. coli) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Growth in stock | | | | Growth on GTKA | | | |
| | Concentration of disinf. solution (%) | | | Control | Concentration of disinf. solution (%) | | | Control |
| | 2 | 1 | 0,1 | | 2 | 1 | 0,1 | |
| 1 | - | +/- | + | + | - | +/- | + | + |
| 2 | - | +/- | + | + | - | - | + | + |
| 3 | - | +/- | + | + | - | - | + | + |
| 4 | - | +/- | + | + | - | +/- | + | + |
| 5 | - | +/- | + | + | - | - | + | + |

Legend: + fog, ring, sediment, positive growth; - negative growth; +/- faint, odd growth of bacteria

Table 3: The results of testing the effectiveness of disinfection appliance Dusept on the micro-organisms of milk by qualitative method with carrier

| Experiment No. | Micro-organisms of milk | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Growth in stock | | | | Growth in GTKA | | | |
| | Concentration of disinf. solution (%) | | | Control | Concentration of disinf. solution (%) | | | Control |
| | 2 | 1 | 0,1 | | 2 | 1 | 0,1 | |
| 1 | + | + | + | + | - | + | + | + |
| 2 | + | + | + | + | - | + | + | + |
| 3 | + | + | + | + | - | + | + | + |
| 4 | + | + | + | + | - | + | + | + |
| 5 | + | + | + | + | - | + | + | + |

In the experiment with milk-like material, 2% concentration of Dusept was not effective. Using the liquid medium in test tubes after cultivation in a thermostat (30°C) some growth of the micro-organisms of milk was registered. It means that the 10-minute- action of 2% solution of Dusept was not sufficient and the micro-organisms entrapped on the carrier could survive. We assume that in addition to

the short exposition time (10 min) it could be also the structure of milk, which could affect the survival of the micro-organisms.

At the inoculation of the rinsing saline with 2% Dusept-solution into GTKA, none of the colonies of micro-organisms occurred.

The micro-organisms swilling into the saline didn't survive the 2% concentration of Dusept. Regarding the findings of both qualitative methods, in case of a long-term disinfection at low concentrations (1% and 0,1%) resistance of micro-organisms on the disinfectant appliance Dusept was created. Then the resistant organisms could also survive the application of increased concentrations. We can assume that this is the reason why the producer recommends using 5% concentration with minimum 30 min operation time. Several authors have been observing the micro-organism resistance by using short time of exposition [12,13] Their results suggest that the micro-organisms entrapped on the carrier showed high resistance in the suspension, hereby there did not occur any marked differences among the used exposition times. The testing methods we have been using in our research of bactericide effects of disinfectant appliances were advised by a number of authors. [7,13].

We were also examining the effectiveness of the disinfectant appliance on surfaces with dried microbial contamination. The quantitative method was used.

Table 4 provides the results of evaluation of effect of the solution of Dusept on $G^+$ and $G^-$ bacteria. We used Dusept with 0,5 concentration which was left to react for 4 min. After the application we found out that the amount of the micro-organisms entrapped on a glass carrier were in lowered order of $10^{2.}$ The disinfectant appliance Dusept wasn't effective on mixture of $G^+$ and $G^-$ bacteria in chosen concentration (5%), because its action survived average $4.10^2$ KTJ of mixture of $G^+$ and $G^-$ micro-organisms. This concentration survived mainly $G^-$ bacteria, that's why the producer recommends 0,8 % concentration of Dusept.

Table 4: The results of testing the effectiveness of disinfecting appliance Dusept on mixture of $G^+$ and $G^-$ bacteria by quantitative method

| Experiment No. | Mixture of G+ and G- bacteria | | |
|---|---|---|---|
| | Bo (KTJ/ml) | K (KTJ/ ml) | S (KTJ/ml) |
| 1 | $48.10^7$ | $25.10^2$ | 18 |
| 2 | $62.10^7$ | $43.10^2$ | 18 |
| 3 | $32.10753.10^7$ | $21.10^2$ | 168 |
| 4 | $53.10^7$ | $27.10^2$ | 5 |
| 5 | $55.10^7$ | $25.10^2$ | 0 |
| $\overline{x}$ | $50.10^7$ | $30,2.10^2$ | 41,8 |
| s | $11,25.10^7$ | $8,78.10^2$ | 7,09 |
| $x_{min}$ | $32.10^7$ | $21.10^2$ | 0 |
| $x_{max}$ | $62.10^7$ | $43.10^2$ | 168 |
| v(%) | 22,5 | 29 | 16,9 |

Legend:
Bo- number of KTJ in 1 ml of 24-hour bacteria-culture spreaded on a glass plate
K- number of KTJ in the control experiment (dross of the plate with no application of the solution)
S- number of KTJ in 1 ml after dross of the plate and application with 5%concentration of disinfectant appliance Dusept with 4minute-action time

The qualitative method was also used when examining the survival of micro-organisms of heat-treated milk, which was left to dry on the surface of a plate. 2% concentration of Dusept was used and it was left to react for 20 min. The results are listed in Table 5. Application of 2% solution of Dusept didn't survive any of the micro-organisms. We confirmed that 2% concentration of disinfectant appliance Dusept is effective and it is sufficient for common disinfection and also for dairy areas.

Table 5: The results of testing the effectiveness of disinfecting appliance Dusept on the organisms of milk by quantitative method

| Experiment No. | Number of micro-organisms in milk | | |
|---|---|---|---|
| | Mo (KTJ/ml) | K (KTJ/ml) | S (KTJ/ml) |
| 1 | $1{,}7.\ 10^4$ | 53 | 0 |
| 2 | $2{,}0.\ 10^4$ | 59 | 0 |
| 3 | $5{,}6.\ 10^4$ | 60 | 0 |
| 4 | $7{,}0.\ 10^4$ | 72 | 0 |
| 5 | $4{,}4.\ 10^4$ | 44 | 0 |
| $\bar{x}$ | $4{,}1.\ 10^4$ | 57,6 | - |
| s | $2{,}29.\ 10^4$ | 10,26 | - |
| $x_{min}$ | $1{,}7.\ 10^4$ | 44 | - |
| $x_{max}$ | $7{,}0.\ 10^4$ | 72 | - |
| v (%) | 55,8 | 17,8 | - |

Legend:
Mo – number of KTJ in 1ml of spreaded milk on a glass plate
K - number of KTJ in check test (dross of the plate without application of solution)
S - number of KTJ in 1 ml after the dross of the plate and after application of disinfectant solution Dusept with 2% concentration with 20- minute action time

## CONCLUSION

We were determining effectiveness of disinfecting appliance Dusept on mixture of $G^+$ and $G^-$ bacteria and micro-organisms contained in milk using 3 methods. Different effective concentrations of Dusept were determined depending on the used method as well as the examined micro-organisms.

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