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Short Communication
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Verification of Effectivness of Conservants K 145 and K 702

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Introduction

In our work we dealt with microbial contamination of cosmetics products. Microorganisms have important role in our lives. They are used in food processing and in pharmacy, but their pathogenic triebes can mean health risk for costumers. Hundred thousands of people use cremes every day. That's why we think, that this topic is important and results can be relevant for practice. In cosmetics products is sterility not expected, but some of specific microorganisms are controlled. These microorganisms can mean health risk for users. As model microorganism we chose yeast *Candida glabrata*. Genus *Candida* can cause oral and genital infections. We could not work with pathogenic triebes so we chose nonpathogenic. We also chose bakteria *Escherichia coli*, which is an indicator of fecal contamination. If *E. coli* is in product, it means, that there are lacks of hygiene. Conservants should have same effect against contamination caused by all species of bacterias and yeasts [1,2]. Our aim was verificaion of effectiveness of conservants K 145 and K 702 against contamination caused by mentioned microorganisms. Principle of effect of conservants are chemical reactions with microorganisms. Conservant K 145 has these active substances: 2-Bromo-2-Nitropropane-1.3-Diol, Methylchloroisothiazolinone, Methylisothiazolinone. Conservant K 702 has these active substances: Phenoxyethanol, Benzoic Acid, Dehydroacetic Acid, Ethylhexylglycerin, Polyaminopropyl Biguanide. Every conservant used in the European union has its own maximum authorised concentration. For conservant K 145 this vaule is 0.2%, for conservant K 702 it is 1%. Conservant K 145 is not recommended for products used for mucous membranes and lips and also for toothpastes. Conservant K 702 can be used for leave-on as well as rinse-off products [3,4].

Experimental

We used several methods in our work. The first one was MIC test. By this test is ascertained minimal concetration of conservant, which is effective against microbial contamination. We prepared aqueous solutions of conservants with different concentrations. For K 145 had solutions concentrations 0.05%; 0.1% and 0.2%. For K 702 concentrations were 0.2%; 0.6% and 1%. To this solutions we added 50 µl of *E. coli* and 50 µl of *Candida glabrata* inoculum. After 30 minutes we took first sample and spread it on SCDA and LB agar. Next samples we took after 2, 47 and 49 hours. We cultivated all samples on 30°C for 48 hours. It appplies the highest number, the more intensive contamination. Zero means no microorganisms. So two highest concentrations were effective after 49 hours. Next method which we used was Schülke Koko test. By this test is monitored effect of conservants during repeated contamination. As samples we used basis for creme production and creme created by ourselves. To this unpreserved samples we added different amount of conservants. This way we created different concentrations of concervants in products (Figures 1 and 2). For K 145 we made concentrations 0.05%; 0.1%, 0.2% and 0.3%. For K 702 concentrations were 0.2%; 0.6%, 1% and 1.35%. To this way conservanted cremes we added 50 µl of *E. coli* and 50 µl of *Candida glabrata* inoculum. After one week we took first samples, diluted it to 10^{-5} and spread dillutions 10^{-3} and 10^{-5} on SCDA and LB agar. We cultivated all samples on 30°C for 5 days. We took samples once a week for 6 weeks. In optimal case the procuct have to be without microbial contamination for 6 weeks. If this happened, it can be said, that the product has a minimum storage life for 30 months [5]. We ascertained, that concentrations 1.35% for K 702 and 0.3% for K 145 were unequivocally effective during the whole test. Concentrations 0.2% for K 145 and 1% for K 702 (maximum authorised concentrations) were effective too, but less reliable. Last used method was qPCR. By this method is verificated presence of microorganism by its DNA. In this way of PCR is undergoes reaction directly monitored by fluorescent pigments. Fluorescence is measured in every cyclus of reaction. Its intensity is proportional to concentration of present DNA. It is determined a value of C_T, which means a point where is statistically greatest the value of fluorescence. It applies the more of DNA in

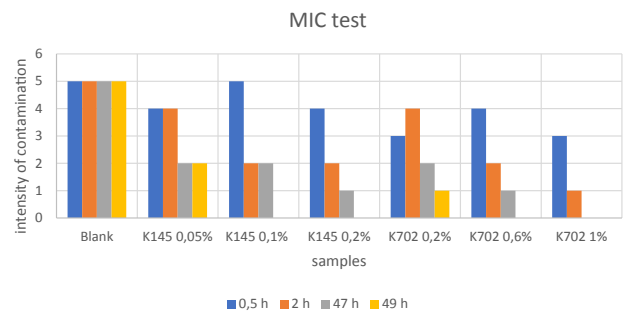

Figure 1: Results of MIC test.

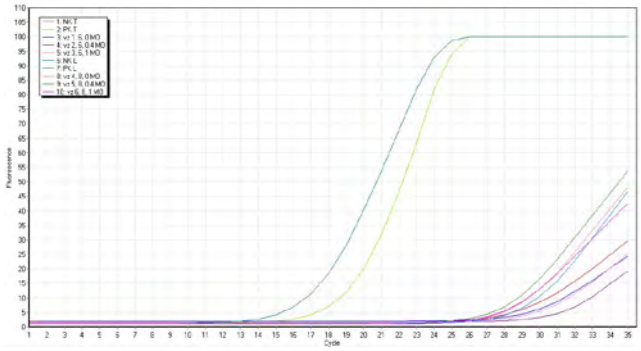

Figure 2: Amplification curve of PCR for yeasts. Dependence of number of cycles on fluorescence intensity.

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sample, the previous detection of DNA and the lowest C_T value. After PCR reaction is used the Melt Analysis (Table 1). By this method is ascertained nucleotide composition of PCR products. Following melting curve is determined temperature of melting for specific DNA and then verified, if it is in PCR product [6,7]. We used this method for commercial product Perfecta Pharmacy Femina Med, which is gel used for intimate hygiene. It contains probiotics and prebiotics, and also conservant K 145. We used barches n. 15174600 (minimum storage life to June of 2018) and n. 152366000 (minimum storage life to August of 2018). At first we isolated DNA by magnetics mediums. Then we put samples, positive and negative controls to cycler. We used primers for yeasts (concretely Oli-F and Oli-R). Positive and negative controls had not surprising results and in all samples was no yeast DNA. So the conservant K 145 was effective.

Number	Colour	Name	Type of sample	C_T
1		NK T	Negative Control	30.36
2		PK T	Positive Control	18.08
3		vz 1, 6/0 MO	Unknown	31.57
4		vz 2, 6/0.4 MO	Unknown	32.85
5		vz 3, 6/1 MO	Unknown	31.47
6		NK L	Negative Control	28.83
7		PK L	Positive Control	16.05
8		vz 4, 8/0 MO	Unknown	28.42
9		vz 5, 8/0.4 MO	Unknown	28.06
10		vz 6, 8/1 MO	Unknown	28.39

Explanation: PK -positive control, NK -negative control, 8/1- product with minimum storage life to 08/2018, inoculum 1%, 8/0.4 -product with minimum storage life to 08/2018, inoculum 0.4%, 8/0 -product with minimum storage life to 08/2018, inoculum 0%, 6/1- product with minimum storage life to 06/2018, inoculum 1%, 6/0.4 - product with minimum storage life to 06/2018, inoculum 0.4%, 6/0 - product with minimum storage life to 06/2018, inoculum 0%.

Table 1: Results of qPCR for yeasts.

Discussion

In our work we verified effectivness of conservants K 145 and K 702. We used microbial tests MIC and Schülke Koko and also molecular biology method qPCR. From microbial tests follows that it is not necessary to use maximum authorised concentration of conservant to products. Lower concentration of conservant is effective against microbial contamination too. From PCR follows that conservant K 145 is efffective in commercial product too.

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