INTRODUCTION

The microbial safety of foods is a major concern to the food industry as well as the consumers. Undesirable microflora may cause changes in taste, smell or structure of various food products. Food safety and health concerns resulting from ingestion of contaminated food are of great importance. Undesirable microflora can be eliminated e.g. by heat treatment or aseptic packaging. Addition of food preservatives is also very effective. Antimicrobial compounds such as sorbic or benzoic acids and their derivatives have been recently replaced by substances that commonly occur in nature and have high biodegradability. Monoacylglycerols have been reported to possess inhibitory activities against a wide range of micro-organisms [1–3]. These lipids being present in natural products such as milk are assumed, at least in lower concentration to be non-toxic to mucosas [4]. They can be added in small amounts (less than 1% wet weight) to food as emulsifying agents so it is technically feasible to add these substances to food products.

The present study was carried out with the objective of determining the efficacy of monoacylglycerols with hydrocarbon chain length varying from ten to fourteen carbon atoms for inactivation of Fusarium culmorum, Aspergillus niger and Bacillus subtilis.

EXPERIMENTAL

Microbial cultures and media. Bacillus subtilis DMF 2006 (1% v/v inoculum) was cultivated at 30°C in liquid Nutrient broth (100 ml, Oxoid, UK) in the presence of different concentrations of tested substances. The inoculum grew for 16 hours at 30°C in Nutrient broth.

The spores of Aspergillus niger DMF 0501 and Fusarium culmorum DMF 0103 were cultivated at 25°C in Malt Extract Broth (Oxoid, UK) in microtitate plates (100 µl) for 14 days. Suspension of spores (10 µl) was washed off from the Malt Extract Agar after 5 days and diluted to the final amount 1.10^3/ml of spores in the growth media.

Assay of antimicrobial activity. The amount of colony forming units (CFU) of Bacillus subtilis was
determined every 24 h during 240 h of cultivation by determination of optical density by spectroscopy ($A = 620 \text{ nm}$). The growth of fungi was detected in microtitrate plates with microscopical detection of spores.

**Chemicals.** 1-Monoacylglycerols were synthesized at the Department of Dairy and Fat Technology, Institute of Chemical Technology Prague, by the reaction of isopropylideneglycerol with methylesters of fatty acids and subsequent degradation of the isopropylidene group [5]. The purity of tested substances was higher than 96%. 1-Monoacylglycerols were dissolved in ethanol and diluted to desired concentration. The final ethanol concentration in growth medium was always 2%, which did not interfere with viability of microbial strains.

**RESULTS AND DISCUSSION**

**Detection of antibacterial properties**

The inhibitory effects of 1-monoacylglycerols on bacterial growth were studied at concentration between 0.01 to 0.1 mg/ml. Addition of 1-monoacylglycerol at the concentration of 0.05 mg/ml causes inhibition of growth of tested microorganisms (Figure 1). The highest inhibition of Bacillus subtilis was caused by 1-undecanoylglycerol, 1-dodecanoylglycerol and 1-tridecanoylglycerol (Figure 1). These substances caused stagnation of lag – period of growth of bacteria. They did not affect the speed of growth in exponential – period and the amount of biomass at the end of cultivation. To sum up the results Inhibitory index (II) was used.

$$II = 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \%$$

$A_{\text{sample}}$ – the area under growth curves (240 h of cultivation) of treated samples

$A_{\text{control}}$ – the area under growth curves (240 h of cultivation) of untreated samples

**Detection of antifungal properties**

The inhibitory effects of monoacylglycerols on fungal growth were studied at concentrations
between 0.01 to 0.5 mg/ml in microtitrate plates with multispectrophometrical (Figure 3) and microspopical (Figure 4) detection. Figure 3 shows the microtitrate plate with *Fusarium culmorum* in presence of 0.5 mg/ml of monoacylglycerols after 10 days of cultivation. There was no growth in the presence of 1-decanoylglycerol, 1-undecanoylglycerol and 1-dodecanoylglycerol (grey spots – fourth, fifth and sixth columns).

1-Monoacylglycerols disrupted the cellular organization [6] and caused shrinking of the spores (Figure 4). Decreased volume of spores was evaluated with software Lucia (Table 1).

The lower concentrations of monoacylglycerols, which inhibit the growth of *Fusarium culmorum* and *Aspergillus niger*, were tested as well. The inhibitory concentration was found to be in the range of 0.07 to 0.1 for 1-decanoylglycerol and 1-undecanoylglycerol, and in the range of 0.1 to 0.5 mg/ml for 1-dodecanoylglycerol.

### CONCLUSIONS

1-Monoacylglycerols with chain lengths varying from 10 to 14 carbons were found to have strong inhibitory effect on microorganisms. 1-unde-
canoylglycerol, 1-dodecanoylglycerol and 1-tridecanoylglycerol were found to exhibit the highest inhibitory effect on growth of Bacillus subtilis DMF 2006. 1-Decanoylglycerol, 1-undecanoylglycerol and 1-dodecanoylglycerol cause inhibition of fungi and reduction of the spore volume.

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References