Monoacylglycerols as Food Additives with Antimicrobial Properties

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Abstract: The antimicrobial activities of monoacylglycerols (MAGs) with different lengths of hydrophobic hydrocarbon chains (1-decanoylglycerol, 1-undecanoylglycerol, 1-dodecanoylglycerol, 1-tridecanoylglycerol, 1-tetradecanoylglycerol) were investigated. Food undesirable bacterial strain *Bacillus subtilis* DMF 2006 and mould strains *Aspergillus niger* DMF 0501 and *Fusarium culmorum* DMF 0103 were used as indicator strains in order to determine antimicrobial properties of monoacylglycerols. The strongest antibacterial effect was caused by 1-undecanoylglycerol, 1-dodecanoylglycerol, 1-tridecanoylglycerol. These substances inhibited growth of *Bacillus subtilis* DMF 2006 for more then 10 days of incubation at the concentration of 0.05 mg/ml. Concentrations of 0.05 and 0.07 mg/ml of 1-decanoylglycerol, 1-undecanoylglycerol, 1-dodecanoylglycerol reduced the mycelium formation of *Fusarium culmorum* and *Aspergillus niger.* Higher concentrations (0.1 and 0.5 mg/ml) of these three substances inhibited spore germination. Knowledge of these properties brings new approach in food preservation where the substitute of chemical substances, such as sorbic and benzoic acids and their salts is in demand.

Keywords: antimicrobial activity; *Aspergillus niger*; *Bacillus subtilis*; *Fusarium culmorum*; 1-monoacylglycerols

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 biodegrability. 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 monoacyglycerols 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 (λ = 620 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 microorganims (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 exponencional – period and the amount of biomass at the end of cultivation. To sum up the results Inhibitory index (II) was used.

$$II = 1 - (A_{sample}/A_{control}) \times 100\ (\%)$$

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

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

Detection of antifungal properties

The inhibitory effects of monoacylgycerols on fungal growth were studied at concentrations

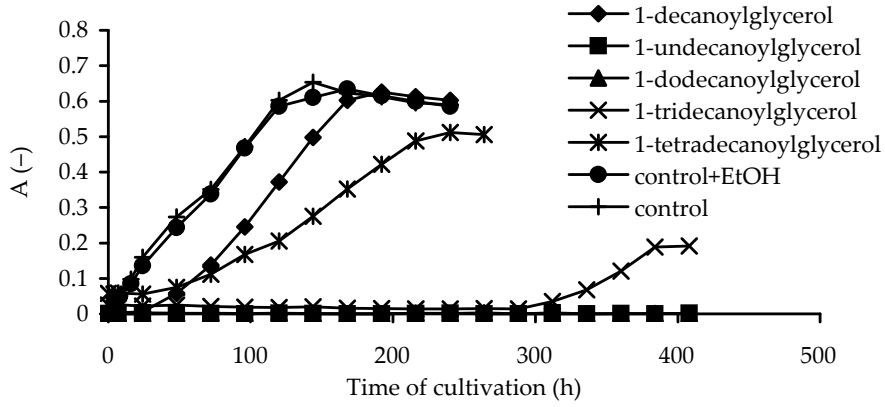


Figure 1. Growth curves of *Bacillus subtilis* in the presence of 0.05 mg/ml of 1-monoacylglycerols

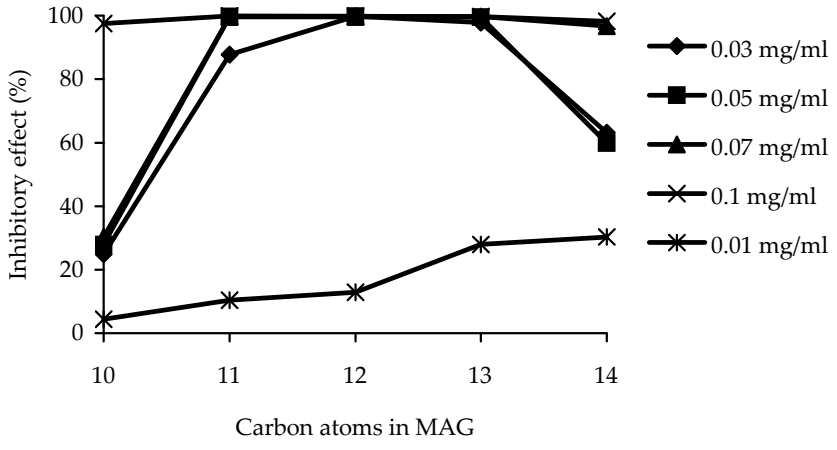
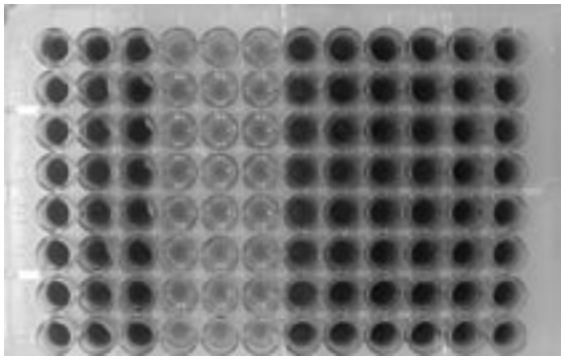


Figure 2. The dependence of inhibitory effect (on the growth of *Bacillus subtilis*) of 1-MAGs on the length of hydrocarbon chain in MAG



Column 1 2 3 4 5 6 7 8 9 10 11 12

Column
(1) K – control
(2, 3, 10) R1, R2 – control + EtOH
(4) MKG – 1-decanoylglycerol
(5) MUG – 1-undecanoylglycerol
(6) MLG – 1-dodecanoylglycerol
(7) MTG – 1-tridecanoylglycerol
(8) MMG – 1-tetradecanoylglycerol
(9) MPG – 1-monohexadecanoylglycerol
(11, 12) Dimod, Emg D – comercial emulsifiers

Figure 3. 10 days of cultivation of *Fusarium culmorum* in microtitrate plates in the presence of 0.5 mg/ml of monoacylglycerols

between 0.01 to 0.5 mg/ml in microtitrate plates with multispectrophometrical (Figure 3) and microspopical (Figure 4) detection. Figure 3 shows the microtitate 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-

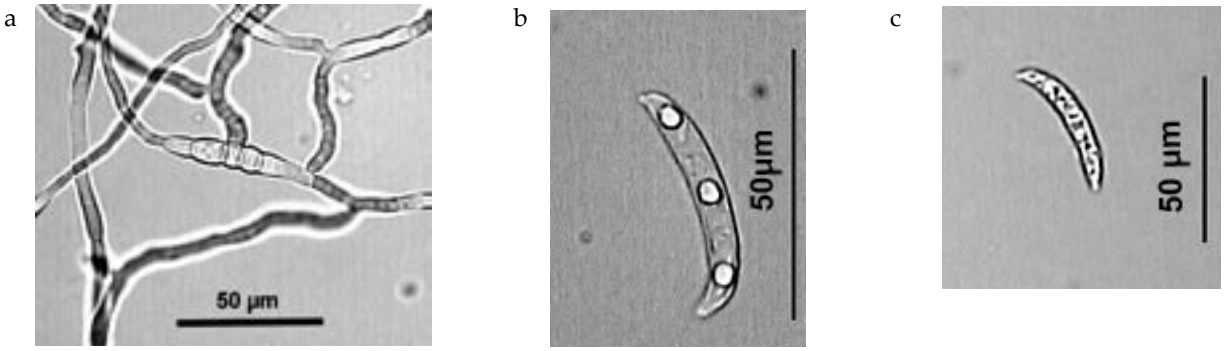


Figure 4. Microscopical detection of *Fusarium culmorum* in the presence of 0.5 mg/ml of monoacylglycerol after 24 hours of cultivation (a – control, b – 1-undecanoylglycerol, c – 1-dodecanoylglycerol); zoom 400 times

Table 1. Decreased volume of spores in the presence of 0.5 mg/ml of monoacylglycerols

	2nd day of cultivation	15th day of cultivation
1-Decanoylglycerol	8.8%	22.6%
1-Undecanoylglycerol	18.1%	33.5%
1-Dodecanoylglycerol	25.8%	13.2%
1-Tridecanoylglycerol	–	–
1-Tetradecanoylglyceol	–	–

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.

Acknowledgement: This work was supported by the Czech Science Foundation Grant Agency of the Czech Republic, Grant No. 525/03/0374.

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