Inhibition of *Aspergillus niger* DMF 0801 by Monoacylglycerols Prepared from Coconut Oil

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Abstract

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The objectives of the present study were to test the antifungal properties (inhibition of radial growth, inhibition of the mould spore germination) of lauroylglycerol and mixtures of monoacylglycerols synthesised from coconut oil (MIX-I and MIX-II) against *Aspergillus niger* DMF 0801. The content of monoacylglycerols in lauroylglycerol, MIX-I and MIX-II was 99.9% (w/w), 97.7% (w/w) and 75,1% (w/w), respectively. The content of 1-lauroylglycerol in MIX-I and MIX-II was calculated from the content of lauric acid and content of monoacylglycerols. The inhibition of the radial growth of *Aspergillus niger* DMF 0801 by lauroylglycerol was stronger than that caused by MIX-I and MIX-II. The inhibition effect of spore germination caused by lauroylglycerol and MIX-I was nearly the same. The inhibition of spore germination increased with increasing content of monoacylglycerol and also with increasing 1-lauroylglycerol content in monoacylglycerols. The level of spore germination inhibition was related to the purity of tested substances. The results of this study indicate that monoacylglycerols made from coconut oil have antifungal activity.

Keywords: antifungal; monoacylglycerol; coconut oil; lauroylglycerol

Lauric acid and some of its derivatives have a certain level of antifungal activity (KABARA 1993). Lauroylglycerol (monolaurin) possessing the highest antimicrobial effectiveness as well as emulsifying activity has an excellent potential for food production applications (KABARA 1993). The antimicrobial spectrum of lauroylglycerol is broad including the species of bacteria, e.g. *Bacillus cereus* (ABABOUCH *et al.* 1994), *Pseudomonas* sp. (BAUTISTA *et al.* 1993) and moulds such as *Aspergillus* sp. (MANSOUR *et al.* 1996), *Penicillium* sp. (MANSOUR *et al.* 1996; PLOCKOVÁ *et al.* 1999), *Cladosporium* sp., *Fusarium* sp., *Alternaria* sp. (PLOCKOVÁ *et al.* 1999).

The fungal contamination of food products is a serious problem. Particularly, the production of mycotoxins toxic to biological systems is the major negative impact of fungal growth in foods (SCOTT 1989). The fungal growth in the food industry is controlled mainly by prevention of mould contamination, nevertheless, addition of antimycotic substances is the principal way of preventing mould growth. Sorbic acid, benzoic acid and their salts are commonly used preservatives in foods (CHIPLEY *et al.* 1993), moreover, many new ways of fungal growth control such as lactic acid bacteria metabolites (PLOCKOVÁ *et al.* 2001) are under testing procedures at present.

In previous studies we tested the antifungal activity of lauric acid derivatives against *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. (PLOCKOVÁ *et al.* 1999; ŘIHÁKOVÁ *et al.* 2001a, b). When tested against fungal spores, we found two different types of antifungal effects. The first type involved the inhibition of spore germination and the second one was the inhibition of the radial growth of *Aspergillus niger* (ŘIHÁKOVÁ *et al.* 2001b). As it was published previously (ŘIHÁKOVÁ *et al.* 2001a), 1-lauroylglycerol and 1-lauroyldiglycerol inhibited the spore germination. Because of the high cost of the preparation of pure substance for industry applications, we prepared two

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mixtures of acylglycerols of a coconut oil to test their antifungal effect. Coconut oil contains 90% saturated fatty acids, and of these, lauric acid accounts for 45–48% and caprylic acid, capric acid, and myristic acid account for 30–36%; they would be expected to have the antimicrobial activity (WANG *et al*. 1993). Thus, the coconut oil is a potentially interesting substrate for synthesis of antimicrobial monoacylglycerols. The inhibition of *Listeria monocytogenes* by MAG coconut oil produced by solid-phase glycerolysis catalysed by lipase PS-30 from *Pseudomonas* sp. and purified by hexane fractionation has been studied until now (WANG *et al*. 1993).

The main objective of this research study was to investigate a possibility of using the coconut oil as a source of monoacylglycerols with antifungal activity.

MATERIALS AND METHODS

Preparation of MAG of coconut oil. To test the antimicrobial effect of 1-lauroylglycerol mixed with other naturally occurring MAG, two MAG mixtures of coconut oil were synthesised. These two mixtures differed in the content of 1-lauroylglycerol and content of lauric acid.

The synthesis of MIX-I was the same as the synthesis of 1-lauroylglycerol (CHANDRAN & BHATNAGAR 1968). Methyl esters of fatty acids of coconut oil were prepared by alkaline catalysed methanolysis (FILIP *et al.* 1992) of coconut oil and refined by vacuum distillation. MIX-II was prepared by alkaline catalysed glycerolysis (SONNTAG 1982) of coconut oil. Natrium glycerolate was used as a catalyst (it was prepared by dilution of natrium in glycerol, the molar ratio of oil–glycerol was 1:6). After cooling, the reaction mixture was dissolved in diethyl ether and washed to neutral reaction.

Determination of lauric acid content. The fatty acid (FA) composition was determined by gas chromatography (ISO 5508, Prague 2000). The glass packed column was used (3 × 2 400 mm, filled with ethylene glycoladipate 15% (w/w) on Chromaton NAW-DMCS-0.125–0.160 mm, nitrogen as carrier gas). The areas of FA methyl esters were integrated by APEX software (DataApex, s.r.o., Version 1.0, Prague 1994) and expressed as percentage of FA (DGF Methoden C-IV 10a, Stuttgart 1987). Lauric acid content was 45.0% in MIX-I and 30.7% in MIX-II.

Determination of monoacylglycerol (MAG), diacylglycerol (DAG) and triacylglycerol (TAG) content. The glyceride mixture composition was determined by Thin Layer Chromatography with Flame Ionisation Detection (TLC-FID) (IATROSCAN TH/10). Chromarod SII (54 ± 9 μm silica layer thickness) was used and the mobile phase composition was *n*-hexane/diethyl ether/formic acid (95:5:1). Scanning speed was 0.42 cm/s, hydrogen pressure 190 kPa and air flow 15 l/min (RANNÝ 1987). Peak areas of glycerides were integrated by APEX software (DataApex, s.r.o., Version 1.0, Prague 1994) and expressed as percentage (Fig. 1). The content of MAG in lauroylglycerol, MIX-I, and MIX-II was 99.9% (w/w), 97.7% (w/w) and 75,1% (w/w), respectively. The content of DAG in MIX-I or MIX-II was 1.9% (w/w) and 24.0% (w/w), respectively. The concentration of TAGs in both mixtures was minimal. The content of 1-lauroylglycerol in MIX-I and MIX-II was calculated from the content of lauric acid and the content of MAG (Fig. 1).

Mould strains. A mould strain used in this study was obtained from the collection of Department of Dairy and Fat Technology (ICT Prague, CR). The mould strain was maintained on a slant agar (Malt Extract Agar, OXOID, GB) and subcultured once a month (cultivation at room temperature for 5–7 days). For all tests only fresh cultures were used. Suspension of spores from the fresh culture was prepared for each test by washing the slant agar with 5 ml of sterile saline with Tween 80 (0.85% [w/w] NaCl, 0.01% [w/w] Tween 80, 1000 ml distilled water).

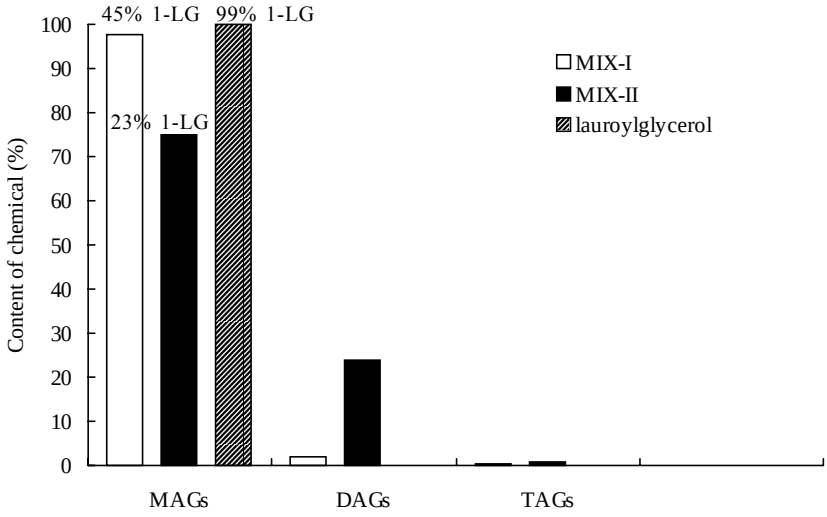

Fig. 1. The content of monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG) at tested MIX-I, MIX-II, and lauroylglycerol (% 1-LG show content of 1-lauroylglycerol in portion of MAGs of tested mixtures)

IFR gel cassette system. Culture media, inocula and the gel cassette were prepared as previously (BROCKLEHURST *et al.*1995, 1996; ŘIHÁKOVÁ *et al*. 2001) and the inhibition of the fungal spore germination and the radial growth were investigated.

RESULTS AND DISCUSSION

Lauroylglycerol has been reported to have the highest activity among several lipid derivatives evaluated for antifungal effects against fungi (KABARA 1993). Furthermore, the costs of the pure 1-lauroylglycerol preparation are high and when used at a higher concentration it gives a soapy flavour to the product (BRANEN *et al.* 1980). Other MAGs rich in 1-lauroylglycerol that are made from vegetable or animal oils and fats could be useful as preservatives. With respect to the fatty acid composition, coconut oil could be one of the most important sources of lauric acid esters. In our work two samples of MAGs differing in the content of 1-lauroylglycerol were prepared by two methods, and consecutively tested against *Aspergillus niger* DMF 0801 at a concentration scale 0.5–2.0 mg/ml. The results are compared with the effect of lauroylglycerol (1-lauroylglycerol, purity > 99%) that was published previously (ØIHÁKOVÁ *et al.* 2001a).

Although the radial growth of *Aspergillus niger* DMF 0801 was slightly inhibited by the presence of all tested concentrations of MIX-I or MIX-II, no difference between the inhibition caused by MIX-I or MIX-II was found (Fig. 2). Interestingly, a large difference was found for the inhibition of spore germination in *Aspergillus niger* DMF 0801 (Fig. 3). The inhibition of spore germination caused by lauroylglycerol was nearly total at concentrations 0.5 mg per ml and higher (ŘIHÁKOVÁ *et al.* 2001a). Similar inhibition was found in the presence of MIX-I at concentrations higher than 1.0 mg/ml. No tested concentration of MIX-II induced a significant inhibition of spore germination.

When compared with lauroylglycerol (ŘIHÁKOVÁ *et al*. 2001a), the reduction in the radial growth of *Aspergillus niger* DMF 0801 by lauroylglycerol was stronger than

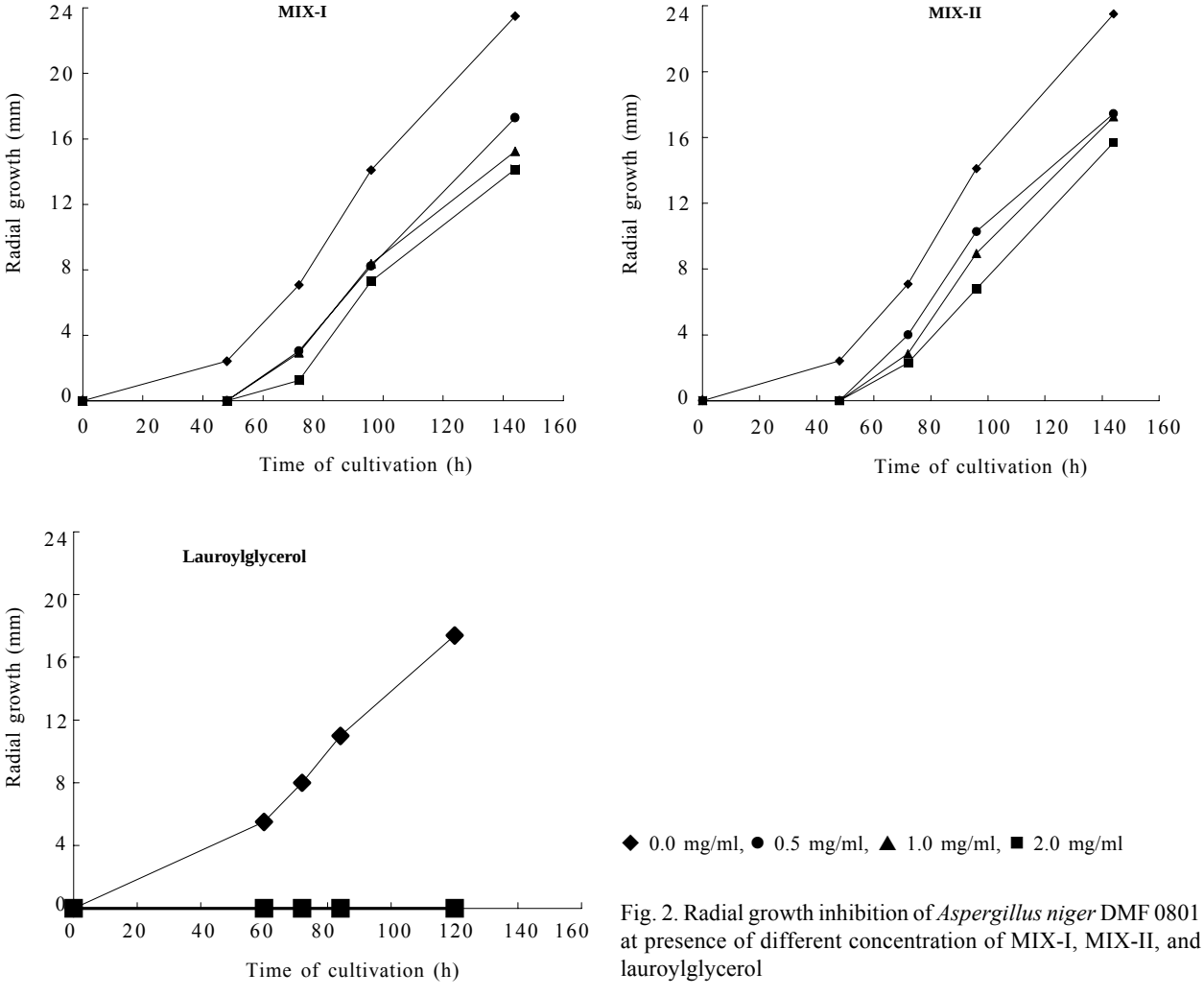

Fig. 2. Radial growth inhibition of *Aspergillus niger* DMF 0801 at presence of different concentration of MIX-I, MIX-II, and lauroylglycerol

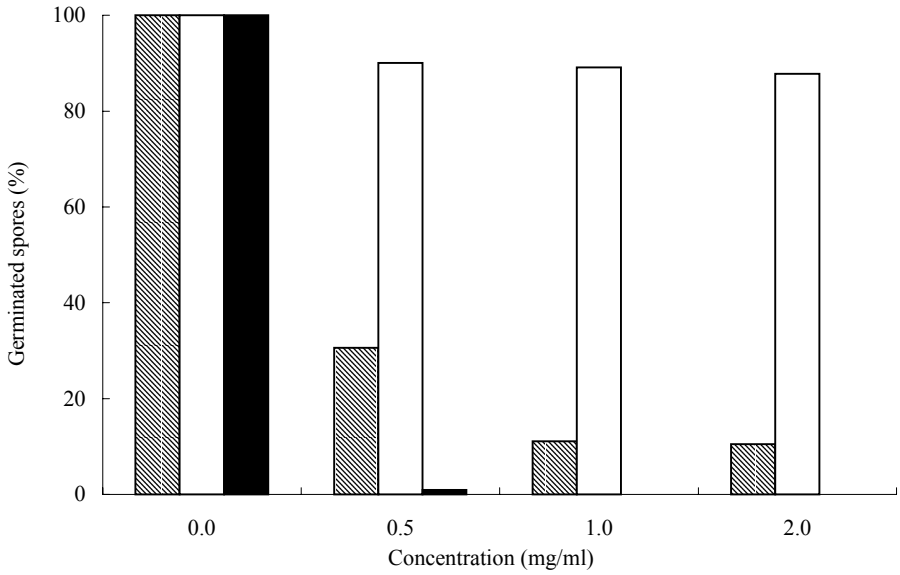

Fig. 3. The inhibition of the spore germination of *Aspergillus niger* DMF 0801 at presence of different concentrations of MIX-I (▨), MIX-II (□) and lauroyl-glycerol (■)

that caused by MIX-I and MIX-II. No differences in the radial growth inhibition of *Aspergillus niger* DMF 0801 caused by MIX-I and MIX-II were found for the tested concentration range although these two mixtures differed in the content of MAGs and DAGs (Fig. 1).

The inhibition effect on spore germination by lauroylglycerol and MIX-I was nearly the same. It is obvious that the inhibition of spore germination increased with increasing content of MAGs and also with increasing content of 1-lauroylglycerol in MAGs (Figs. 1 and 3). It can be concluded that the intensity of inhibition of spore germination in *Aspergillus niger* DMF 0801 is related to the purity of tested substances even though the MIX-I and MIX-II inhibition of the radial growth of *Aspergillus niger* DMF 0801 whose germination was not inhibited and produced colony forming spores seems not to be related to the content of 1-lauroylglycerol in the tested mixtures of MAGs.

To get satisfactory interpretation of this phenomenon it is necessary to carry out additional examinations of the antifungal activity of MAGs and DAGs present in MIX-I and MIX-II (that means monoacylglycerols and diacylglycerols typical of MIX-I and MIX-II prepared from coconut oil) against *Aspergillus niger* DMF 0801 not only *per se* but also to test the antifungal activity of each possible combination of monoacylglycerol and diacylglycerol esters against *Aspergillus niger* DMF 0801 that MIX-I and MIX-II included. The inhibition effect of other fatty acids and monoacylglycerols present in MIX-I and MIX-II was not examined in this study although the antibacterial effect of lauric acid, capric acid and monocaprin was published (KATO & SHIBASAKI 1975; BERGSSON *et al.* 2001a, b).

Our results are in good agreement with those of WANG *et al.* (1993), who tested the inhibition of *Listeria monocytogenes* by MAGs of coconut oil produced by solid-phase glycerolysis catalysed by lipase PS-30 from *Pseudomonas* sp. They published that fractionated MAGs showed a slightly more inhibiting effect against *Listeria monocytogenes* than unfractionated MAGs. MAGs prepared from coconut oil were more effective against *Listeria monocytogenes* than lauroylglycerol.

According to the results of this study the mixture of MAGs made from coconut oil could potentially be used as preservatives with antifungal effect instead of lauroylglycerol in industry applications. In future it could be useful to test the antifungal activity of MAGs prepared from palm kernel oil or babassu oil that are oils with high content of lauric acid.

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Souhrn

ŘIHÁKOVÁ Z., FILIP V., PLOCKOVÁ M., ŠMIDRKAL J., ČERVENKOVÁ R. (2002): **Inhibice** *Aspergillus niger* DMF 0801 **monoacylglyceroly připravenými z kokosového oleje.** Czech J. Food Sci., **20**: 48–52.

Testovali jsme antifungální vlastnosti (inhibici růstu kolonie, inhibici germinace spor) lauroylglycerolu a směsí monoacylglycerolů připravených z kokosového oleje (MIX-I a MIX-II) na *Aspergillus niger* DMF 0801. Obsah monoacylglycerolů v lauroylglycerolu byl 99,9 % hm., v MIX-I 97,7 % hm. a v MIX-II 75,1 % hm. Obsah 1-lauroylglycerolu (1-LG) v MIX-I a MIX-II byl vypočítán z obsahu laurové kyseliny a z obsahu monoacylglycerolů. Inhibice růstu kolonií spor *Aspergillus niger* DMF 0801 v přítomnosti lauroylglycerolu byla silnější než inhibice růstu kolonií spor *Aspergillus niger* DMF 0801 způsobená přítomností MIX-I a MIX-II. Inhibice germinace spor lauroylglycerolem a směsí MIX-I byla téměř stejná. Čím více se zvyšoval obsah monoacylglycerolu ve vzorku, tím silnější byla inhibice germinace spor. Stejný efekt měla rostoucí koncentrace 1-lauroylglycerolu. Účinek testovaných látek v inhibici germinace spor souvisel s jejich čistotou. Získané výsledky indikují, že monoacylglyceroly připravené z kokosového tuku měly antifungální aktivitu.

Klíčová slova: antifungální; monoacylglyceroly; kokosový tuk, lauroylglycerol

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