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The Influence of Fatty Acids and their Derivatives on the Growth of *Kluyveromyces*, *Saccharomyces*, and *Candida* Species

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Abstract: The antifungal properties of medium-chain fatty acids and their esters with glycerol were investigated. Fatty acid chain lengths of ten to twelve carbon atoms were examined, namely decanoic, undecanoic and dodecanoic acids and their 1-monoglycerides. *Kluyveromyces marxianus* var. *marxianus*, *Saccharomyces cerevisiae* and *Candida maltosa* were used as indicator yeast strains. Inhibition of yeast growth was detected using spectrophotometry, agar plates, flow cytometry, and microscopy. It was observed that the inhibitory effects decreased with increasing chain length. The minimum inhibitory concentration of fatty acids and 1-monoglycerides was found to vary between 0.05 and 0.10 mg/ml. The most sensitive yeast strain was *Saccharomyces cerevisiae*. *Kluyveromyces marxianus* var. *marxianus* and *Candida maltosa* were less sensitive to the antimicrobial effect of tested substances. Knowledge of these properties of these compounds brings new alternatives for food preservation.

Keywords: Antifungal activity · Fatty acid · 1-Monoglycerides · Yeast

Introduction

Yeast fermentation is used in the manufacturing of foods, such as bread, wine, vinegar and surface-ripened cheese. The most industrially important yeast is of the genus *Saccharomyces*, especially the species *S. cerevisiae*. For preparation of dairy products such as kefir, *Kluyveromyces marxianus* var. *marxianus* is used. However some yeasts are contaminants, like *Candida maltosa* which is found in yoghurt. These ascospore-forming yeasts are readily bred for desired characteristics, but yeasts are undesirable when they cause spoilage to fruit juice, sauerkraut, syrup, meat, wine, dairy and other food products [1]. Fermentation is usually stopped either though filtration or pasteurization. The latter is limited to certain foods and since it is a heat treatment, it can denature proteins. Filtration is limited to clear liquids. Neither process can be applied to certain foods, so safe and effective fungicides are needed to control yeast growth. Medium-chain free fatty acids and their corresponding 1-monoglycerides have been found to have a broad spectrum of microbial activity [2][3]. These lipids are commonly found in natural products, for example, in milk, and therefore are likely to be nontoxic to mucosas, at least at low concentrations. In 1996 lauric acid was recognized by the WHO as a GRAS – generally recognized as safe.

1-Monoglycerides are used as emulsifiers in the food industry, so it is technologically feasible to use these substances in food products. Decanoic acid was identified among the compounds, other than ethanol, that are produced during ethanolic fermentation by yeasts and are toxic to the yeasts themselves [4][5]. Decanoic acid is currently added as adjuvant of sulphur dioxide for biological stabilization of sweet wines. As shown by Krebs *et al.* [6], the undissociated toxic form of the weak acid enters the cell by passive diffusion across the plasma membrane and dissociates in neutral cytoplasm causing the decrease of intracellular pH. The insertion of liposoluble acids from inside the plasma membrane is thought to decrease the spatial organization of the membrane [7], affecting its function as a selective barrier [8] and as a matrix for enzymes [7][9]. This work was carried out in order to determine if the tested fatty acids or their 1-monoglycerides have an influence on the growth of *Candida maltosa*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* and therefore could be useful for prevention of food spoilage.

Materials and Methods

Microorganisms and Growth Conditions

Candida maltosa YP 1, *Kluyveromyces marxianus* var. *marxianus* DMF 1005 and *Saccharomyces cerevisiae* DBM 181 were provided from the Collection of the Department of Dairy and Fat Technology, Institute of Chemical Technology, Prague. Stock cultures were maintained on malt extract agar (Oxoid, UK), stored at 1 °C and subcultured monthly (5 d at 25 °C). For each experiment a fresh culture in malt extract broth pH 5.4 (Oxoid, UK) was used.

Chemicals

Fatty acids were purchased from Fluka (purity >97% (GLC)), 1-monoglycerides were synthesized at the Department of Dairy and Fat Technology, ICT, Prague (purity >97% (GLC)). Stock solutions of each

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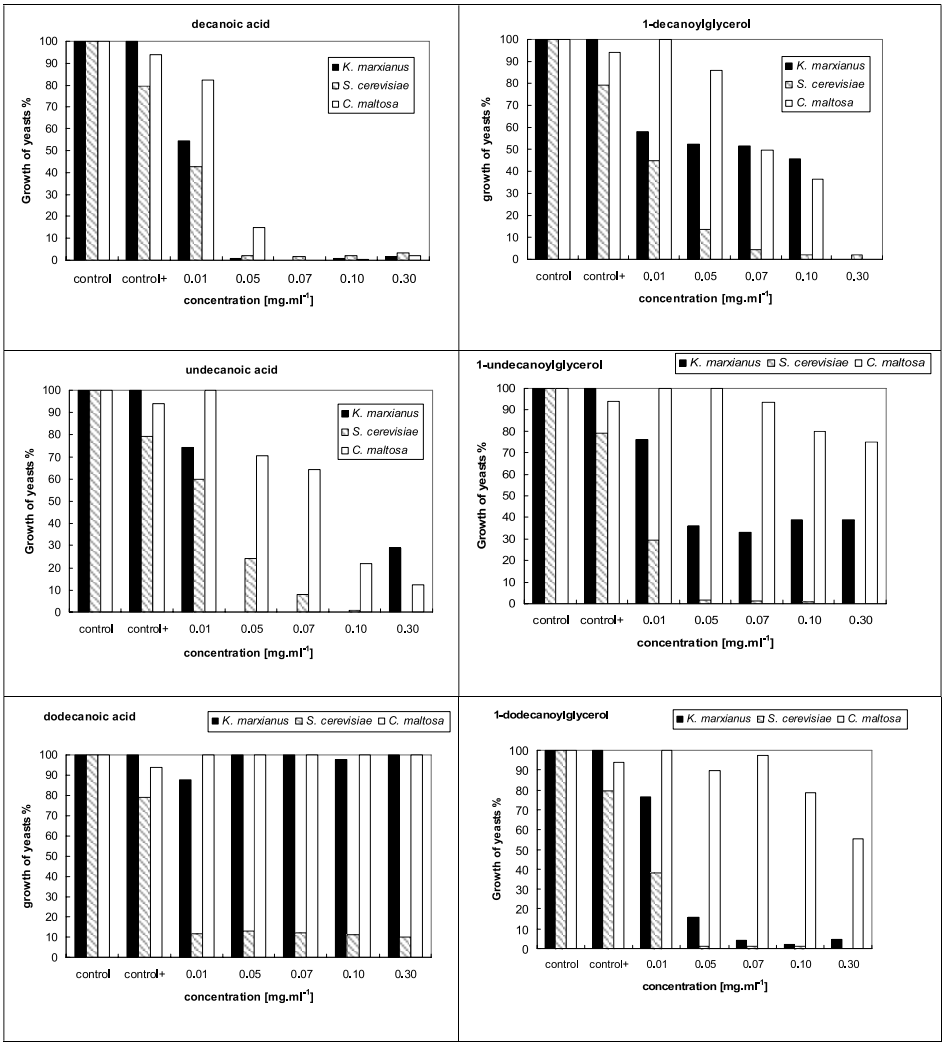

Fig. 1. The growth of yeasts in the presence of fatty acids and their 1-monoglycerides (42 h incubation, concentration of tested substances 0.01–0.3 mg.ml^{-1}).

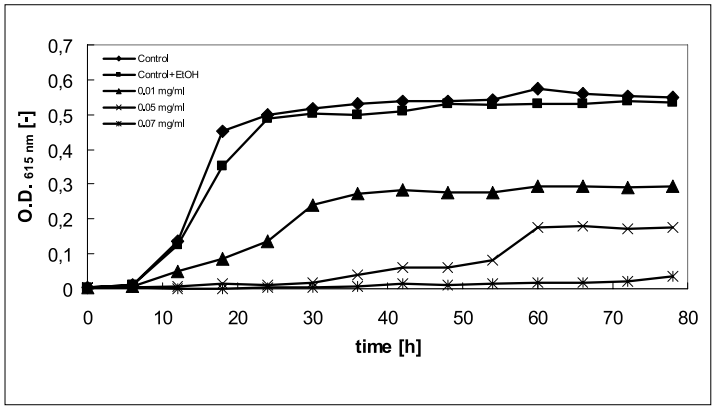

Fig. 2. Growth curves of *S. cerevisiae* in the presence of 1-decanoylglycerol: specific growth rate [h^{-1}]: control: 0.18, control +: 0.17, 0.01 mg.ml^{-1}: 0.06, 0.05 mg.ml^{-1}: 0.03.

compound were prepared by dissolution in ethanol and stored at 5 °C.

Determination of Antifungal Activity

Microtiter plates with a well capacity of 300 µl were used. Inoculum (1%) was diluted in Malt Extract Broth pH 5.4 (100 µl) supplemented by tested substances at a desired concentration and were incubated at 25 °C for 42 h. The optical density (615 nm) of each well was read at 18 and 42 h with a microtiter plate spectrophotometer (Unicam Helios gamma/delta, Spektronic Unicam, UK). The growth of yeasts was expressed in % as a ratio of O. D.x/O. D.o *100. (O.D.x = optical density of inoculated sample, O. D.o = optical density of Malt Extract Broth without inoculum). 100 µl from wells where growth was inhibited after 72 h were spread on malt extract agar in Petri plates that were incubated for 3 d and CFU.ml^{-1} was counted. The yeast cell viability was detected by optical microscope (DMLS, Leica, Germany) in the presence of methylene blue solution (dead cells are colored in blue) – the cell shape was evaluated by using the Lucia G software, and by flow cytometric analysis (PAS-III flow cytometer, Partec GMBH, Munster, Germany) in the presence of propidium iodine [10].

Results and Discussion

The use of ethanol as a solvent for tested substances has no significant influence on the growth of microorganisms (control +, Fig. 1). The inhibitory effect of fatty acids and 1-monoglycerides decreases with increasing length of carbon chain (except for the growth of *K. marxianus* in the presence of 1-monoglycerides where the increasing carbon chain length causes the inhibitory effect to increase) (Fig.1).

Decanoic and undecanoic acid caused higher inhibition of yeasts than their 1-monoglyceride analogues. The inhibitory effect of dodecanoic acid was lower compared to its 1-monoglyceride analogue. In the tested group of yeasts *Saccharomyces cerevisiae* was the most sensitive (for this reason it was chosen for future experiments) and *Candida maltosa* was the least sensitive. Data summarized in Fig. 2 show the effect of tested substances on the growth curves where the lag-phase was prolonged; there is an exponential inhibition of the maximum specific growth rate and the biomass yield decreases with increasing concentration of tested substances [11]. Simultaneously, the number of viable cells detected by microscope and by flow cytometry (Fig. 3, Table) decreased. The results from flow cytometry are in agreement with observations made by microscopy. The volume of cells which were not inhibited in the presence of the tested substances, increased during the incubation. The volume of inhibited cells did not change, and damage to the vacuoles was observed. Transfer of cells incubated for 72 h in the presence of tested substances onto fresh malt extract agar did not augment the number of viable cells (Table). This suggests that the native effect of those substances is not reversible [12].

These data indicate that these medium-chain fatty acids and their 1-monoglycerides could be used as food additives to selectively control the growth of yeasts.

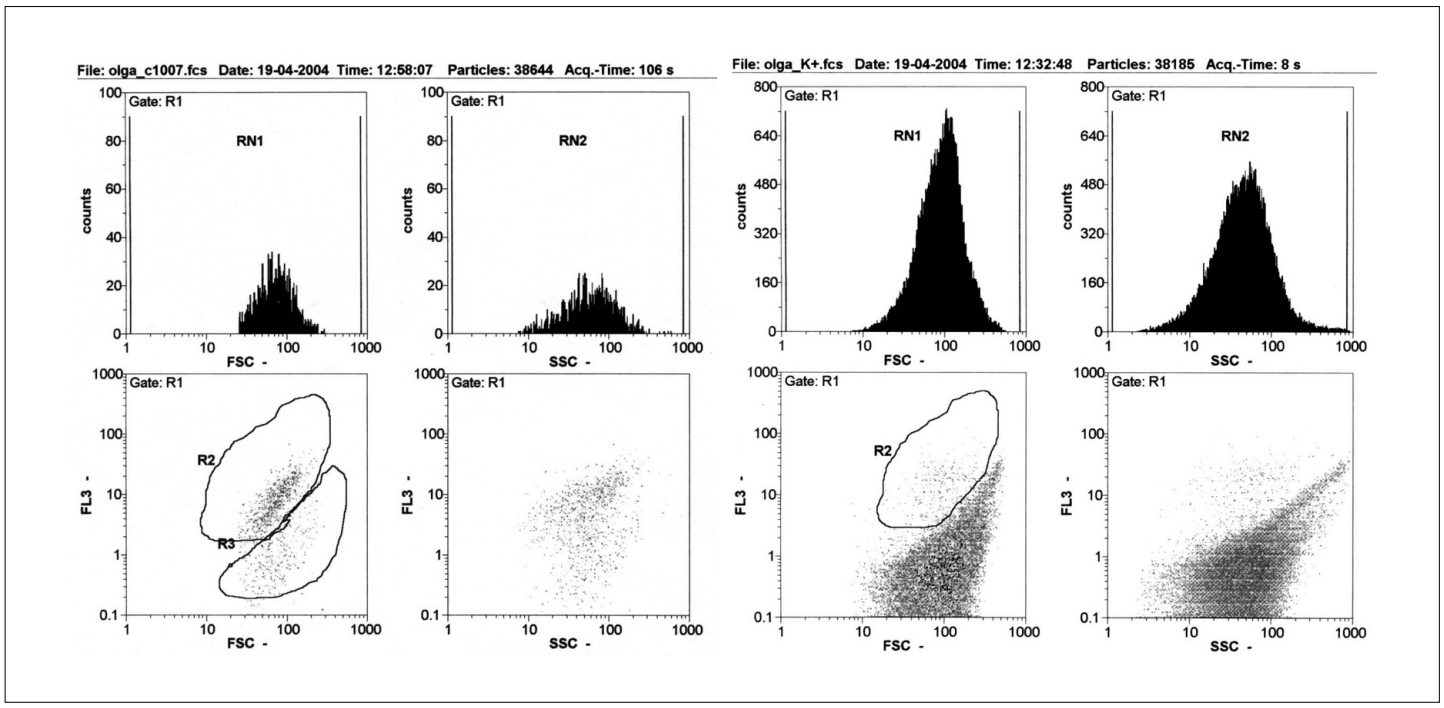

Fig. 3. Results (histograms) from flow cytometry (decanoic acid 0.07 mg.ml^{-1} and control): FSC = cell size; Count = number of cells; SSC = granularity of cells; FL3 = fluorescence intensity; R2 = area of dead cells; R3 = area of viable cells

Table. Comparison of flow cytometry and CFU.ml^{-1} methods for assessing *S. cerevisiae* growth after 72 h of cultivation in the presence of tested substances.

	c [mg.ml^{-1}]	viable cells R3 [%]	dead cells R2 [%]	CFU.ml^{-1}
control	0.00	98.8	1.2	2.7*10^7
C10	0.01	98.7	1.3	4.8*10^7
	0.05	35.7	64.3	0
	0.07	34.0	66.0	0
C11	0.01	77.1	22.9	1.8*10^6
	0.05	35.4	64.6	0
	0.07	19.5	80.5	0
MAG 10	0.01	98.6	1.4	4.3*10^6
	0.05	83.1	16.9	3.3*10^6
	0.07	76.0	24.0	0.8*10^6
MAG 11	0.01	97.6	2.4	3.8*10^6
	0.05	30.1	69.9	0
	0.07	32.5	67.5	0

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