Monoacylglycerols as Fruit Juices Preservatives

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Abstract

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Limiting or preventing the growth of undesirable microbial flora in food products is one of the main goals of food microbiology. A number of preservation methods have been designed to extend the shelf-life of the food products by reducing the microbial growth while new antimicrobials are still examined. Monoglycerides are naturally occurring compounds with inhibitory activity against various microorganisms. We evaluate the antimicrobial effects of 8 monoglycerides *in vitro* and in fresh unpasteurised apple juice. Out of all monoglycerides tested, monocaprin (MAG C10:0) and monolaurin (MAG C12:0) showed the best ability to supress or prevent the growth of filamentous fungi *in vitro.* The addition of these monoglycerides to apple juice resulted in a decrease in total viable counts of bacteria and yeasts. Monocaprin was able to stop completely the growth of bacteria and yeasts at a concentration of 250 μg/ml, and to prevent microbial spoilage of apple juices for at least two weeks.

Keywords: monoglycerides; antimicrobial activity; bacteria; yeasts; filamentous fungi; apple juice

The current worldwide drive for a healthier lifestyle has led to a rising demand for fresh foods, free from additives, with a high nutritional value, possessing antioxidant and free-radical scavenging properties, and intended for consumption both at home and in catering establishments. Many fruits contain a variety of minor ingredients, particularly vitamins and minerals, as well as carbohydrates, which are the predominant solid components. Several components with antioxidant activity are found in fruit juices. These include ascorbic acid, tocopherols, β-carotene, and flavonoids. It should be noted that the changes and losses, particularly of the minor components of juices, occur under adverse conditions of the processing technologies. In this way, minimally processed fruit juices offer great advantages for the consumers. The industry with minimal processing of fruit and vegetables needs an appropriate selection of raw materials and operation of improved sustainable strategies for reducing the losses and providing high quality and safe commodities. The most important target for keeping the overall quality of these commodities is a decrease in microbial spoilage flora as that causes both decay and safety problems.

Monoglycerides (MAGs) or their derivatives are widely used emulsifiers in the food industry. Being commonly found in natural products, such as milk, MAGs are generally considered as non-harmful and safe agents with no adverse reactions, non-toxic to mucosa, at least at low concentrations, which makes them suitable for wide industrial

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applications (Bergsson 1998, 2001). Their use is known in the production of margarine, ice creams, yoghurts or frozen desserts, they are also used in bakery applications where they increase the durability of bread and have a favourable influence on the rheological properties of the dough (Mouloungui *et al.* 1998; Buňka *et al.* 2007).

In addition, many studies published recently have described inhibitory effects of fatty acids and their monoglycerides on the growth of microorganisms. Physicochemical and functional properties, as well as antimicrobial activity of MAGs, depend on the number of carbon atoms and double bonds present in the fatty acid chain (Kabara *et al* 1972; Conley & Kabara 1973; Wang & Johnson 1992). The exact mode of the action against microorganisms is not yet precisely clear and several hypotheses have been suggested. One of them is based on the amphiphilic character of MAG molecule and assumes their penetration and incorporation into cytoplasmic membrane with subsequent disruption of its permeability and nutrient transport (Nair 2004; Dufour 2007). An alternative hypothesis involves the penetration of fatty acids, their dissociation in the inner cell environment, and an increase of intracellular acidity (Sun *et al.* 1998; Nair *et al.* 2005).

The inhibitory potency of MAGs against gram-positive bacteria is well documented (Kabara *et al.* 1972; Schlievert *et al.* 1992; Oh & Marshal 1993; Branen & Davidson 2004; Buňková *et al.* 2011). Gram-negative bacteria were shown to be more resistant (Skřivanová 2006; Buňková *et al.* 2011), but exception have been reported, e.g. *Helicobacter pylori* (Petschow *et al.* 1996; Preuss *et al.* 2005), *Neisseria gonorrhoeae* (Bergsson *et al.* 1998), *Klebsiella pneumoniae* (Růžička *et al.* 2003). Monoglycerides have also proved to prevent or limit the growth of yeasts and filamentous fungi (Bergsson *et al.* 2001; Růžička *et al.* 2003; Buňková*et al.* 2010).

Literature survey indicates MAGs antimicrobial activity with a relatively broad spectrum. In our previous study, we compared antibacterial effecs of seven monoglycerides on food-borne pathogens or spoilage bacteria (Buňková 2011). The aim of this study was, hence, to determine the inhibitory effects of a similar group of monoglycerides against micromycetes *in vitro*, to select MAGs with the best microbicidal efficacy and evaluate their potential to be applied in unpasteurised juices to decrease the growth of microbial spoilage flora.

MATERIAL AND METHODS

Microorganisms. The inhibitory effects of the selected 1-monoglycerides were tested with the following filamentous fungi: *Alternaria alternata* DFTM 202, *Aspergillus niger* DFTM 238, *Mucor racemosus* DFTM 335, *Penicillium roqueforti* DFTM 078. All strains of micromycetes were obtained from the collection of the Department of Food Technology and Microbiology of Tomas Bata University in Zlin (Czech Republic).

Filamentous fungi were aseptically sampled and incubated on Fungal agar (HiMedia, Mumbai, India) at 25 ± 1°C for 7–14 days.

Solutions and chemicals. Monoglycerides were prepared by direct addition of the corresponding fatty acids to glycidol (2,3-epoxy-1-propanol) (both Sigma-Aldrich, St. Louis, USA) according to the procedure published by Janiš *et al.* (2000). The reaction was performed under fixed conditions of pressure and temperature in an open glass reactor equipped with a magnetic stirrer and temperature-stabilised jacket capable of maintaining the reaction temperature required for the reaction within ± 0.5°C limit. The reaction temperature for each fatty acid ranged from 90–96°C; chromium(III) acetate hydroxide (Sigma-Aldrich, St. Louis, USA) was used as a catalyst. Crude reaction products were purified by threefold filtration and recrystallisation from ethanol. The purity of all the MAGs used was better than 99% as determined by HPLC. Monoglycerides of the following acids were prepared: caprylic acid (MAG C8:0), capric acid (MAG C10:0), undecanoic acid (MAG C11:0), undecenoic acid (MAG C11:1), lauric acid (MAG C12:0), myristic (MAG C14:0), palmitic (MAG C16:0) and oleic acids (MAG-C18:1).

Stock solutions of the MAGs tested (100 ml) were prepared at a concentration of 25 g/l (w/v) in absolute ethanol (LachNer, Neratovice, Czech Republic). They were sterilised by filtration (Millipore with the porosity of 0.22 μm) and stored in closed test tubes at a temperature of 4°C.

Antifungal activities of MAG in vitro. The corresponding quantities of MAG solutions were added to the individual doses of heat-sterilised Fungal Agar and dosed aseptically into Petri dishes. The growth was observed at the following concentrations of each MAG: 250, 750, 1000, and 1500 μg/ml. Fungal inoculum was taken with a needle from the peripheral growth zone of the stock cultures. Petri dishes supplemented with MAG were in-

oculated by four times pinning. The samples were incubated at 25 ± 1°C for 14 days. The colony radii were measured after 7 and 14 days of incubation. The MIC values were determined in Petri dishes displaying no visible growth with the lowest MAG concentration.

Antimicrobial activity of MAG in apple juice. The samples of fresh unpasteurised apple juice were aseptically dosed in 50 ml quantities into sterile glass jars with sealable lids and stored at 6 ± 2°C. One series of the juice samples were enriched with MAG of capric or lauric acid (MAG C10:0, MAG C12:0) in the concentration range of 50–1500 µg/ml. The control series without MAGs was also included. All samples were prepared in triplicate. The growth of microorganisms in apple juice was monitored for 4 weeks with regular sampling every 7 days of storage. The apple juice samples for microbiological analysis were aseptically removed and appropriately diluted with sterile saline solution. For the determination of microbial counts, the treated juices were diluted and plated out on plate count agar (PCA) for determining the total aerobic or facultative anaerobic plate counts, de Man, Rogosa, and Sharpe (MRS) agars for determining the lactic acid bacteria, ENDO agar for enterobacteria count, and Fungal agar for yeasts and moulds plate counts. The plates were incubated at 37°C for 48 h (bacteria), or at 25 ± 1°C for 14 days (yeasts and moulds), and the results were expressed as colony forming units per 1 ml of the sample (CFU/ml).

The results of microbiological analyses were statistically evaluated by means of Kruskal-Wallis test and Wilcoxon test. The significance level used in the test was 0.05. Unistat® 5.5 software (Unistat, London, UK) was used for statistical evaluation.

RESULTS AND DISCUSSION

Antifungal activity of MAGs *in vitro*

The results of antifungal activity on culture media showed (Table 1) that MAGs of fatty acids with 8–12 carbon atoms were able to prevent the growth of all micromycetes tested except for *M. racemosus* DFTM 335, which proved to be resistant to MAG C8:0 and MAG C11:0. The high tolerance of this fungus to MAG activity was also reported by Růžička (2003). Buňková (2010) found that the growth of some penicillia including *P. roqueforti* was strongly inhibited by MAG C10:0 and MAG C12:0 while a weak inhibitory action on *A. niger* was recorded. In our study, the behaviour of these strains with respect to MAGs showed similar features. From all MAGs tested, MAG C10:0 and MAG C12:0 showed the best ability to suppress or prevent the growth of filamentous fungi *in vitro.*

Antimicrobial activity of MAGs in unpasteurised apple juice

Based on the results of *in vitro* assay for the evaluation of antimicrobial activity of MAGs with 8–18 carbon atoms in fatty acid chain, two monoglycerides: MAG of capric acid (MAG C10:0) and MAG of lauric acid (MAG C12:0) were chosen for the determination of the activity against microbial contaminants in fresh unpasteurised apple juice.

The inhibitory effect on the growth of bacteria in apple juice was observed after the application of both monoglycerides tested. During the cultivation in the presence of MAG C10:0 at concentrations

Table 1. Effect of monoglycerides against filamentous fungi

	MIC (µg/ml)			
	Alternaria alternata	*Aspergillus niger*	*Mucor racemosus*	*Penicillium roqueforti*
MAG C8:0	1000	1500	NI	1000
MAG C10:0	250	1000	1500	750
MAG C11:0	1000	1500	NI	1500
MAG C11:1	1500	1500	1500	1500
MAG C12:0	750	1500	1500	1000
MAG C14:0	1500	> 1500 (NI)	> 1500 (NI)	1000
MAG C16:0	1000	> 1500 (NI)	> 1500 (NI)	> 1500 (NI)
MAG C18:1	1500	> 1500 (NI)	> 1500 (NI)	> 1500 (NI)

MIC – minimal inhibitory concentration; NI – no inhibition

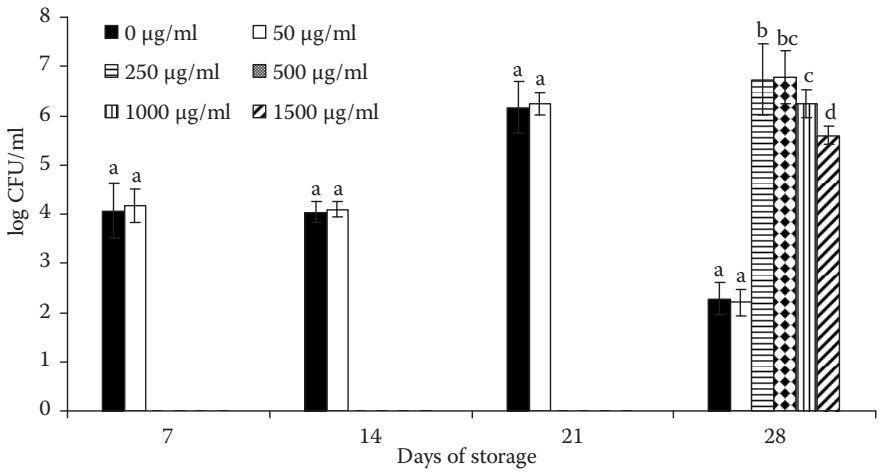

Figure 1. Effect of monocaprin (MAG C10:0) on the growth of bacteria in unpasteurized apple juice. The total aerobic or facultative anaerobic plate count is expressed as log CFU/ml of apple juice sample

The values reflecting statistically significant differences in plate counts are designated with different letters in indexes ($P < 0.05$); samples taken after 7, 14, 21, and 28 days of storage were evaluated by means of Kruskal-Wallis test separately

of 250–1000 µg/ml, no growth of bacteria occurred for 3 weeks of storage (Figure 1). MAG C10:0 at a concentration of 50 µg/ml did not suppress bacterial growth and CFU values remained comparable to those of the control sample without MAG. Similar trends could be observed with MAG C12:0, however, the inhibitory action lasted only 7 days of storage.

MAG C10:0 also showed a significant inhibitory effect on the yeasts present in apple juice with total inhibition at concentrations equal or higher than 250 µg/ml for 2 weeks of storage and reduction in yeast counts in the 3rd and 4th weeks of storage (Figure 2). In comparison with the monoglyceride of capric acid, the application of MAG of lauric acid (MAG C12:0) led to a decrease in CFU values, but complete inhibition was not observed, not even in the first sampling period after 7 days of storage.

Filamentous fungi were detected in low counts not exceeding 10^2 CFU/ml in the control apple juice sample throughout the storage period. In the presence of MAG C10:0, the total counts of filamentous fungi decreased with no growth observed at MAG C10:0 concentrations higher than or equal to 250 µg/ml. In comparison with MAG C10:0, monolaurin (MAG C12:0) exhibited a weaker inhibitory action with complete inhibition of filamentous fungi at 500 µg/ml concentration.

Throughout the 28-day storage, no enterobacteria were detected in the apple juice samples whereas lactic acid bacteria were isolated at low counts in the 4th week.

The antimicrobial effect of MAGs might not be strong enough to justify their application alone as general-purpose preservatives. On the other hand, the employment of MAG C10:0 in specific applications to prevent or limit the growth of Gram-positive bacteria, spore forming bacteria, and yeasts seems to be promising, regarding their natural origin. In addition, antimicrobial activity could be enhanced by combination of MAGs with

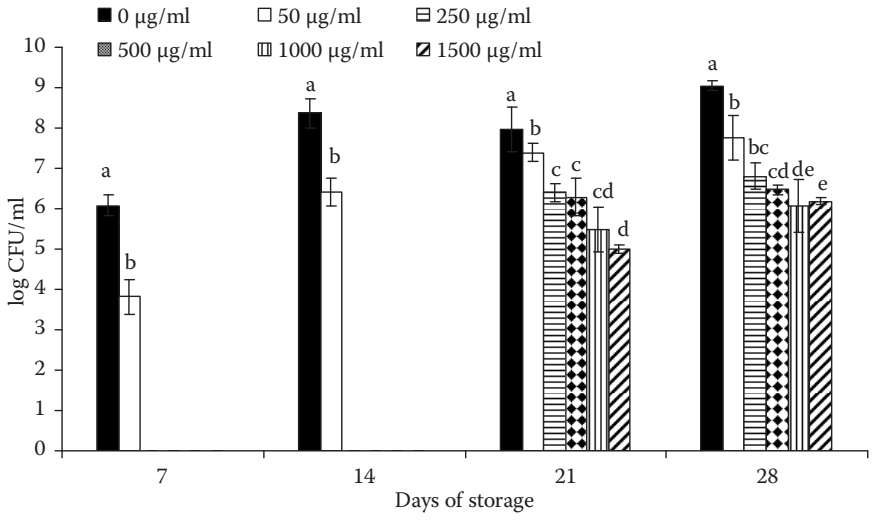

Figure 2. Effect of monocaprin (MAG C10:0) on the growth of yeasts in unpasteurised apple juice. The total yeasts plate count is expressed as log CFU/ml of apple juice sample

The values reflecting statistically significant differences in plate counts are designated with different letters in indexes ($P < 0.05$); samples taken after 7, 14, 21, and 28 days of storage were evaluated by means of Kruskal-Wallis test separately

other antimicrobial substances or factors limiting the growth of undesirable microorganisms.

CONCLUSION

In this study, different ability to limit the growth of microorganisms was recorded for individual monoglycerides differing in the type of fatty acid bound to the glycerol backbone. Monoglycerides with 10 and 12 carbon atoms showed satisfactory antimicrobial activities and were further studied for the possible application in fresh juices. The addition of monoglycerides to unpasteurised apple juice resulted in a decrease in total viable counts of bacteria, yeasts, and . The monoglyceride of capric acid (MAG C10:0) was found to be more efficient of both tested substances as it was able to stop completely the growth of bacteria and yeasts at a concentration of 250 µg/ml. MAG C10:0 prevented microbial spoilage of apple juices for at least two weeks.

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