Antimicrobial Effects of Fatty Acid Fructose Esters

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Abstract

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Antimicrobial effects of various fatty acids and their esters have been extensively studied. Esters with saccharides (glucose, sucrose) have been found to have a broad spectrum of microbicidal activity. The objective of this study was to investigate the susceptibility of four microbial strains (*Bacillus cereus, Escherichia coli, Saccharomyces cerevisiae*, and *Fusarium culmorum*) to the antimicrobial properties of fatty acid (capric, lauric, myristic, and palmitic) fructose esters. Microorganisms were cultivated in liquid media supplemented with various concentrations of the tested agents. A spectrophotometric method was used for the quantitative detection of the microbial growth. Both the cultivation and measuring of the absorbance was carried out in microtiter plates. Our results indicate that the addition of the tested compounds strongly reduce the number of viable microorganisms. Higher concentrations caused microbicidal effect. The inhibitory action decreased rapidly as the chain length increased. Caprinoylfructose proved to be the most active.

Keywords: antimicrobial effects; fatty acid esters; acylfructoses; microtiter plates

At present foodstuffs without synthetic additives are preferred. One possibility how to decrease the amount of preservatives is to substitute them with compounds based on fatty acids and their derivatives. Antimicrobial effects of various fatty acids and their esters have been extensively studied in recent years. They have been found to have a broad spectrum of antimicrobial activity. These lipids are commonly found in natural products and are, therefore, likely to be non-toxic (Bergsson *et al.* 2001). Fatty acid esters with glycerol, glucose, fructose, and sucrose are used as surfactants due to their surface activity. Sugar fatty acid esters, synthesised from renewable resources such as fatty acids and carbohydrates, are biodegradable and have broad applications in the food industry. Other fields of application include cosmetics, detergents, oral-care products, and medical supplies (Ferrer *et al.* 2005).

The bactericidal activity of these lipids depends on their nature, e.g. chain length, and on the microbial strain involved. Generally, Gram-positive bacteria are more sensitive whereas Gram-negative bacteria are not (Sprong *et al.* 2001). This phenomenon may be caused by the difference between the outer membrane of the cell wall of Gram-positive and Gram-negative bacteria (Sun *et al.* 2003). Recently, however, some lipid-sensitive Gram-negative bacteria have been described. Distinct experimental conditions, such as the test medium used, pH, lipid concentrations, and probably also the microbial strains tested, may be responsible for the observed differences in lipid sensitivity of microorganisms (Sprong *et al.* 2001). The mechanism by which these lipids kill microorganisms is not known but electron microscope studies indicate that they disrupt cell membranes.

Cell membrane is the primary target of their effect (Kabara 1993; Avis & Bélanger 2001).

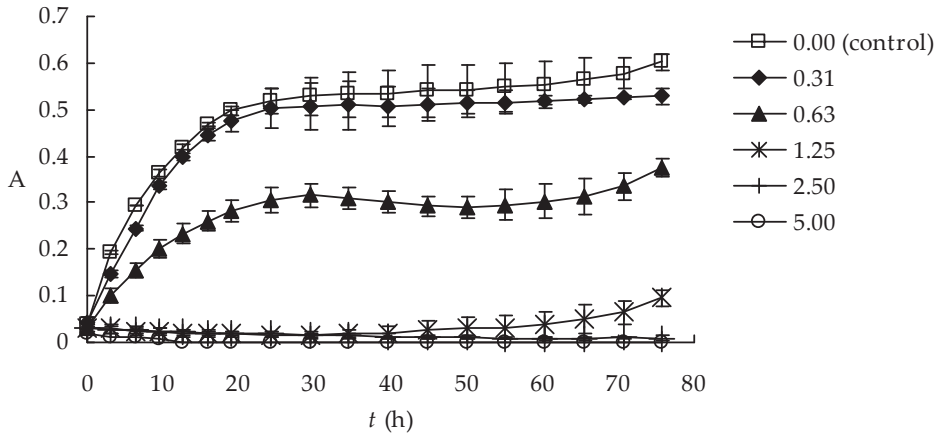
The objective of this study was to investigate the susceptibility of four microbial strains (*Bacillus cereus* DMF 2001, *Escherichia coli* DMF 7503, *Saccharomyces cerevisiae* DMF 2880 and *Fusarium culmorum* DMF 0103) to the antimicrobial properties of several fatty acid fructose esters. These microorganisms are natural contaminants in food industry.

MATERIALS AND METHODS

Microbial cultures and media. *Bacillus cereus* DMF 2001, *Escherichia coli* DMF 7503, *Saccharomyces cerevisiae* DMF 2880 and *Fusarium culmorum* DMF 0103 were used as indicator strains. Cultivation media were purchased from Oxoid Ltd. (Hampshire, UK). The strains were cultivated on slant agar (*B. cereus, E. coli* – Nutrient agar, 30°C, 24 h; *S. cerevisiae* – Malt extract agar, 30°C, 72 h; *F. culmorum* – Malt extract agar, 25°C, 7 days). Bacterial and yeast strains were inoculated into the liquid medium (*B. cereus, E. coli* – Nutrient broth, 30°C, 24 h; *S. cerevisiae* – Malt extract broth, 30°C, 72 h). Then their absorbance was modified to 0.1 (λ = 650 nm – bacteria, λ = 630 nm – yeast). Harvesting of fungal spores was done by flooding fungal culture (*F. culmorum*) after 7 days with sterile physiological saline with Tween 80 (8.5 g NaCl, 1 ml Tween 80, 1000 ml distilled water) and rubbing with a sterile inoculating loop. Spore density was determined microscopically using a Buerker counting chamber. The spore solution was diluted to obtain a 1×10^5/ml concentration of spores in the growth medium (Malt extract broth).

Chemicals. Fructose esters with fatty acids – capric (C10), lauric (C12), myristic (C14), and palmitic (C16) at concentrations from 0.31 mmol/l to 5.00 mmol/l were used. Acylfructoses were enzymaticaly synthesised at the Department of Dairy and Fat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, by the lipase-catalysed acylation of fructose. Fatty acid esters were prepared by the reverse hydrolysis in nonaqueous medium. The direct esterification of fructose and fatty acids was performed with a molar ratio of 2:1 in the presence of 2-methyl-2-butanol as organic solvent – adjuvant at 60°C using commercial immobilised lipase (Novozym 435) from *Candida antarctica* B (Šabeder *et al.* 2005). The purity of fructose fatty acid monoesters isolated via column chromatography was higher than 99% (TLC). Monoacylfructoses were dissolved in ethanol and diluted to the desired concentration. The final ethanol concentration in the growth medium was always 2% which did not interfere with viability of the microbial strains. Stock solutions of fatty acid esters were diluted to the desired concentration in the appropriate liquid media (*B. cereus, E. coli* – Nutrient broth; *S. cerevisiae, F. culmorum* – Malt extract broth).

Assay of antimicrobial activity. A spectrophotometric method, using an automatic cultivator/reader PowerWave XS (Bio Tek Instruments, Winooski, USA) was used for the quantitative detection of the microbial growth. Microorganisms



Control is without the inhibitory compound; A – absorbance of the sample with microbial inoculum reduced by that of the blank determination (λ = 650 nm); *t* – time of incubation

Figure 1. Growth curves of *Bacillus cereus* in the presence of various concentrations (mmol/l) of caprinoylfructose

were cultivated in liquid media supplemented with various concentrations (0.31, 0.63, 1.25, 2.50, and 5.00 mmol/l) of the tested compounds. Inoculum or spore suspension was added to flasks containing liquid media with different concentrations of the tested agents. Sterile 96-well microtitrate plates were filled from these flasks (200 µl in each well). Medium without any addition of acylfructoses was used as a control. Liquid media with the addition of the tested compound but without the addition of microbial inoculum served as a blank. After substraction its absorbance from the absorbance of the samples, where the inoculum was added, absorbance of pure microorganisms was obtained. After shaking the plates for 10 s, the absorbance (λ = 650 nm – *B. cereus, E. coli*, λ = 630 nm – *S. cerevisiae, F. culmorum*) was determined every 3 h during the first 24 h of incubation and then every 5 h during the rest of the 72 h in case of *B. cereus, E. coli* and every 24 h during 240 h of cultivation in case of *S. cerevisiae* and *F. culmorum*. All the analyses were performed in triplicate and the experimental data represent the average of them. Growth curves of the tested strains that were cultivated with antimicrobial agents were obtained. The integrals of the curves were then counted and compared with the control being without the addition of inhibitory compounds. To sum up the results Inhibitory index (II) was used (Eq. 1):

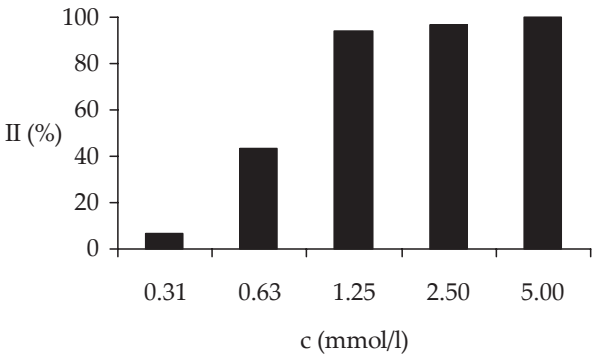


Figure 2. The inhibitory index (II) of *Bacillus cereus* in the presence of various concentrations (mmol/l) of caprinoylfructose (Inhibitory index was calculated from Eq. 1, the respective areas being evaluated from Figure 1)

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

where:

A_{sample} – area under growth curves of the treated samples

$A_{control}$ – area under growth curves of the untreated samples

RESULTS AND DISCUSSION

Our results indicate that the addition of the tested compounds reduce the number of viable microorganisms. Acylfructoses caused stagna-

Bacillus cereus DMF 2001					
Compound	*c* (mmol/l)				
	0.31	0.63	1.25	2.50	5.00
Caprinoylfructose	**6.4**	**43.6**	**93.7**	**96.9**	**100.0**
Lauroylfructose	**64.3**	**73.8**	**74.6**	**84.8**	**87.7**
Myristoylfructose	**43.8**	**46.8**	**67.2**	**84.7**	**86.2**
Palmitoylfructose	**–34.7**	**–13.9**	**–7.6**	**–1.7**	**2.1**

Saccharomyces cerevisiae DMF 2880					
Compound	*c* (mmol/l)				
	0.31	0.63	1.25	2.50	5.00
Caprinoylfructose	**63.3**	**71.9**	**76.9**	**78.5**	**88.3**
Lauroylfructose	**9.5**	**16.7**	**19.2**	**19.5**	**23.8**
Myristoylfructose	**1.0**	**5.2**	**8.8**	**10.0**	**18.3**
Palmitoylfructose	**1.2**	**1.2**	**–3.5**	**–9.4**	**22.3**

Escherichia coli DMF 7503					
Compound	*c* (mmol/l)				
	0.31	0.63	1.25	2.50	5.00
Caprinoylfructose	**16.8**	**23.3**	**26.4**	**45.7**	**51.7**
Lauroylfructose	**6.1**	**25.5**	**27.7**	**28.6**	**31.2**
Myristoylfructose	**7.8**	**19.6**	**27.9**	**28.3**	**31.9**
Palmitoylfructose	**11.1**	**13.0**	**15.2**	**24.4**	**38.3**

Fusarium culmorum DMF 0103					
Compound	*c* (mmol/l)				
	0.31	0.63	1.25	2.50	5.00
Caprinoylfructose	**7.0**	**15.8**	**99.2**	**100.0**	**100.0**
Lauroylfructose	**7.4**	**13.5**	**18.4**	**23.0**	**81.7**
Myristoylfructose	**21.3**	**38.4**	**63.4**	**80.1**	**99.0**
Palmitoylfructose	**9.0**	**19.7**	**26.1**	**28.0**	**50.4**

Higher growth	Inhibition (%)					
0–20%	0–10	10–25	25–50	50–90	> 90	100

Inhibitory effect of every fructose ester and microorganism was expressed by means of the Inhibitory index (II) calculated from Eq. 1

Figure 3. Inhibitory effects of all tested compounds used at different concentrations on tested microbial strains

tion of the lag-period of the bacterial growth. No microbial growth was detected when higher concentrations of caprinoylfructose were used (Figure 1). Percentual inhibition effect for every concentration was obtained (Figure 2). Figure 3 shows the efficiency of all compounds used at different concentrations on the tested microbial strains. The darker the colour the higher was the inhibition effect. Caprinoylfructose showed the highest antimicrobial activity. On the other hand palmitoylfructose increased the growth of some microorganisms. This can be explained by the fact that esters of long chain fatty acids may serve as a carbon source for some microorganisms.

CONCLUSION

In the present work antimicrobial properties of several fatty acid fructose esters (caprinoylfructose, lauroylfructose, myristoylfructose, and palmitoylfructose) against *Bacillus cereus* DMF 2001, *Escherichia coli* DMF 7503, *Saccharomyces cerevisiae* DMF 2880 and *Fusarium culmorum* DMF 0103 were studied. Caprinoylfructose showed the highest antimicrobial activity. The inhibitory effect decreased rapidly as the chain length increased. Further investigations are in purpose in order to validate these results in real systems.

References

Avis T.J., Bélanger R.R. (2001): Specificity and mode of action of the antifungal fatty acid *cis*-9-heptadecenoic acid produced by *Pseudozyma flocculosa*. Apllied and Environmental Microbiology, **67**: 956–960.

Bergsson G., Arnfinnsson J., Steingrímsson Ó., Thormar H. (2001): *In vitro* killing of *Candida albicans* by fatty acids and monoglycerides. Antimicrobial Agents and Chemotherapy, **45**: 3209–3212.

Ferrer M., Soliveri J., Plou F.J., López-Cortés N., Reyes-Duarte D., Christensen M., Copa-Patiño J.L., Ballesteros A. (2005): Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosum* and *Candida antarctica* B, and their antimicrobial properties. Enzyme and Microbial Technology, **36**: 391–398.

Kabara J.J. (1993): Antimicrobials in Food. Marcel Dekker, New York: 307–341.

Sprong R.C., Hulstein M.F.E., Van der Meer R. (2001): Bactericidal activities of milk lipids. Antimicrobial Agents and Chemotherapy, **45**: 1298–1301.

Sun C.Q., O'Connor C.J., Roberton A.M. (2003): Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. FEMS Immunology and Medical Microbiology, **36:** 9–17.

Šabeder S., Habulin M., Knez Ž. (2005): Lipase-catalyzed synthesis of fatty acid fructose esters. Journal of Food Engineering, **77**: 880–886.

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