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Comparison of Anti-*Vibrio* Activities of Potassium Sorbate, Sodium Benzoate, and Glycerol and Sucrose Esters of Fatty Acids

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The effects of fatty acids and their glycerol and sucrose esters, potassium sorbate, and sodium benzoate on growth of *Vibrio parahaemolyticus* in laboratory media at pH 6.7 were evaluated. The minimum concentrations at which inhibition by esters of glycerol could be detected were lowest for monolaurin (5 μg/ml) and monocaprin (40 μg/ml); these concentrations were lower than those observed for inhibition by lauric and capric acids, respectively. Inhibitory action of sucrose caprylate was detected at 40 μg/ml, whereas sucrose caprate was effective at 100 μg/ml; sucrose esters of lauric, myristic, and palmitic acids were ineffective at 100 μg/ml. Potassium sorbate and sodium benzoate inhibited growth at concentrations as low as 30 and 300 μg/ml, respectively, and enhanced the rate of thermal inactivation of *V. parahaemolyticus* at slightly higher concentrations. Fatty acid esters of glycerol and sucrose offer potential as perservatives for slightly acid or alkaline low-fat foods which do not lend themselves to the full antimicrobial action of traditional food preservatives such as potassium sorbate and sodium benzoate.

The antibacterial and antifungal properties of fatty acids have been studied extensively. Generally, the most active chain length for saturated fatty acids is C_{12}, whereas the most active monounsaturated fatty acid is $C_{16:1}$, and the most active polyunsaturated fatty acid is $C_{18:2}$ (4). The antimicrobial activity of lauric acid (C_{12}) can be enhanced when esterified to a variety of polyhydric alcohols. Several reports indicate that glycerol monolaurate may be inhibitory only to gram-positive microorganisms (2, 5, 8); however, other studies (7, 10, 11, 14) have shown that glyerol esters of capric and lauric acids have strong activity against gram-negative bacteria, e.g., *Escherichia coli, Serratia marcescens, Proteus vulgaris, Salmonella typhimurium,* and *Pseudomonas aeruginosa,* when in the presence of citric or phosphoric acids.

The sucrose ester of lauric acid is reported to inhibit the growth of *E. coli* to a greater extent than lauric acid itself (6). Sucrose dicaprylate and sucrose monolaurate have been demonstrated to be active against gram-negative and -positive bacteria, as well as fungi (7, 8). However, other workers (2) failed to demonstrate inhibition by lauric acid or its sucrose derivatives against *E. coli* or other gram-negative organisms.

Salts of sorbic and benzoic acids are well established as additives for preserving foods and beverages. Both acids are most effective in undissociated form, and, on a weight-to-weight basis, sorbate is generally more effective than is benzoate. Since the pK_a of sorbic acid is 4.76 compared to 4.19 for benzoic acid, the preservative action of sorbic acid can be demonstrated at higher pH. *Pseudomonas* species, largely responsible for spoilage of refrigerated fresh seafoods, meats, and dairy products, are susceptible to the action of sorbate (3, 12). These foods have a pH near neutrality. From a public health standpoint, the presence of *Vibrio parahaemolyticus* on seafoods is of particular concern. The use of sorbic acid to retard the rate of growth of this bacterium in crabmeat and flounder adjusted to pH 6.2 has been reported (13).

The development and use of safe antimicrobial preservatives in foods continue to be of great interest to the processing industry. The present study was designed to evaluate the effectiveness of fatty acid esters of glycerol and sucrose to control the growth of *V. parahaemolyticus,* and to compare these derivatives to the traditional food preservatives, potassium sorbate and sodium benzoate. The effects of these compounds on the rate of thermal inactivation of *V. parahaemolyticus* was also studied.

MATERIALS AND METHODS

Source of compounds. Fatty acids and their esters of glycerol were purchased from Sigma Chemical Co., St. Louis, Mo. Caprylic (C_8), capric (C_{10}), lauric (C_{12}),

and myristic (C_{14}) acids and their monoesters (monocaprylin, monocaprin, monolaurin, and monomyristin, respectively) were evaluated. Sucrose esters (caprylate, caprate, laurate, myristate, and palmitate [C_{16}]) were a gift from Ryoto Co. Ltd., Tokyo, Japan. The purity of fatty acids was >95%; however, the ratio of mono-, di-, and triesters varied considerably among the test compounds (Table 1). Food grade potassium sorbate (Monsanto Industrial Chemicals Co., St. Louis, Mo.) and sodium benzoate (Pfizer Inc., New York, N.Y.) were used throughout the study.

Preparation of solutions. Fatty acids and their esters of glycerol and sucrose were dissolved in 95% ethanol whereas potassium sorbate and sodium benzoate were dissolved in water. All of these solutions were filter-sterilized and added to broth immediately before initiating tests. Control tests were carried out using 95% ethanol not containing fatty acids or derivatives.

Conditions for culturing. *V. parahaemolyticus* 8700 (O4:K11, Kanagawa positive) was cultured at 30°C in tryptic soy broth containing 3% NaCl (TSBS, pH 7.0). A 16- to 18-h culture was diluted 1:100 in 0.1 M potassium phosphate buffer containing 3% NaCl (salt buffer, pH 7.0), and 1 ml was inoculated into 100 ml of TSBS (pH 6.7) (250-ml flask) to which test solutions had been added. Cultures were continuously agitated at 30°C on a rotary shaker (150 rpm, 4-cm radius). Aliquots were withdrawn over a period of 9 h, and absorbancy at 620 nm was determined at 20-min intervals. An arbitrary scheme was established for designating the degree of inhibition of growth by test chemicals (Table 2). Since the addition of ethanol alone caused some lag in growth compared to TSBS containing no added solution, an arithmetic difference of <6 between the percentage transmittance of control and test cultures during the period of 4.5 to 7.5 h post-inoculation was interpreted as no inhibition.

Thermal inactivation studies. One milliliter of diluted cell suspension was transferred to 100 ml of TSBS (47°C), with and without test compounds. Samples were withdrawn over a 3-h period of continuous agitation in a constant-temperature (47°C) water bath, diluted in salt buffer, and surface-plated (0.1 ml) on thiosulfate-citrate-bile salts-sucrose agar. Colonies were counted after 20 to 24 h, and viable population per milliliter of heating broth versus time was plotted on a semi-log graph. The decimal reduction times at 47°C (D_{47} = time in minutes at 47°C required to reduce the viable population by 90% or one log cycle) were then calculated for *V. parahaemolyticus* exposed to various concentrations of test compounds. Data represent means of three or more independent trials.

RESULTS

Fatty acids and their glycerol esters. The minimum inhibitory concentrations (MICs) for fatty acids and their glycerol esters toward *V. parahaemolyticus* are shown in Table 3. Caprylic acid and monocaprylin were least effective, with MICs of 100 μg/ml. Capric and myristic acids were slightly more inhibitory (MIC = 60 μg/ml); however, monocaprin had greater activity whereas monomyristin had less activity compared to respective free fatty acids. Lauric acid had the same inhibitory action as did capric and

TABLE 1. *Composition of sucrose esters*

Compound[a]	Composition of sucrose ester (%)		
	Mono-	Di-	Tri-
Sucrose caprylate	31	43	26
Sucrose caprate	27	40	33
Sucrose laurate	79	19	2
Sucrose myristate	76	21	3
Sucrose palmitate	72	23	4

[a] Purity of fatty acids was >95%. According to Ryoto Co. Ltd., Tokyo, Japan.

TABLE 2. *Arbitrary system for designating the degree of inhibition of growth of V. parahaemolyticus*

Arithmetic difference[a]	Degree of inhibition	Sign designation
0–5	None	−
6–20	Slight	+
21–50	Moderate	++
51–90	Great	+++
>90	Total	++++

[a] Maximum difference between percentage transmittance of control and test cultures after 4.5 to 7.5 h of incubation.

TABLE 3. *Inhibitory effects of fatty acids and their monoesters of glycerol on growth of V. parahaemolyticus*

Compound	Degree of inhibition[a] at:					
	5[b]	10	20	40	60	100
Caprylic acid	ND[c]	ND	−	−	−	+
Monocaprylin	ND	ND	−	−	−	+
Capric acid	ND	ND	−	−	+	+
Monocaprin	−	−	−	+	+	++++
Lauric acid	ND	ND	−	−	+	++
Monolaurin	+	++	++++	++++	++++	++++
Myristic acid	ND	ND	−	−	+	+
Monomyristin	ND	ND	−	−	−	+

[a] See Table 2.
[b] Concentration in micrograms per milliliter.
[c] ND, Not determined.

myristic acids but monolaurin was inhibitory to *V. parahaemolyticus* at concentrations as low as 5 μg/ml.

Sucrose esters. The sucrose esters tested are listed in Table 4. At 100 μg/ml, esters of lauric, myristic, and palmitic acids had no inhibitory effect on rate of growth of *V. parahaemolyticus*. Activity was observed for sucrose caprate (MIC = 100 μg/ml) and sucrose caprylate (MIC = 40 μg/ml). Compared to free acids (Table 3), sucrose esters of lauric and myristic acids were less active whereas sucrose caprylate exhibited markedly higher inhibition.

Traditional food preservatives. Table 5 lists the results using potassium sorbate, sorbic acid, and sodium benzoate. Potassium sorbate was more inhibitory (MIC = 30 μg/ml) than was sorbic acid (MIC = 70 μg/ml), probably due to the enhanced solubility of the salt form. Of the three compounds evaluated, sodium benzoate was least inhibitory (MIC = 300 μg/ml).

Combinations of compounds. The inhibitory effects of monolaurin and sucrose caprylate combined with potassium sorbate and sodium benzoate are summarized in Table 6. Comparison of these data with those obtained from experiments designed to evaluate each compound independently (Tables 3, 4, and 5) indicates that these fatty acid esters of glycerol and sucrose do not appear to act synergistically with potassium sorbate or sodium benzoate to inhibit the growth of *V. parahaemolyticus*. There was no additive affect.

Thermal inactivation studies. The D_{47} values for *V. parahaemolyticus* heated in TSBS with and without added potassium sorbate and sodium benzoate are listed in Table 7. The presence of 50 μg of potassium sorbate per ml resulted in a significant ($P < 0.05$) reduction in the thermal death time. Although a significant decrease in D_{47} value was not observed in TSBS containing 200 μg of sodium benzoate per ml compared to the control, a significant decrease in the time required to inactivate 90% of the cells was noted in TSBS containing 500 μg/ml. Results from tests conducted to determine and compare the effects of glycerol and sucrose esters on thermal inactivation rates were inconclusive due to the influencing effect of ethanol. These data are not presented.

TABLE 4. *Inhibitory effects of fatty acid esters of sucrose on growth of V. parahaemolyticus*

Compound	Degree of inhibition[a] at:			
	20[b]	40	60	100
Sucrose caprylate	−	++	++	++++
Sucrose caprate	−	−	−	++
Sucrose laurate	−	−	−	−
Sucrose myristate	−	−	−	−
Sucrose palmitate	−	−	−	−

[a] See Table. 2.
[b] Concentration in micrograms per milliliter.

TABLE 5. *Inhibitory effects of common food preservatives on growth of V. parahaemolyticus*

Compound	Degree of inhibition[a] at:									
	10[b]	20	30	40	50	70	100	300	600	1,000
Potassium sorbate	−	−	+	+	++	++	++	+++	+++	+++
Sorbic acid	−	−	−	−	−	+	++	+++	ND	ND
Sodium benzoate	ND[c]	ND	ND	ND	ND	−	−	+	++	+++

[a] See Table 2.
[b] Concentration in micrograms per milliliter.
[c] ND, Not determined.

TABLE 6. *Inhibitory effects of combinations of monolaurin, sucrose caprylate, and common food preservatives on growth of V. parahaemolyticus*

Compound	Concn (μg/ml)	Degree of inhibition[a] when combined with:					
		Potassium sorbate at:			Sodium benzoate at:		
		20[b]	50	100	100[b]	300	600
Monolaurin	4	+	++	+++	−	+	++
	8	+	++	+++	+	+	++
	12	++	++	+++	++	++	++
Sucrose caprylate	20	−	+	++	−	+	++
	40	++	++	++	+	++	++
	60	++	++	++	++	++	++

[a] See Table 2.
[b] Concentration in micrograms per milliliter.

TABLE 7. *Effects of common food preservatives on decimal reduction times at 47°C (D_{47} values) for V. parahaemolyticus*

Compound	Concn (μg/ml)	D_{47} value[a] (min)
Potassium sorbate	0	25.3 a
	20	23.3 a
	50	16.3 b
	100	12.0 b
Sodium benzoate	0	26.0 a
	200	23.3 ab
	500	16.7 bc
	1000	14.0 c

[a] In comparing D_{47} values within each test compound, values not followed by the same letter are significantly different ($P \leq 0.05$).

DISCUSSION

The inhibition of growth of *V. parahaemolyticus* by fatty acids in the C_8 to C_{14} range is not too surprising in light of existing data on the bactericidal activities of several of these fatty acids on other gram-negative bacteria. The stronger anti-*Vibrio* activity of monocaprin and, especially, monolaurin confirms reports by Kato and Shibasaki (7, 10) that fatty acids esters of glycerol do exhibit retarding effects on gram-negative bacteria. These researchers demonstrated that citric and polyphosphoric acids had an enhancing effect on the antibacterial action of monocaprin and monolaurin against several gram-negative bacterial species. They concluded that in *E. coli* a significant amount of lipopolysaccharide from cell walls was released by treatment with acids, thus stimulating the transport of monolaurin into the cell. In another study conducted by the same workers (8), monocaprin and monolaurin were reported to have little or no bacteriostatic activity toward gram-negative bacteria when tested in media not containing added citric or polyphosphoric acids. Kabara and associates (2, 5) tend to support the latter study, reporting that various fatty acid esters of glycerol, if active at all, are inhibitory toward gram-positive but not gram-negative bacteria. The positive responses of *V. parahaemolyticus*, a marine bacterium with considerable tolerance to salt (up to 10%) and intolerance to acid conditions (1), to monocaprin and monolaurin at concentrations less than that of respective free fatty acids and in a culturing medium at pH 6.7, establishes a potential for use of these compounds as preservatives in low-fat foods with slightly acidic or perhaps alkaline pH. The presence of citric or polyphosphoric acids does not appear to be a requisite for demonstrating inhibitory activity of esters of fatty acids and glycerol against all gram-negative bacteria.

Results from tests designed to evaluate sucrose esters confirm a report (6) indicating that such compounds do inhibit the growth of gram-negative bacteria. Sucrose laurate was inhibitory to *E. coli* at 100 μg/ml. However, of the esters evaluated here, increased activity was correlated with decreased carbon number in the fatty acid, i.e., the MIC was lowest for sucrose caprylate, higher for sucrose caprate, and not detected (>100 μg/ml) for the remaining sucrose esters tested. Relative activity may be associated with the ratio and relative amounts of mono-, di-, and triesters as well as chain length. Sucrose dicaprylate has been shown to be inhibitory to *E. coli* whereas sucrose monocaprylate was not (7). A higher percentage of di- and triesters was present in sucrose derivatives of caprylic and capric acids compared to lauric, myristic, and palmitic acids evaluated in the present study (Table 1). Conley and Kabara (2) failed to demonstrate inhibitory activity ofsucrose esters of fatty acids ($\geq C_{12}$) against gram-negative bacteria.

Positive effects of potassium sorbate were noted at concentration as low as 30 μg/ml. This was somewhat surprising, since the pH of the broth used to test the compound was 6.7, the approximate maximum for the active, undissociated form. The relatively reduced or nonexistent effect of sorbic acid at comparable concentrations was probably due to lower solubility in water. The sensitivity of *V. parahaemolyticus* may be greater in liquid laboratory media than in crabmeat and flounder. Robach and Hickey (13) reported that 500 μg of potassium sorbate per g delayed initiation of growth of three strains of *V. parahaemolyticus* (including strain 8700) in seafood homogenates.

The lack of synergistic effects of monolaurin and sucrose caprylate in combination with potassium sorbate or sodium benzoate on *V. parahaemolyticus* may indicate that the mechanism of action of each of these compounds is different. Results from experiments reported here do not indicate an additive affect.

It was hoped that a comparison could be made among various test compounds with respect to their effects on thermal inactivation of *V. parahaemolyticus*. Kato and Shibasaki (9) showed that monocaprin and monolaurin had a strong enhancing effect on the thermal destruction of *E. coli* and *P. aeruginosa*. Because *V. parahaemolyticus* was extremely sensitive to ethanol at elevated temperatures, we were not able to carry out experiments using fatty acid esters of glycerol and sucrose. Although sucrose esters are soluble in water and could have been tested in this manner, previous growth studies involved the use of ethanol as a carrier to facilitate com-

parison with glycerol esters. The traditional food preservatives, however, definitely enhanced thermal inactivation of *V. parahaemolyticus*.

In summary, the inhibitory effects of several fatty acid esters of glycerol and sucrose on growth of a gram-negative bacterium, *V. parahaemolyticus*, have been demonstrated. At pH 6.7, the effects of monolaurin, monocaprin, and sucrose monocaprylate are substantial, approaching the level of action exhibited by potassium sorbate and exceeding the extent of activity of sodium benzoate at similar concentrations. Fatty acid esters of glycerol and sucrose offer potential as microbial inhibitors for slightly acid or alkaline low-fat foods which may not lend themselves to the full preservative action of potassium sorbate or sodium benzoate. The antimicrobial effectiveness of esters as influenced by lipid components in foods would appear to be a serious drawback to their use on a practical scale, but additional research needs to be conducted to confirm this suspicion.

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