Inhibition of *Listeria monocytogenes* by Monoacylglycerols Synthesized from Coconut Oil and Milkfat by Lipase-Catalyzed Glycerolysis

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Monoacylglycerols (MAGs) synthesized from coconut oil and milkfat were evaluated in brain heart infusion (BHI) broth and in pasteurized milk for antimicrobial activity against the Scott A strain of *Listeria monocytogenes*. MAGs were produced by solid-phase glycerolysis catalyzed by lipase PS-30 from *Pseudomonas* sp. and purified by hexane fractionation. The purities of MAGs from coconut oil and milkfat before hexane fractionation were 74.6% and 54.5% and after were 97.5% and 95.7%, respectively. Fractionated coconut MAGs were slightly more inhibitory against *L. monocytogenes* than unfractionated MAGs. *L. monocytogenes* was inactivated by hexane-fractionated coconut MAGs at 250–400 μg/mL in pasteurized skim, at 500–750 μg/mL in 2% milk, and at 750–1000 μg/mL in whole milk at 4 °C, but the MAGs were less inhibitory at 13 °C and at room temperature. MAGs prepared from coconut oil were more effective than monolaurin against *L. monocytogenes* in BHI and pasteurized milk, whereas MAGs made from milkfat did not inhibit *L. monocytogenes* in pasteurized milk. The composition of fatty acids present in the coconut MAGs was associated with the anti-listerial activity, and lauric acid (C_{12}) was the most active fatty acid of the series C_8–C_{14}. Certain combinations of the MAGs, particularly monocaprin and monolaurin, showed synergistic activity against *L. monocytogenes*. Our results indicate that MAGs synthesized from coconut oil could be used to control *L. monocytogenes* in certain dairy products or in other foods that contain reduced fat.

INTRODUCTION

Monoacylglycerols (MAGs) are commonly used as emulsifying agents in various foods (Krog, 1990). Certain naturally occurring MAGs have also been reported to inhibit Gram-positive bacteria, viruses, yeast, fungi, and tumor cells (Kato et al., 1967; Shibasaki and Kato, 1978; Chipley et al., 1981; Kato, 1981; Welsh et al., 1981; Thormar et al., 1987). Considerable attention has recently been directed to improving methods of MAG synthesis for use as surfactants (Holmberg and Osterberg, 1988; McNeill et al., 1990, 1991a,b), but little attention has been given to evaluation of synthesized MAGs specifically for antimicrobial properties. Prospective advantages of using lipases in synthesis of MAGs as antimicrobial agents include incorporation of desired fatty acids and positional selectivity of lipases.

Monolaurin has been investigated extensively as an antimicrobial agent for foods and cosmetics (Shibasaki and Kato, 1978; Kabara, 1984). Other MAGs including monocaprin and monocaprylin were also reported to have antimicrobial activity in certain foods (Shibasaki and Kato, 1978). Coconut oil contains 90% saturated fatty acids, and of these, 45–48% are lauric acid and 30–36% are other short- and medium-chain fatty acids which would be expected to have antimicrobial activity. Thus, coconut oil is a potentially interesting substrate for synthesis of antimicrobial MAGs.

Listeria monocytogenes continues to be a pathogen of concern in the dairy industry. This pathogen can survive at low pHs and high salt concentrations (Farber and Peterkin, 1991) and can grow in a variety of dairy products held at low temperatures. Outbreaks of listeriosis have been associated with the consumption of dairy products (Fleming et al., 1985; James et al., 1985; Farber and Peterkin, 1991; Schuchat et al., 1992). In a previous study, we found that *L. monocytogenes* in autoclaved skim milk was sensitive to killing by monolaurin, particularly at low temperatures (Wang and Johnson, 1992). In this study, MAGs synthesized by enzymatic glycerolysis of coconut oil and milkfat were evaluated for anti-listerial activity.

MATERIALS AND METHODS

Enzymatic Preparation of MAGs. Commercial lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) from *Pseudomonas fluorescens* (type PS-30, 33.8 units/mg) was purchased from Amano International Enzyme Co. (Troy, VA). Milkfat (anhydrous) was obtained from Level Valley Dairy (West Bend, WI). All solvents (HPLC grade) and formic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Coconut oil, glycerol, fatty acids (>99% pure), and lipase from *Candida cylindracea* (1100 units/mg) were obtained from Sigma Chemical Co. (St. Louis, MO).

For production of MAGs (Yang et al., 1993), a mixture of 20 mL of melted milkfat or coconut oil, 8 g of glycerol, and 160 μL of water was combined in a tightly capped 125-mL Erlenmeyer flask. The reaction mixtures were preincubated at 35 °C in a rotary G-24 incubator shaker (New Brunswick Scientific Co., Edison, NJ) with constant shaking at 300 rpm until the contents reached 35 °C. The reaction was initiated by adding 250 mg of lipase powder and was carried out for 24 h at 35 °C, and then the temperature was decreased to 25 °C for 3–4 days. The product mixture was dissolved in chloroform and then passed through a funnel containing sodium sulfate and the filter paper (Whatman No. 1) to remove the lipase powder and excess glycerol. The products were concentrated by solvent evaporation.

Solvent Fractionation and HPLC Analysis. The product mixture was suspended in hexane at room temperature to fractionate MAGs (which remained insoluble) from soluble diacylglycerols (DAGs), free fatty acids (FFAs), and triacylglycerols (TAGs). The glycerolysis products before and after solvent fractionation were analyzed by normal-phase HPLC on a system consisting of two Waters 501 pumps, a Varex ELASD IIA laser light-scattering mass detector (Varex Corp., Burtonsville,

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MD), and an Econosil silica column (250 mm × 4.6 mm i.d., 5-μm particle size) obtained from Alltech Associates, Inc. (Arlington Heights, IL). The column temperature was controlled at 30 °C with a column heater. Mobil phase A consisted of hexane/chloroform/formic acid (60:40:0.2 v/v), and mobile phase B consisted of hexane/acetone/chloroform (25:35:65 v/v) (Yang and Chen, 1991). The flow rate was 1.5 mL/min. Samples were dissolved in chloroform prior to injection. The content of MAGs was expressed on a mass basis in terms of peak area percentage in the sample mixture.

Free Fatty Acid Analysis of MAGs. Fatty acid profiles of MAGs made from milkfat and coconut oil were determined by HPLC according to the method of Garcia et al. (1990) with slight modification. Samples analyzed included MAGs prepared from milkfat and purified by solvent fractionation and MAGs prepared from coconut oil before and after solvent fractionation. Fifty milligrams of MAGs was hydrolyzed with 50 mg of lipase from *C. cylindracea* in 2 mL of sodium phosphate buffer (pH 7.4) with overnight shaking at 22–25 °C. The liberated fatty acids were extracted three times with ethyl ether (3 mL) and dried over sodium sulfate. The ether extract was filtered through a 0.45-μm nylon membrane, and the fatty acids were derivatized to form *p*-bromophenacyl esters. A sample (15 μL) was loaded onto an RP-18 column (Lichrosorb, RP-18, 250 mm × 4.6 mm i.d., 5-μm spherical packing) and chromatographed using a flow rate of 1 mL/min and a constant temperature of 30 °C. The absorbance of the eluting stream was monitored at 254 nm. Program control and data acquisition were performed using Baseline 810 software (Waters Associates, Milford, MA). The relative concentrations of free fatty acids were expressed as percentage of peak area. The values reported are the mean of duplicate determinations.

Assay of Antimicrobial Activity. *L. monocytogenes* strain Scott A was used in this study. Stock cultures were maintained by monthly transfers on brain heart infusion (BHI) agar slants (Difco Laboratories, Detroit, MI) and stored at 4 °C. Cells from BHI agar slants were subcultured in BHI broth in test tubes (1.0 × 12.5 cm) that were incubated at 37 °C for 18 h without shaking. One loopful was inoculated into 10 mL of BHI broth. This was then used to inoculate BHI broth (pH 6.0) that contained 99% pure monoglycerides from Sigma and MAGs synthesized by glycerolysis from coconut oil and milkfat. The initial population of *L. monocytogenes* was 10^3–10^4 colony forming units (cfu)/mL. *L. monocytogenes* was enumerated by plating dilutions on duplicate BHI agar plates each day for 6 days. The minimum listericidal concentration of monoglycerides was defined in this study as the lowest concentration at which no colonies grew on BHI agar after 2 days of incubation.

For inhibition tests in milk, tubes containing 10 mL of pasteurized whole, 2%, or skim milk (obtained from the University of Wisconsin—Madison dairy plant) were used. Monocaprylin (MC_8), monocaprin (MC_{10}), monolaurin (MC_{12}), monomyristin (MC_{14}), monopalmitin (MC_{16}), monostearin (MC_{18}), and monoolein ($MC_{18:1}$) (each >99% pure) (Sigma) and lyophilyzed MAGs made from coconut oil and milkfat were dissolved in absolute ethanol and added to the milk to give final concentrations of 250, 500, or 750 μg/mL (final concentrations of ethanol were 0.5%, 1%, or 2%). The milk tubes were inoculated with 1000–3000 cfu/mL of *L. monocytogenes* Scott A and were incubated at 4 or 13 °C or room temperature (23 °C). *L. monocytogenes* was enumerated by plating on duplicate modified Oxford agar plates (MOX) (Curtis et al., 1989) which were incubated at 37 °C for 48 h. Enrichments were also performed by the USDA/FSIS method when direct counts were not obtained on MOX agar. All experiments were performed in duplicate and were replicated at least once.

RESULTS

Glycerolysis Products and Fatty Acid Compositions. Milk and coconut MAGs were produced by solid-phase glycerolysis catalyzed by lipase PS-30 from *P. fluorescens*. The composition of glycerolysis products including FFAs, MAGs, DAGs, and TAGs was analyzed by normal-phase HPLC (Table I). The contents of MAGs from coconut oil and milkfat before hexane fractionation were 74.6% and 54.5% and after were 97.5% and 95.7%, respectively. As a result of fractionation, the contents of coconut and milk DAGs decreased from 17.0% and 27.8% to 2.4% and 3.8%, respectively. TAGs or FFAs were decreased to less than 0.5%. Therefore, hexane fractionation effectively removed most of the FFAs, DAGs, and TAGs from MAGs.

Table I. Composition of Glycerolysis Products from Coconut Oil and Milkfat before and after Hexane Fractionation Determined by HPLC Analysis[a]

sample	composition (% wt)			
	TAGs[a]	FFAs	DAGs	MAGs
coconut (NF)[b]	7.6	0.8	17.0	74.6
conconut (F)	0.1	nd[c]	2.4	97.5
milkfat (NF)	14.4	3.3	27.8	54.5
milkfat (F)	0.5	nd	3.8	95.7

[a] Values are means from duplicate samples from independent analyses. [b] NF; nonfractionated; F, fractionated. [c] nd, not detected.

Table II. Fatty Acid Composition of MAGs Synthesized with Lipase PS-30 from Coconut Oil and Milkfat

major free fatty acid[a]	composition (%)		
	coconut (NF)[b]	coconut (F)[b]	milk (F)
C_4	nd[c]	nd	2.2
C_6	0.5	3.6	1.0
C_8	12.1	2.9	0.5
C_{10}	9.3	4.5	2.1
C_{12}	45.5	41.6	4.0
C_{14}	14.3	24.7	17.8
C_{16} + $C_{18:1}$	15.1	16.9	52.2
C_{18}	2.7	5.0	18.2
$C_{18:2}$	0.5	0.8	2.0

[a] C_4, butanoic; C_6, hexanoic; C_8, octanoic; C_{10}, decanoic; C_{12}, dodecanoic; C_{14}, tetradecanoic; C_{16}; hexadecanoic; C_{18}, octadecanoic; $C_{18:1}$, *cis*-9-octadecenoic; $C_{18:2}$, *cis*-9,12-octadecadienoic acids. [b] F, fractionated; NF, not fractionated. [c] nd, not detected. [d] Values are means of duplicate samples from two independent determinations.

Fatty acid compositions of glycerolysis products before and after fractionation were determined by HPLC (Table II). The fatty acid compositions of coconut oil and milkfat after glycerolysis and without hexane fractionation were similar to the natural fatty acid compositions of coconut oil and milkfat. However, hexane fractionation resulted in differences in the distribution of certain fatty acids. After hexane fractionation, coconut glycerolysis products contained 95% MAGs with higher contents of myristic (C_{14}) and stearic acids ($C_{18:0}$) and lower contents of caprylic (C_8) and capric acids (C_{10}) than the glycerolysis products without fractionation (Table II). Similar changes in fatty acid compositions were also observed in milkfat glycerolysis products due to selective enrichment by solvent fractionation of MAGs with the resulting fatty acid composition.

In Vitro Inhibition of *L. monocytogenes* by MAGs. Individual MAGs (99% pure) including MC_8, MC_{10}, MC_{12}, and MC_{14} obtained commercially and MAG mixtures synthesized from coconut oil and milkfat were tested singly in BHI broth at pH 6 for inactivation or inhibition of *L. monocytogenes*. Of the MAGs screened, several inactivated *L. monocytogenes*, including MC_{10}, MC_{12}, MC_{14}, and fractionated coconut MAGs at concentrations of 75, 25, 50, and 10 μg/mL, respectively (Table III). Among the MAGs of the series MC_8–MC_{14}, MC_{12} was most inhibitory against *L. monocytogenes*. MC_8 was the most water-soluble among these compounds tested and could be used at a high concentration (≤300 μg/mL) without causing turbidity in the culture media, but it had only a bacteriostatic effect at 300 μg/mL. In contrast, MC_{14} was the least water-soluble and when used at concentrations greater than 100 μg/mL, it caused turbidity in the media,

Table III. Inhibition of *L. monocytogenes* by MAGs in Brain Heart Infusion Broth Incubated at 37 °C[a]

monoacylglycerol	min bactericidal concn (μg/mL)	monoacylglycerol	min bactericidal concn (μg/mL)
MC_8	NK[b]	MC_{18}	NI
MC_{10}	75	$MC_{18:1}$	NI
MC_{12}	25	$MC_{18:2}$	NI
MC_{14}	50	coconut MAGs	10
MC_{16}	NI	milk MAGs	NI

[a] Growth was measured by plating cells daily for up to 6 days. When listericidal, complete killing was observed after 24 h and in subsequent platings. The cells in the control incubations containing 2% ethanol grew from 10^3 to $\sim 10^3$ cfu/mL. [b] NK, compound at 300 μg/mL was inhibitory but did not inactivate the cells. [c] NI, not inhibitory at 300 μg/mL.

Table IV. Effect of Mixtures of MAGs on Inhibition of *L. monocytogenes* in BHI Broth (pH 6.0) for 6 Days at 37 °C[a]

monoacylglycerol[b] (concn, μg/mL)	effect on growth[c]
combination of two MAGs	
MC_{10} (50) + MC_{12} (25)	I[d]
MC_{10} (50) + MC_{14} (25)	I
MC_{12} (25) + MC_{14} (25)	I
MC_{10} (100) + MC_{16} (100)	NI[e]
MC_{10} (100) + MC_{18} (100)	NI
MC_{10} (100) + $MC_{18:1}$ (100)	NI
MC_{12} (25) + MC_{16} (100)	NI
MC_{12} (25) + MC_{18} (100)	I
MC_{12} (25) + $MC_{18:1}$ (100)	NI
MC_{14} (50) + MC_{16} (100)	NI
MC_{14} (50) + MC_{18} (100)	NI
MC_{14} (50) + $MC_{18:1}$ (100)	NI
combination of three MAGs	
MC_{10} (50) + MC_{12} (25) + MC_{16} (50)	I
MC_{10} (50) + MC_{12} (25) + MC_{18} (50)	I
MC_{10} (50) + MC_{12} (25) + $MC_{18:1}$ (50)	I
MC_{10} (50) + MC_{14} (25) + MC_{16} (50)	I
MC_{10} (50) + MC_{14} (25) + MC_{18} (50)	I
MC_{10} (50) + MC_{14} (25) + $MC_{18:1}$ (100)	I
MC_{12} (25) + MC_{14} (25) + MC_{18} (100)	I
MC_{12} (25) + MC_{14} (25) + $MC_{18:1}$ (50)	I
MC_{12} (25) + MC_{16} (100) + $MC_{18:0}$ (100)	NI
MC_{12} (25) + MC_{16} (100) + $MC_{18:1}$ (100)	NI
MC_{12} (25) + MC_{18} (100) + $MC_{18:1}$ (100)	NI

[a] The initial population of *L. monocytogenes* was 10^3–10^4 cfu/mL. [b] Numbers in parentheses represent the concentration of monoglyceride tested. [c] Each combination was tested in three independent tubes. [d] I, inhibitory; no colonies of *L. monocytogenes* grew on BHI agar after 6 days at 37 °C. [e] NI, noninhibitory; *L. monocytogenes* grew to $>10^8$ cfu/mL.

which may explain its poor inhibitory activity against *L. monocytogenes* in BHI broth (Table III). The optimum listericidal concentration for MC_{14} was approximately 50 μg/mL in BHI broth.

Of the mixed MAGs tested, only coconut MAG inactivated *L. monocytogenes* (Table III). The interaction among mixtures of MAGs in BHI broth was studied (Table IV). The results indicated that the combination of MC_{12} and MC_{10} had a synergistic inhibitory effect on *L. monocytogenes* and that the combination of MC_{14} with MC_{10} or MC_{12} enhanced the anti-listerial activity. Furthermore, the combination of MC_{14}, MC_{10}, and MC_{12} not only enhanced the anti-listerial activity but also decreased neutralization of inhibition by MC_{16} or $MC_{18:1}$ in vitro (Table IV).

Inhibition of *L. monocytogenes* in Milk by MAGs. MAGs that were listericidal in BHI were evaluated for inhibitory activity in pasteurized milk. Of the MAGs tested, MC_{10}, MC_{12}, and fractionated coconut MAG showed strong inhibitory activity in pasteurized and skim milks (Figures 1a and 2a). Either monolaurin or monocaprin alone at concentrations greater than 200 μg/mL inactivated *L. monocytogenes* in skim milk (Figure 1a). Monocaprin at concentrations greater than 200 μg/mL was more effective than monolaurin in killing *L. monocytogenes* in skim, 2%, and whole milks (Figure 1a–c). This may due to the better water solubility of MC_{10} than MC_{12} and higher effective concentrations in the serum phase of pasteurized milks. Hexane-fractionated coconut MAGs inactivated *L. monocytogenes* in pasteurized skim at 250–400 μg/mL, in 2% milk at 500–750 μg/mL and in whole milk at 750–1000 μg/mL at 4 °C (Figure 2a–c). The anti-listerial activity of coconut MAGs was nearly equivalent to that of MC_{10} in milk (Figures 1 and 2). Coconut MAGs were more active than MC_{12} at concentrations greater than 200 μg/mL in pasteurized milks. The effectiveness of MC_{10}, MC_{12}, and coconut MAG against *L. monocytogenes* varied inversely with the fat content of the milks. Fractionated coconut MAGs were slightly more inhibitory against *L. monocytogenes* than MAGs without fractionation (Figure 2). The anti-listerial activities of coconut MAGs, MC_{10}, and MC_{12} in milk were temperature dependent (Figure 3). *L. monocytogenes* was more strongly inhibited at 4 °C than at 13 °C or ambient temperature. Interestingly, the combination of MC_{10} (100 μg/mL) with MC_{12} (100 μg/mL) had an additive inhibitory effect on *L. monocytogenes*, and the combination of MC_{14} (200 μg/mL) and MC_{10} (200 μg/mL) enhanced the anti-listerial activity in skim milk at 4 °C (Figures 4 and 5). Higher concentrations of monoglycerides were needed to inhibit *L. monocytogenes* in pasteurized milks than in autoclaved milks, possibly due to changes in the composition of the milk during autoclaving.

DISCUSSION

Of several lipids evaluated for antimicrobial activity against Gram-positive bacteria, monolaurin has been reported to have the greatest activity (Shibasaki, 1982) and would seem to have the greatest potential for use as an additive in foods and pharmaceuticals. However, MC_{12} forms complexes with macromolecules found in foods including starches, proteins, and lipids and is rendered less active in the presence of these substances (Shibasaki, 1982; Wang and Johnson, 1992). To avoid this problem, lower molecular weight monoglycerides such as MC_8 and MC_{10} have been used to control spoilage yeasts in soy sauce and wiener sausages (Hatanaka et al., 1978; Shibasaki and Kato, 1978). Furthermore, monolaurin is costly to prepare and at a high concentration has a soapy flavor. Other MAGs could be useful as antimicrobials for food preservation. In the present study, enzymatic conversion of suitable natural oils into effective MAGs was attempted to produce an antimicrobial mixture. Purified (>99%) monolaurin and MAGs (>95% pure calculated on monoester basis) prepared by lipase-catalyzed glycerolysis from coconut and milkfat were evaluated in BHI broth and pasteurized milk for anti-listerial activity. Our results showed that the coconut MAG mixture was more effective than monolaurin alone for inhibition of *L. monocytogenes* both in vitro and in pasteurized milk (Table III and Figure 2).

The composition of fatty acids present in the MAGs affected the anti-listerial activity. The mixture contains compounds of varying solubility which may be active in the aqueous and fatty phases of the food. Cells of *L. monocytogenes* probably vary in their hydrophobicities but may predominate in the aqueous phase. The different physical properties of the MAGs and variations in cell surface hydrophobicities may explain why the mixed

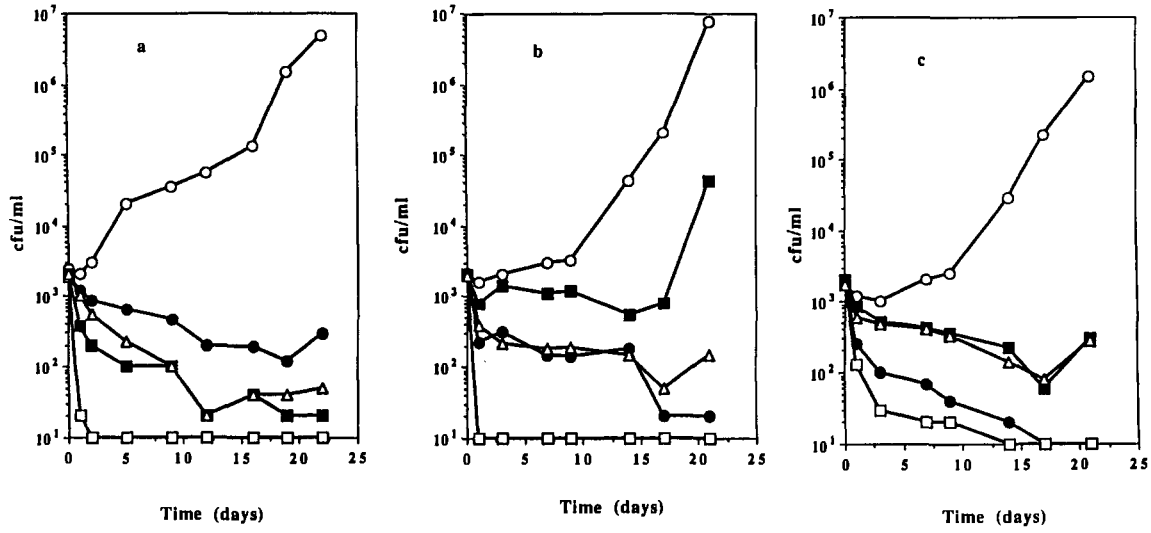

Figure 1. Effect of MC_{10} and MC_{12} on inhibition of *L. monocytogenes* in pasteurized skim (a), 2% (b), and whole milks (c) at 4 °C. Data represented are the average of two determinations in duplicate samples. Symbols: (a) control (○), MC_{10} (200 μg/mL) (●), MC_{10} (400 μg/mL) (□), MC_{12} (200 μg/mL) (■), MC_{12} (400 μg/mL) (△); (b) control (○), MC_{10} (500 μg/mL) (●), MC_{10} (1000 μg/mL) (□), MC_{12} (500 μg/mL) (■), MC_{12} (1000 μg/mL) (△); (c) control (○), MC_{10} (750 μg/mL) (●), MC_{10} (1000 μg/mL) (□), MC_{12} (750 μg/mL) (■), MC_{12} (1000 μg/mL) (△).

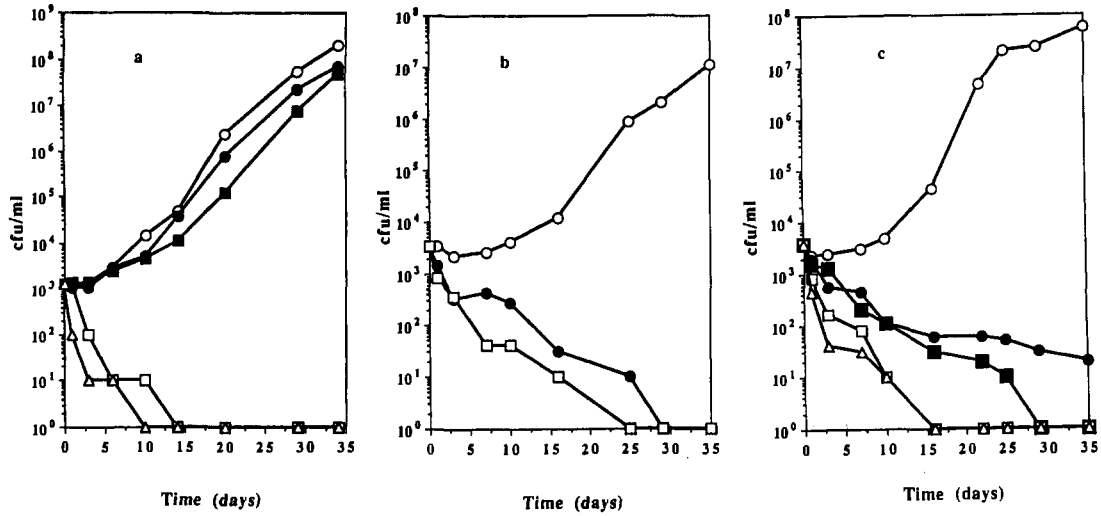

Figure 2. Inhibitory effect of coconut MAGs (F, fractionated; NF, nonfractionated) on *L. monocytogenes* in pasteurized skim (a), 2% (b), and whole milks (c) at 4 °C. Data represented are the average of two determinations in duplicate samples. Symbols: (a) control (○), MAG (NF) 100 μg/mL (●), MAG (NF) 250 μg/mL (□), MAG (F) 100 μg/mL (■), MAG (F) 250 μg/mL (△); (b) control (○), MAG (NF) 500 μg/mL (●); MAG (F) 500 μg/mL (□); (c) control (■), MAG (NF) 750 μg/mL (△), MAG (NF) 1000 μg/mL (○), MAG (F) 750 μg/mL (●), MAG (F) 1000 μg/mL (□).

coconut MAGs were more effective than monolaurin alone for the inhibition of a population of *L. monocytogenes*. The combination of MC_{14} with MC_{10} or MC_{12} enhanced the anti-listerial activity and decreased neutralization by MC_{16} and $MC_{18:1}$.

Coconut MAGs contained relatively high quantities (74–83%) of anti-listerial MAGs (C_8–C_{14}), including 43–48% C_{12}, the most active fatty acid in the series, and relatively low quantities (15–17%) of C_{16} and $C_{18:1}$ which neutralized the inhibitory activity. In contrast, milkfat contained higher amounts (56–60%) of C_{16} and $C_{18:1}$, lesser quantities (18–22%) of active medium-chain fatty acids, and only 3–5% of C_{12}. Hence, the differences in fatty acid compositions of coconut and milk MAGs may explain why milk MAG was not effective against *L. monocytogenes*.

Tsuchido et al. (1981) considered the thermodynamic characteristics of the bactericidal action of monolaurin and predicted the bactericidal activity would be more effective in low-temperature long-time treatments than in high-temperature short-time treatments. They also suggested that monolaurin could be used as a food additive (emulsifier) to reduce the heat treatment required to achieve commercial sterility of foods. In this study, we found that MAGs prepared from coconut oil had higher anti-listerial activity at 4 °C than at 13 or 23 °C, similar to our previous study on monolaurin alone (Wang and Johnson, 1992). At lower temperatures bacteria change the phospholipid composition of the cytoplasmic membrane, affecting its fluidity (Sinensky, 1974). The cytoplasmic membrane at low temperatures may be more susceptible to penetration by MAGs. Our results suggest that coconut MAGs could be particularly valuable for the preservation of refrigerated foods.

The mechanism of antibacterial action of MAGs is not established. Because of the lipophilic properties of MAGs, it is possible that they insert into the plasma membrane and inhibit enzymes involved in energy production or transport of nutrients. Vadehra and Wahi (1985) reported that monolaurin caused extensive damage of membranes, leakage of intracellular protein and nucleic acids, and

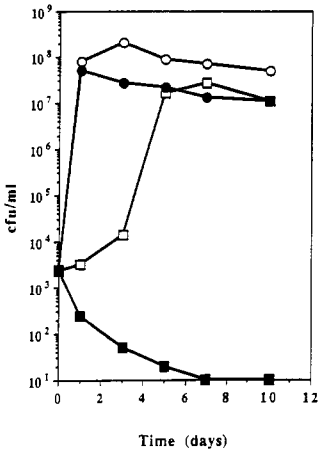

Figure 3. Temperature effect on inhibition of *L. monocytogenes* in pasteurized skim milk by coconut MAGs at 250 μg/mL. Data represented are the average of two determinations in duplicate samples. Symbols: control (○); MAG (23 °C) (●); MAG (13 °C) (□); MAG (4 °C) (■).

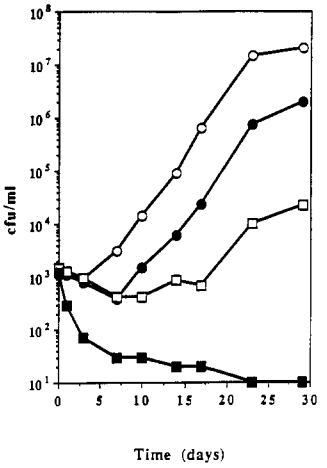

Figure 4. Additive effect of MC_{10} and MC_{12} on inhibition of *L. monocytogenes* in pasteurized skim milk at 4 °C. Data represented are the average of two determinations in duplicate samples. Symbols: control (○); MC_{10} (100 μg/mL) (●); MC_{12} (100 μg/mL) (□); MC_{10} (100 μg/mL) + MC_{12} (100 μg/mL) (■).

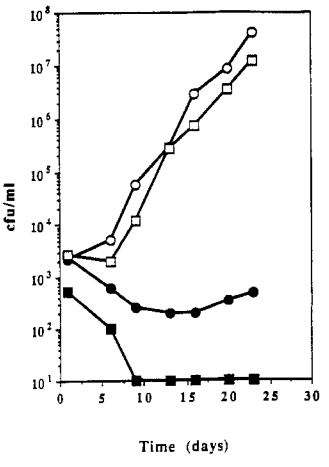

Figure 5. Enhancement in anti-listerial activity of MC_{10} by MC_{14} in pasteurized skim milk at 4 °C. Data represented are the average of two determinations in duplicate samples. Symbols: control (○); MC_{10} (200 μg/mL) (●); MC_{14} (200 μg/mL) (□); MC_{10} (200 μg/mL) + MC_{14} (200 μg/mL) (■).

decreased activities of certain enzymes. The detrimental effects of monolaurin against microorganisms are neutralized in media by certain other surfactants such as Tweens or Spans (containing fatty acids, mostly C_{18} and $C_{18:1}$) (Baker et al., 1983). In this study, it was observed that the anti-listerial activities of MC_{10} or MC_{12} were neutralized by MC_{16}, MC_{18}, or $MC_{18:1}$ and that their activity was potentiated by MC_{14} at low concentrations (25–75 μg/mL). Therefore, it is likely that the composition of fatty acids in a surfactant would contribute to disruption or stabilization of the cell membrane. It was previously found that fats decreased the antimicrobial activity of MAGs and that DAGs or unsaturated long-chain MAGs ($MC_{18:1}$ and $MC_{18:2}$) had no anti-listerial activity (Wang and Johnson, 1992). Hence, it is important to minimize the interference by DAGs and TAGs and other interfering ingredients in foods to achieve maximum control of undesirable organisms. Our results indicate that MAGs would be most suitable for use in reduced-fat foods.

In conclusion, we have found that specific MAGs synthesized from coconut oil by lipase-catalyzed glycerolysis could be used as inhibitory agents in foods against *L. monocytogenes*, particularly in reduced-fat foods. Their multifunctional properties may be exploited to impart emulsifying as well as antimicrobial properties and to increase the shelf life of foods.

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