

Inhibition of Bacteria by Lactulose Preparations

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Lactulose syrups were similar to sucrose syrups in water activity-lowering effects but were more inhibitory toward test microorganisms. Heat-treated commercial lactulose syrups were most inhibitory, whereas non-heat-treated pure lactulose was only slightly more inhibitory than sucrose.

Humectants are included in intermediate moisture foods to produce the required water activity (A_w) that prevents microbial growth. Two of the most commonly used humectants, glycerol and sucrose, impart undesirable flavors to foods and have been implicated as causative factors in liver enlargement (10), heart disease, or diabetes (5a, 13a). Because of the need for replacements for glycerol and sucrose (15), we decided to examine the synthetic disaccharide lactulose (4-*O*- β -D-galactopyranosyl-D-fructose) as a potential humectant for foods. We previously determined the sweetness of this sugar at various concentrations (13). This report compares the antimicrobial effects of lactulose and lactulose syrups relative to sucrose. Mütting et al. (9) and Liem (K. S. Liem, U.S. patent 3,562,388, February 1971) reported that lactulose preparations, in vitro and in vivo, respectively, exhibit an antibacterial effect on certain intestinal microorganisms. These workers, however, used commercial lactulose syrups designed for the therapy of liver disorders (2, 4) and which often contained other sugars, flavorings, and in some cases antimicrobial preservatives (12). In our study, we used pure, crystalline lactulose, as well as a variety of lactulose syrups, to determine if these antimicrobial properties are due to lactulose itself or to other compounds present in minor amounts.

Cephulac (Richardson-Merrell), a 50% (wt/wt) lactulose syrup, was purchased from a local pharmacy and contained no preservative. Lactulose syrup A was prepared by the calcium hydroxide method of Montgomery and Hudson (8) and then fractionated on a coconut charcoal column (3) with aqueous ethanol to yield a light brown syrup containing 87.3% lactulose, 6.3% lactose, 5.7% galactose, and 0.7% tagatose. Syrup B was prepared by the reaction of α -lactose monohydrate with triethylamine (F. W. Parrish, U.S. patent 3,514,327, May 1970), with subsequent charcoal fractionation to yield a yellow

syrup containing 71% lactulose, 25% lactose, and 4% tagatose. Syrup C was prepared by the reaction of α -lactose monohydrate with sodium aluminate by the procedure of Hodge (personal communication). The syrup contained 75% lactulose, 8% tagatose, 14% galactose, and 3% lactose. All sugars were quantitatively determined by high-pressure liquid chromatography on a μ /Bondapak/Carbohydrate column (Waters Associates) with authentic sugars as standards.

Crystalline lactulose was prepared from syrup C by the procedure of Oosten (11). The criteria of purity were melting point (168 to 171°C), unchanged mixed melting point with authentic crystalline lactulose (Aldrich), and retention time identical to that of the authentic sugar by high-pressure liquid chromatography.

The sugar solutions (based on their lactulose or pure sucrose content) were tested for microbial inhibition at concentrations of 0, 14, 21, 28, 35, 42, 49, 56, and 63% unless otherwise indicated. These levels were obtained by adding, after autoclaving, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 g of 70% (wt/wt) sugar solutions (pH adjusted to 7.0) to autoclaved (15 lb/in²; 15 min) tubes (16 by 150 mm) containing 0.5 ml of a 10-fold normal concentration of tryptic soy broth and quantities of sterile water to bring the volume to 5 ml after the addition of the sugar solutions. The tubes were shaken by a Vortex mixer and inoculated by needle with 24-h cultures of test bacteria grown in tryptic soy broth at 30°C. Incubation was at 30°C for all organisms. For *Clostridium botulinum*, 0.1% sodium thioglycollate was added to the medium, and incubation was in an anaerobic incubator (National Appliance Co.) previously evacuated to 63.5 cm, with replacement of the vacuum by N₂. The presence (+) or absence (-) of microbial growth was determined by visual inspection after 18 h or 2 or 3 days. The highest sugar concentration at which a test organism was observed to grow was called the maximum growth-permitting concentration (MGPC).

Another set of experiments was conducted as above except that sugar solutions were sterilized

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by filtration through 0.22- μ m porosity filter membranes.

A_w was determined with the Electric Hygrometer (Aminco). Approximately 50 to 70 ml of each sugar solution was placed in a pint mason jar with a lithium chloride sensor. After equilibration at 26.6°C for 20 to 24 h in a forced draft incubator, readings were taken. The sensors were transferred to jars containing saturated KNO_3 , and readings were taken after a 20- to 24-h equilibration. Correction factors obtained were applied to the A_w values of the sugar solutions.

When test microorganisms were incubated for 10 days in media containing increasing concentrations of sucrose (Table 1), most organisms showed no growth at greater than 42 to 49% sugar concentrations. The A_w values for these growth-inhibiting sucrose concentrations were in general agreement with growth-limiting A_w values previously reported for *Escherichia coli* (5, 15), *Staphylococcus aureus* (1, 6, 7, 14), *Pseudomonas aeruginosa*, and *Salmonella typhimurium* (7).

The procedure used to test sucrose was then applied to several lactulose syrups. Lactulose syrup A (Table 1) was considerably more inhibitory than sucrose toward all microorganisms tested. Only a 14 to 21% (wt/wt) concentration of lactulose in this syrup was necessary to cause inhibition of growth. The results in this table are for after 10 days incubation. At 18 h, however, in the case of lactulose, inhibition was complete in all except the control tubes. In the sucrose tubes, on the other hand, the MGPC was nearly

the same at 18 h and 10 days; the greatest change noted was a one-tube (7%) difference. This greater inhibition was not caused by lowered A_w because sucrose and lactulose syrup A appeared to be nearly identical in A_w -lowering effects over the critical range tested.

It is noteworthy that various lactulose syrups, although similar in carbohydrate composition, were different in their microbial inhibition. Lactulose syrup B produced the same results as syrup A, but syrup C was less inhibitory. For instance, the 10-day MGPC for *E. coli* was 28% for syrup B and 42% for syrup C. Similarly for *S. aureus*, the MGPC for syrups A and B was 28%, whereas it was 35% for syrup C. The antimicrobial effects of these syrups, therefore, appeared to have been due to unidentified compounds present in varying amounts in the syrups, rather than to lactulose, per se. To test this possibility, the antimicrobial activities of several sucrose samples and lactulose syrups were compared to that of pure recrystallized lactulose. With *S. aureus*, the MGPC for pure lactulose (Table 2), 25% (wt/wt), was similar to those of several sucrose samples (35% [wt/wt]). With the same organism, the MGPC values for a commercial lactulose syrup, Cephulac, and lactulose syrup B, as expected from the earlier study, were much lower. This strongly implies that both the laboratory preparation and Cephulac possess antimicrobial properties associated with minor constituents of the syrups.

The identity of these antimicrobial compounds is at present unknown. We explored the

TABLE 1. Inhibition^c of microorganisms by lactulose syrup A^b and sucrose

Culture	Sugar	Inhibition ^c at the following sugar concn (% [wt/wt]):									
		0	14	21	28	35	42	49	56	63	
<i>E. coli</i>	Syrup A	++	++	+-	--	--	--	--	--	--	--
	Sucrose	++	++	++	++	++	++	++	--	--	--
<i>S. aureus</i>	Syrup A	++	++	+-	--	--	--	--	--	--	--
	Sucrose	++	++	++	++	++	++	++	++	++	++
<i>P. aeruginosa</i>	Syrup A	++	--	--	--	--	--	--	--	--	--
	Sucrose	++	++	++	++	++	++	++	++	--	--
<i>S. typhimurium</i>	Syrup A	++	+-	--	--	--	--	--	--	--	--
	Sucrose	++	++	++	++	++	++	++	++	--	--
<i>C. botulinum</i>	Syrup A	++	++	--	--	--	--	--	--	--	--
	Sucrose	++	++	++	++	--	--	--	--	--	--
A_w	Syrup A	ND ^d	ND	ND	ND	ND	ND	0.95	0.94	0.92	0.89
	Sucrose							0.95	0.94	0.93	0.90

^a Incubation was at 30°C for 10 days.

^b Concentrations are based on the lactulose content of the syrup or on pure sucrose. The syrups were separately autoclaved.

^c Duplicate tubes; +, growth; -, no growth.

^d ND, Not determined.

possibility that the inhibition observed may have resulted from antimicrobial artifacts introduced during lactulose syrup preparation. This possibility was dismissed when sucrose treated in the same way as lactose during isomerization and purification exhibited no more inhibition than did untreated sucrose.

Because lactulose syrups A, B, and C, as well as Cephulac, are all prepared via the base-catalyzed isomerization of lactose, it is possible that alkaline degradation products of sugars may be largely responsible for the microbial inhibition.

The crystalline lactulose used in the previous experiments was autoclaved separately from the medium. Less inhibition was observed in another experiment (Table 3) when the lactulose was membrane filtered rather than autoclaved. This suggests that thermally derived reaction products of lactulose or lactulose syrups may be involved in the microbial inhibition.

TABLE 2. Effect of sucrose and lactulose on *S. aureus*^a

Prepn	A _w (at a 56% concn [wt/wt])	MGPC ^b (% [wt/wt])
Locally purchased light brown sugar	0.92	35
Philippine raw sucrose	0.92	35
Crystalline sucrose	0.92	35
Lactulose syrup B	0.92	2.5
Crystalline lactulose	0.91	25
Commercial lactulose (Cephulac)	0.91	1.25

^a Tubes were incubated at 30°C for 24 h.

^b Increments (based on lactulose or sucrose content of the syrups) were 1.25, 2.5, 5, 10, 15, 20, 25, 30, 35, and 40% (wt/wt), with the exception of the lactulose preparations, which were diluted 1:10 of the above concentration, i.e., 0.125 to 4.0%. The sugar solutions were autoclaved separately from the medium.

TABLE 3. Effect of autoclaved versus filter-sterilized lactulose preparations on the growth^a of *S. aureus*

Sugar concn ^b (% [wt/wt])	Inhibition ^c with:			
	Pure lactulose		Syrup A	
	Heated ^d	Filtered ^e	Heated ^d	Filtered ^e
7	++	++	++	++
14	++	++	---	+-
21	++	++	---	---
28	---	++	---	---
35	---	---	---	---

^a Incubation was at 30°C for 24 h.

^b Concentrations are based on the lactulose content of the syrup or on pure lactulose.

^c See footnote, c, Table 1.

^d Autoclaved for 15 min at 15 lb/in².

^e Membrane filtered (0.22-μm porosity filter).

We are currently characterizing minor components of lactulose syrups that may have antimicrobial activity and studying the mechanism of their formation. These studies will help to further evaluate the safety and practicality of using lactulose in foods. It is not likely that the antimicrobial effect was caused by the sugars lactose, galactose, and tagatose that were also present in the crude syrups as their total concentrations were, respectively, 13% in syrup A, 29% in syrup B, and 25% in syrup C. Syrups A and B had the same MPGC in our tests, whereas syrup C was less inhibitory.

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