

SWEETNESS OF LACTULOSE RELATIVE TO SUCROSE

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ABSTRACT

The sweetness of lactulose over the range of concentration 5–35% (W/V), measured by a trained panel using paired comparison with standard reference solutions of sucrose of varying concentrations, is 48% to 62% of that of sucrose. In addition, sensitivity thresholds and recognition thresholds for sweetness of lactulose and sucrose were determined by a rating-scale method. The sweetness of a mixture containing 10% (W/V) lactulose and 5% (W/V) sucrose showed a synergistic effect of 22%, and a mixture of 5% (W/V) lactulose and 2.5% (W/V) sucrose showed 12% synergism. Partial hydrolysis of lactulose to give a mixture containing 5% (W/V) lactulose, 2.5% (W/V) galactose, and 2.5% (W/V) fructose caused a 6% synergistic effect on sweetness.

INTRODUCTION

THE ANNUAL WHEY production in the United States has been estimated at 36 billion pounds (2.4 billion pounds of solids) (Lough, 1974), approximately half of which is discarded (Johnson et al., 1976). Economic considerations have led Drews (1975) to advocate utilization of whey protein concentrate and lactose derived from whey rather than yeast protein. Isolation of whey protein concentrate by ultrafiltration has been described (McDonough et al., 1971). Deionization of the ultrafiltrate yields α -lactose monohydrate (Parrish et al., 1979) or β -lactose (Kavanagh, 1975), both of which have been used as partial replacements for sucrose in bakery goods (Ash, 1976; Goldman and Short, 1977). However, Ash (1976) found that lactose is not useful when sweetness or high solubility of the sugar components is required.

We thought that lactulose [4-O- β -D-galactopyranosyl- β -D-fructofuranose] (Isbell and Pigman, 1938; Perlin et al., 1973) might possess sweetness and solubility properties which would make it useful in baking and confectionery applications. Isomerization of lactose to lactulose was first accomplished by Montgomery and Hudson (1930) who recognized that lactulose is sweeter than lactose, but not as sweet as sucrose; in addition, these workers found lactulose to be very soluble in water, and that crystallization to produce anhydrous lactulose was difficult. In tests with finely powdered crystalline lactulose, Lee and Birch (1976) confirmed the sweetness observation of Montgomery and Hudson (1930) and found that lactulose was devoid of bitter taste. The work described here presents our findings on the sweetness of lactulose, derived from lactose by isomerization, compared to that of sucrose, together with determinations of sensitivity thresholds and recognition thresholds for sweetness of these sugars.

EXPERIMENTAL

Materials

α -Lactose monohydrate was obtained from Sigma Chemical Co., and sucrose from Fisher Scientific Co. Lactulose [4-O- β -D-galacto-

pyranosyl- β -D-fructofuranose] was prepared from α -lactose monohydrate by isomerization with calcium hydroxide by the procedure of Corbett and Kenner (1954), seed crystals of lactulose being obtained from Aldrich Chemical Co., Inc. Partially hydrolyzed lactulose was obtained by treating a 10% (W/V) lactulose solution (1000 ml) with food-grade β -galactosidase (1g; 40,000 ONPG units) (Enzyme Development Corporation) from *Saccharomyces lactis* for 4 hr at 30°C and pH 6.7, after which time the solution was heated to 70°C for 5 min to inactivate the enzyme. The solution was decolorized with charcoal (Eastman Kodak Company), and spectrophotometric analyses (see later) showed the extent of hydrolysis of lactulose to be 70%. Untreated lactulose was added to give a mixture containing 5% (W/V) lactulose, 2.5% (W/V) galactose, and 2.5% (W/V) fructose. Distilled water for taste testing was obtained from Lehigh Valley Cooperative Farmers.

Analytical methods

Purity of the sugar materials was determined from optical rotation measurements on 2% (W/V) aqueous solutions in a 1 dm tube with a Perkin Elmer Model 141 polarimeter; specific optical rotation data used in the calculation of purity were those reported by Buma and van der Veen (1974) for α -lactose monohydrate, Isbell and Pigman (1938) for lactulose, and Bates (1942) for sucrose. Moisture content was determined on 50–500 mg samples by use of a Photo-volt Aquatest II instrument, and ash content was measured by the AOAC method I (1975).

The presence of other sugars as impurities in the test sugars was tested by thin-layer chromatography on cellulose plates with ethyl acetate/pyridine/water (10/4/3, by volume) used as solvent (Aspinall and Ferrier, 1957), and visualization of the spots was made with orcinol reagent (Klevstrand and Nordal, 1950) for ketose sugars, with aniline oxalate (Horrocks and Manning, 1949) for reducing sugars and sucrose, or with alkaline silver nitrate (Trevelyan et al., 1950) for all classes of sugars. In addition, gas-liquid chromatography of trimethylsilyl derivatives of the sugars (Sweeley et al., 1963) was performed on a 4 ft \times 1/8 in. column of 3% SP-2100 on Supelcoport (100–120 mesh) with temperature programming from 150–210°C and a flame ionization detector. Other parameters were helium flow-rate 30 ml/min, injector temperature 215°C, and detector temperature 220°C.

Spectrophotometric analyses were made with a copper reagent to determine monosaccharides in the presence of disaccharides (Tauber and Kleiner, 1932), with 3,5-dinitrosalicylic acid to measure total reducing sugars (Miller, 1959), and with thiobarbituric acid to determine ketose sugars (Percheron, 1963) to evaluate the purity of the sugars.

Method used for sensory evaluation

Sensory evaluations were made by a forced choice paired comparison method (Pangborn, 1963) in which the judges were asked to determine the sweeter member of each pair comprising a fixed concentration of lactulose or lactose and one of three sucrose solutions of differing concentrations. The choice of method is based on the observation of Stone and Oliver (1969) that paired-comparison is the most exact method because responses are the result of direct comparison between two stimuli.

Panel selection and composition

The panel consisted of in-house personnel with previous experience in sweetness evaluation of sugar syrups. Selection was based on their ability to determine relative sweetness of samples in paired comparison tests with different levels of sucrose; two series of sucrose solutions were presented and each judge was asked to find the samples of equal sweetness. In another test two series of lactose and sucrose solutions were presented to determine the concentrations for equal sweetness; the findings were compared with literature values (Amerine et al., 1965).

The 31 panel members were scientists, engineers, technicians,

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and secretaries. There were 21 males and 10 females ranging in age from 20 to 62. In each session 20 judges were used.

Material evaluation

All tests were performed in the morning. Solutions were prepared 16 hr before being tested to allow mutarotational equilibrium of lactose and lactulose to be established (Pangborn and Gee, 1961). Serving order was randomized within and between pairs. Samples were coded and served at 22°C in 30 ml amounts in odorless plastic cups. Three pairs of samples were tested in each session, one of lactulose or lactose for comparison with each of three concentrations of sucrose. All tests were replicated twice.

Environmental conditions

A separate taste panel room with partitioned booths was used. The room was maintained at 20°C under positive pressure. Air, brought in from outside, was filtered through charcoal. The room was equipped with white fluorescent lights and lights with a choice of colored filters. The white fluorescent lights were used in most of the tests except that when a discernable color difference occurred green lights were used.

Data analysis

The equal sweetness point was found by plotting the percentage of judges who selected the lactulose or lactose solution as sweeter than the sucrose solution versus the sucrose concentration (Pangborn, 1963). The concentration of sucrose was calculated for which 50% of the judges selected the lactulose or lactose solution, and this concentration of sucrose is the equal sweetness point (Fig. 1). This

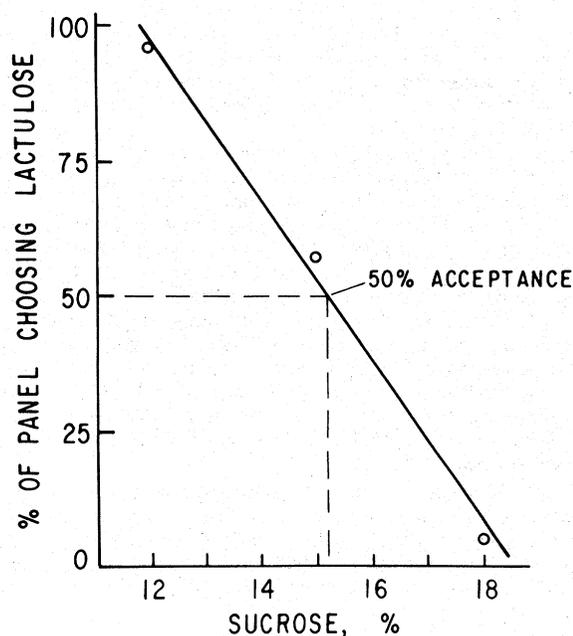


Fig. 1—Concentration of sucrose having sweetness equal to that of lactulose or lactose test solution. Example shown is for 25% (w/v) lactulose.

Table 1—Analyses for α -lactose monohydrate, β -lactulose and sucrose

	α -Lactose monohydrate	β -Lactulose	Sucrose
Specific optical rotation ^a (589 nm)	+52.62°	-50.63°	+66.49°
Purity % ^b	99.82	99.87	99.94
Moisture %	5.12 ^c	0.015	0.0048
Ash %	0.091	0.034	0.001

^a Equilibrium value at 20°C

^b Based on specific optical rotation

^c Includes water of hydration

calculation was made for each concentration of lactulose or lactose tested.

Sensitivity threshold and recognition threshold for sweetness for lactulose and sucrose were made by a modification of the method of Gregson (1962) by limiting the choice of responses to the following:

1. Definitely the same as water.
2. A perceptible taste, cannot say what.
3. A barely recognizable sweet taste.
4. A definitely recognizable sweet taste.

An ascending series was tested for sucrose comprising 0, 0.125, 0.25, 0.50, 1.00 and 2.00% (W/V) sucrose. For lactulose the concentrations were 0, 0.25, 0.50, 1.00, 2.00 and 4.00% (W/V). The percentage of judges selecting responses 1 and 2 was plotted against the solution concentration. The sugar concentration was interpolated for the 50% response level, and this is the sensitivity threshold. Similarly, the 50% response level for selection of responses 1, 2, and 3 is the recognition threshold.

Synergism between lactulose and sucrose was examined by comparing the sweetness of a mixture containing 10% (W/V) lactulose and 5% (W/V) sucrose with standard solutions containing 10, 11, 12 and 13% (W/V) sucrose. The 50% response level was used to calculate the equal sweetness point. Similarly, a mixture of 5% (W/V) lactulose and 2.5% (W/V) sucrose was evaluated, using 4.5, 5.0, 5.5, 6.0 and 6.5 (W/V) sucrose.

The effect on sweetness of partial hydrolysis of lactulose was studied by comparing a mixture containing 5% (W/V) lactulose, 2.5% (W/V) galactose, and 2.5% (W/V) fructose with standard solutions of 6.5, 7.0, 7.5, 8.0, and 8.5% (W/V) sucrose. The 50% response level was used to calculate the equal sweetness point.

RESULTS & DISCUSSION

THE PURITY (based on specific optical rotation data at equilibrium), moisture content, and ash content of the sugars used in the sweetness studies are shown in Table 1. No other sugars were detected as impurities by thin-layer chromatography on cellulose. Gas-liquid chromatography of trimethylsilyl derivatives of the crystalline sugars (Sweeley et al., 1963) showed a single peak for all three sugars. The absence of monosaccharides in lactulose, lactose, and sucrose, whether examined in the crystalline state or from equilibrated, aqueous solution, was shown by application of the spectrophotometric procedure using copper reagent (Tauber and Kleiner, 1932) and by gas-liquid chromatography of the trimethylsilyl derivatives (Sweeley et al., 1963). Two other spectrophotometric analyses were found to be of value in following the transformation of lactose to lactulose and for examining the purity of the crystalline lactulose. With 3,5-dinitrosalicylic acid reagent (Miller, 1959) lactose and lactulose showed identical absorbances at 540 nm, whereas sucrose gave no reaction. With thiobarbituric acid reagent (Percheron, 1963) lactulose and sucrose showed identical absorbances at 432 nm, whereas lactose gave only 3.8% of the absorbance of lactulose or sucrose. The results of all of these analytical procedures provided assurance of the high purity of the sugar materials used in our sweetness studies.

In our sweetness measurements we used aqueous solutions of sugars which had been allowed to stand 16 hr in order to achieve mutarotational equilibrium (Pangborn and Gee, 1961) of lactulose and lactose, whereas other workers (Montgomery and Hudson, 1930; Lee and Birch, 1976) used crystalline sugars applied to the tongue. Since in food applications sugars are used in an aqueous environment, we thought it was more practical to examine the equilibrated, aqueous sugar solutions rather than crystalline or freshly prepared materials.

The concentrations of the three sucrose solutions used for measuring the sweetness of each lactulose solution are shown in Table 2. The sweetness of equilibrated lactulose over the range of concentration 5–35% (W/V) was found to be 48% to 62% of that of sucrose (Table 3).

In the determination of threshold response levels (Greg-

son, 1962), we measured sensitivity thresholds and recognition (for sweetness) thresholds (Amerine et al., 1965) for lactulose and sucrose (Table 4); the latter values agree with the sweetness relationships shown in Figure 2. Our values of 0.36% for sensitivity threshold and of 0.53% for recognition threshold for sucrose (Table 4) are similar to the median values of 0.342% and 0.582%, respectively, reported by Pfaffmann (1959).

Sweetness comparison of lactose (1.5–16%) and sucrose was made in a study which was curtailed when it was found that the data were closely similar to those of Pangborn (1963), whose results are interpolated in Table 3 to allow comparison of the concentrations for equal sweetness of lactulose and lactose. This comparison shows clearly that lactulose is sweeter than lactose over the range of concentration we examined. Synergistic effects on sweetness for mixtures of sugars are well known (Amerine et al., 1965). We observe a synergistic effect in a mixture containing 10% (W/V) lactulose and 5% (W/V) sucrose. On the basis of additivity of sweetness contributions, this combination would have a sweetness equivalent to 10.07% (W/V) sucrose, whereas by sensory evaluation we find sweetness equivalent to 12.2% (W/V) sucrose, i.e., 22% synergism. Similarly, a mixture containing 5% (W/V) lactulose and 2.5% (W/V) sucrose was expected to have a sweetness equivalent to 4.91% (W/V) sucrose, whereas the value obtained by sensory evaluation was 5.5% (W/V) sucrose, i.e., 12% synergism.

We have kept 70% (W/V) aqueous solutions of lactulose at 4°C for 2 yr, and they have shown no formation of crystalline material or color development.

Partial hydrolysis of 10% (W/V) lactulose was performed with food-grade β -galactosidase to give a mixture containing 5% (W/V) lactulose, 2.5% (W/V) galactose, and 2.5% (W/V) fructose. The mixture was equivalent in sweetness by sensory evaluation to 7.44% (W/V) sucrose, whereas the arithmetic sum of the sweetness contributions of the individual sugars gives an expected value of 7.02% (W/V) sucrose based on our value for lactulose (Table 3) and those of Amerine et al. (1965) for galactose and fructose. These values of 7.02 and 7.44% (W/V) differ significantly ($p < 0.05$) and indicate a synergistic effect of 6%.

The greater sweetness and solubility of lactulose compared to lactose make it of interest to study lactulose as a partial replacement for sucrose in food applications, as Ash (1976) has done for lactose. This work is in progress in our laboratories.

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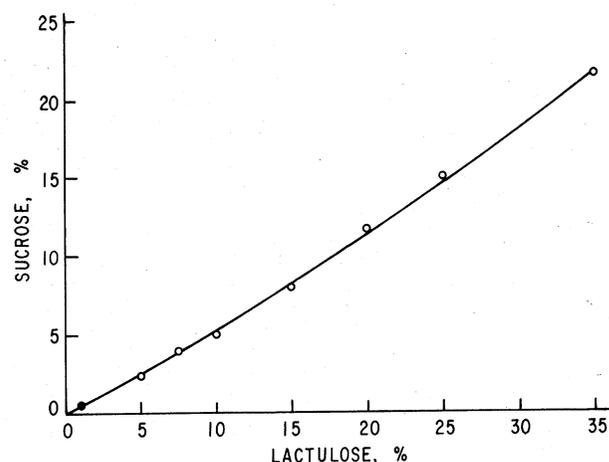


Fig. 2—Concentration of sucrose having sweetness equal to that of lactulose test solution.

Table 2—Concentrations of sucrose solutions used in paired-comparison test of sweetness of lactulose solutions

Lactulose	Concentration % (W/V)		
	Sucrose		
5.00	1.0	2.0	3.0
7.50	3.0	4.0	5.0
10.0	4.0	5.0	6.0
15.0	8.0	9.0	10.0
20.0	9.0	11.0	13.0
25.0	12.0	15.0	18.0
35.0	18.0	21.0	24.0

Table 3—Equal sweetness concentration values for lactose, lactulose, and sucrose

Lactulose	Concentration % for equal sweetness	
	Sucrose ^a	Lactose ^b
5.00	2.41(0.39)	7.48
7.50	4.01(0.31)	11.12
10.0	5.07(0.13)	13.53
15.0	8.04(1.15)	20.27
20.0	11.79(0.99)	28.79
25.0	15.16(0.60)	36.45
35.0	21.84(2.67)	51.63

^a Value in parentheses is standard error for duplicate analyses using 20 judges.

^b Calculated from Pangborn (1963) data.

Table 4—Sensitivity threshold and recognition threshold (for sweetness) of lactulose and sucrose

Sugar	Concentration % (W/V)	
	Sensitivity threshold ^a	Recognition threshold ^a
Lactulose	0.53 (0.30–0.94)	1.22 (0.64–2.34)
Sucrose	0.36 (0.24–0.52)	0.53 (0.41–0.67)

^a Values in parentheses are 95% confidence intervals for duplicate analyses using 20 judges.

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