Sugar reduction of skim chocolate milk and viability of alternative sweetening through lactose hydrolysis

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ABSTRACT

Milk consumption by Americans has not met the standards of the Dietary Guidelines for Americans. Chocolate milk can improve milk consumption, especially by children, due to its color and taste. However, the high sugar content of chocolate milk is a cause for concern about its healthfulness, resulting in its removal from some school lunch programs. It is important to reduce the sugar content of chocolate milk and still maintain acceptability among consumers. It is also important to investigate other natural alternatives to sweetening. The objectives of this study were to identify the different sweetness intensity perceptions of sucrose in water and various dairy matrices, to identify the acceptable reduction in sweet taste for chocolate milk for both young adults (19–35 yr) and children (5–13 yr), and to determine if lactose hydrolysis is a viable alternative. Threshold and power function studies were used to determine the benchmark concentration of sucrose in chocolate milk. The acceptability of sugar reduction from the benchmark concentration for both young adults and children and the acceptability of lactose hydrolyzed chocolate milk (4°C for 24 h) with added lactose for young adults were evaluated. Acceptability results demonstrated that sugar reduction in chocolate milk is possible for both young adults and children as long as it does not exceed a 30% reduction (from 205 mM). Lactose hydrolysis of added lactose was used to achieve the sweetness of sucrose in chocolate milk but required >7.5% (wt/vol) added lactose, which contributed undesirable calories, indicating that lactose hydrolysis may be more suitable for other dairy beverages that require less added sugar. The findings of this study demonstrate consumer acceptance of reduced-sugar chocolate milk and a possible way to use lactose hydrolysis in dairy beverages.

Key words: chocolate milk, power function, sugar reduction, lactose hydrolysis

INTRODUCTION

The Dietary Guidelines for Americans recommend that adult Americans consume 3 cups (710 mL) per day of fat-free or low-fat milk or equivalent milk products (USDA-HHS, 2010). Studies have shown that flavored milk increases milk consumption among both adults and children (Anonymous, 2003; Murphy et al., 2008). Flavored milk provides essential nutrients similar to that of plain milk and other milk products (Murphy et al., 2008) and, because of its pleasurable taste, has gained popularity among children and adults (Kim et al., 2013). In 2011, sales in flavored milk and milk drinks reached \$731 million in the United States (International Dairy Foods Association, 2012). Chocolate is the most popular milk flavor for both children and adults (Boor, 2001; Thompson et al., 2004, 2007; NDC, 2010). Various studies have addressed the relationship between high sugar consumption and health concerns such as obesity (Berkey et al., 2004; Malik et al., 2006); therefore, despite the popularity of chocolate milk and the benefit of increasing milk consumption for children and adults, the high sugar content of chocolate milk has raised health concerns. The Los Angeles school system recently banned flavored milk as part of a child obesity prevention program (Hoag, 2011). However, Quann and Adams (2013) determined that eliminating flavored milk in elementary schools decreased overall milk consumption and, to replace the nutrient deficit from the milk consumption, additional foods with more fat and calories would be required. Therefore, the balance between increasing milk consumption while maintaining low sugar content is important.

Sugar reduction is usually achieved by replacing sugar with nonnutritive sweeteners. However, the disadvantages of these sweeteners are that they are often artificial, which are not label friendly, and often carry metallic or bitter taste and aftertastes (Schiffman et al., 1995). The naturally existing milk sugar lactose has a lower relative sweetness compared with its monosac-

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charide constituents glucose and galactose. Lactase is currently used by the dairy industry to produce lactose-free milk. As such, lactose hydrolysis of milk with added dried dairy ingredients such as lactose or whey permeate might be a label-friendly method to sweeten chocolate milk naturally. Lactose hydrolysis is usually conducted before pasteurization of raw milk or after pasteurization followed by heat treatment to deactivate the enzyme. Sterile lactase can also be added into UHT milk with aseptic packaging, where the lactose will be hydrolyzed during the first few days of storage. If an acceptable sweetness of chocolate milk could be achieved by hydrolyzing added lactose or a high-lactose dairy ingredient such as whey permeate, it would provide an alternative way of sweetening chocolate milk. To our knowledge, no published work has addressed this question.

Various studies have been conducted on the effect of sugar reduction on sensory perception of different foods and beverages but to our knowledge, no studies have described the dose-response relationship of sucrose in different dairy matrices, especially chocolate milk. It is important to understand sweetness perception of chocolate milk and what level of sugar reduction can be achieved without affecting consumer acceptance. The objectives of this study were to identify the sweetness intensity perception of sucrose in water and various dairy matrices, to identify the acceptable sweet taste reduction for chocolate milk for both young adults and children, and to determine if lactose hydrolysis is a viable alternative to sweeten chocolate milk.

MATERIALS AND METHODS

Experimental Overview

Two experiments (experiments 1 and 2) were conducted to achieve the objectives of this study. In experiment 1, the acceptable sugar reduction of skim chocolate milk for both young adults and children was identified. Threshold and power function studies were conducted to determine a representative concentration of sucrose in skim chocolate milk comparable to commercial product. Based on the benchmark concentration, different percentages of sucrose reduction in chocolate milk were evaluated by both young adults and children. In experiment 2, the objective was to determine if lactose hydrolysis was a viable alternative to sweeten skim chocolate milk. The lowest acceptable sweet level from experiment 1 for both young adults and children was chosen as the sweet taste target for lactose hydrolysis experiments. The acceptance of chocolate milk sweetened by lactose hydrolysis compared with sucrose-sweetened chocolate milk was then evaluated by young adults.

Chocolate Milk Manufacture

Chocolate milk base without added sugar used for bench-top studies for power function and lactose hydrolysis studies was manufactured by adding 10.19 g of cocoa powder (NC85, Benjamin P. Forbes Company, Broadview Heights, OH) to 1 L of raw skim milk. The mixture was pasteurized at 69°C for 30 min, and then immediately fed to a 2-stage homogenizer (Panda 2K, Niro Inc., Columbia, MD) at 2,000 kPa (first stage) and 4,000 kPa (second stage). The milk was cooled and stored at 4°C. Sucrose was then added at different concentrations in each experiment. Chocolate milk for consumer tests was manufactured by the North Carolina State University (NCSU) Dairy Education Unit (Raleigh, NC) using skim milk and the same cocoa powder concentrations (described in the Consumer Acceptance sections).

Experiment 1: Sugar Reduction in Skim Chocolate Milk

Threshold Determination. All sensory testing protocols were approved by the NCSU Institutional Review Board. The detection and recognition threshold of sucrose in water, skim milk, whole milk, and skim chocolate milk was determined using a 7-series ascending 3-alternative forced choice method (ASTM International, 1991; Plotto et al., 2004; Drake et al., 2011). Samples (30 mL) were presented in lidded 60-mL soufflé cups with 3-digit codes, and the series was presented in ascending concentration. The sweetness levels were presented with a dilution factor of 3 (0.111 to 81 mM sucrose in water, 0.333 to 243 mM sucrose in milks). In each series, samples were randomized. All samples were equilibrated to 20°C before testing.

Subjects ($n = 40$) were instructed how to evaluate the samples before testing, and the sure/not sure modification detailed by Lawless et al. (2000) was used. Subjects were asked to remove the cup lid and taste each sample, and then to choose the 1 item of the 3 that they thought was different. Subjects were also asked if they recognized the difference. Subjects were asked to give certainty judgments (sure/not sure) for both difference and recognition. The individual best-estimated threshold was determined by calculating the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response, with all subsequent responses correct. If the subject indicated a “not sure” response for the correct choice, a factor of 1.41 was multiplied to adjust for the possibility of a chance correct response (Lawless et al., 2000; Leksrisompong et al., 2010). Group thresholds

were determined by taking the geometric mean of the individual BET values.

Power Function of Sweetness in Different Media. Twelve panelists (9 females, 3 males, aged 21–55 yr) were used for generating power functions of sucrose in water, skim milk, and skim chocolate milk. Each panelist had some previous experience with descriptive analysis (~25–50 h of training), and 5 h of additional training on sweet taste identification and magnitude estimation scaling was conducted. The starting sucrose concentrations in each food matrix were based on the recognition threshold of sucrose. Magnitude estimation scaling (Meilgaard et al., 1999) was used to create the power functions. For magnitude estimation, subjects were instructed to taste a reference sample (100 mM of sucrose in water) each time they evaluated solutions. This reference was assigned a value of 100 in sweet taste intensity. Nine concentrations for sucrose in water (12.87 to 218.79 mM) and skim milk (15 to 255 mM) were chosen, and 7 sucrose concentrations for skim chocolate milk (20 to 260 mM) were chosen. Each panelist evaluated each product in triplicate in separate sessions.

Acceptance of Sugar Reduction in Chocolate Milk by Young Adults. Chocolate milks with sweetness levels 205 mM (current commercial sucrose concentration equivalent), 142.2 mM, 86.6 mM, 52.7 mM, 32.1 mM, and 17.14 mM (recognition threshold of sweetness in skim chocolate milk), which represented 30.6, 57.8, 74.3, 84.3, and 91.5% sucrose reductions from 205 mM, were evaluated by consumers. The concentration levels were chosen from the results of power functions. Sucrose concentrations of 17.14 and 205 mM in chocolate milk were manufactured by the NCSU Dairy Education Unit. Raw milk (156 kg) was mixed with cocoa powder (1.54 kg) and sucrose (0.89 kg for 17.14 mM and 10.63 kg for 205 mM). The mixture was homogenized (2,758 kPa first stage and 3,447 kPa second stage), pasteurized at 79.4°C for 20 s, and then cooled to 4°C. Other sucrose concentrations were prepared by mixing these 2 concentrations.

Consumers were recruited using an online screener using SSI Web (version 8.1.2, Sawtooth Software, Orem, UT) and were from the consumer database of more than 5,000 people maintained by the NCSU Sensory Service Center, with the target number of consumers being 145. The recruitment criteria were 50% male, 50% female, ages 19 to 35 yr, and self-reported consumers of low-fat or skim chocolate milk at least 2 to 3 times per month. Samples were presented monadically, with the order of presentation completely randomized to prevent order of presentation effects. Chocolate milk with 86.6 mM sucrose was chosen arbitrarily as an extra sample tested in addition to the 6 other sucrose

concentrations for statistical purposes. Sixty milliliters of chocolate milk was poured into 3-digit coded, 177.4-mL clear plastic tumblers (Waddington North America Inc., Chelmsford, MA) for consumer evaluation.

Consumers first answered demographic questions and were then presented with a chocolate milk, one at a time, and evaluated each milk based on appearance and taste. Consumers were required to have a 1-min rest with water/cracker rinse between milks. For each product, liking based upon appearance was evaluated first. After consumers consumed the chocolate milk, they evaluated samples for overall liking, flavor liking, sweetness liking, and just about right (**JAR**), cocoa flavor liking and **JAR**, thickness liking and **JAR**, and purchase intent. The liking scale was a 9-point scale where 1 = dislike extremely and 9 = like extremely. The **JAR** scale was a 5-point scale where 1 or 2 = too little, 3 = just about right, and 4 or 5 = too much. Purchase intent was scored on a 5-point scale where 1 or 2 = would not buy, 3 = maybe/maybe not, 4 or 5 = would buy. Data collection was completed using Compusense Five (version 5.2, Compusense, Guelph, ON, Canada). All participants were compensated with a \$10 gift card to a local shopping store.

Acceptance of Sugar Reduction in Chocolate Milk by Children. Parents of children (n = 50 for 5–7 yr, n = 50 for 8–10 yr, n = 50 for 11–13 yr) were recruited from the same NCSU database, with the target number of consumers being 150 children. The recruitment criteria were children that drink chocolate milk at least 2 to 3 times per month. Parents and legal guardians of children in the target age range were prescreened using a screener created in SSI Web and launched into the database. In order for the children to participate in the test, their parents or legal guardians were required to read and sign a consent form that explained the purpose and the logistics of the study. An ingredients and allergens list for the chocolate milk was also provided and the test protocol was approved by the NCSU Institutional Review Board (NCSU IRB 1569-10-7).

Skim chocolate milks with sucrose concentrations at 86.6, 126, 140.6, 162, and 205 mM, which represented 57.8, 38.5, 31.4, 21.0, and 0% sucrose reductions from a current commercial sucrose concentration (205 mM) were tested. The concentrations were chosen from the young adults' acceptance test and manufactured as previously described. Chocolate milks were presented monadically at 4°C with order of presentation completely randomized to prevent order of presentation effects. Three milks were evaluated by 5- to 7-yr-old children (86.6, 140.6, and 205 mM), and all 5 milks were evaluated by 8- to 13-yr-old children. Chocolate milk (60 mL) was poured into 3-digit coded, 177.4-mL

clear plastic tumblers. The children were presented with a chocolate milk, one at a time, and evaluated samples based on taste. For each product, after the children consumed the chocolate milk, 5- to 7-yr-old children evaluated milks for overall liking; 8- to 10-yr-old children evaluated overall liking, sweetness, and chocolate flavor liking; and 11- to 13-yr-old children evaluated overall liking, sweetness liking and JAR, and chocolate flavor liking and JAR. The liking scale used was a 7-point "smiley face" scale where 1 = super bad and 7 = super good (Kroll, 1990; Guinard, 2000). The JAR scale was a 3-point smiley face scale where 1 = too little, 2 = just about right, and 3 = too much.

For 5- to 7-yr-old children, paper ballots were used. One moderator was assigned to each child. Before evaluating milks, the moderator obtained the child's favorite and least favorite food information from the parent and practiced the scale with the child after reading the scale aloud to the child. If the child successfully identified their favorite food with a high score and their least favorite food with a low score, the moderator then proceeded with the child to evaluate chocolate milks. A practice (warm-up) has been suggested to improve a child's performance in sensory testing (Popper and Kroll, 2005). After the children tasted the samples, they then used a pen to circle or point at the face that represented how much they liked it.

Children 8 to 13 yr of age evaluated the milks using a computer with the smiley face scale, and assistance was provided if needed. After evaluating all milks, children were directed to pick which one was their favorite. Parents were instructed to help with demographic questions to indicate the child's sex, age, and chocolate milk consumption frequency at the end. Data collection was completed using Compusense Five (version 5.2, Compusense). All participants were compensated with a \$25 gift card to a local shopping store.

Experiment 2: Alternative Sweetening by Lactose Hydrolysis

Lactose Hydrolysis. Four commercially available enzymes containing β -galactosidase were obtained from 4 distributors. The enzymes included Ha-Lactase (**HL**; Chr. Hansen Inc., New Berlin, WI), GODO-YNL2 Yeast Neutral Lactase (**GL**; Danisco A/S, Copenhagen, Denmark), Lactozyme lactase (**LL**; Sigma-Aldrich, St. Louis, MO), and Maxilact (**ML**; DSM Food Specialties, Heerlen, the Netherlands). All the enzymes were derived from the dairy yeast *Kluyveromyces*. Raw whole milk, pasteurized whole milk, pasteurized skim milk, chocolate milk base, and 5% (wt/wt) and 10% (wt/wt) lactose solutions in phosphate buffer prepared from 2 different manufacturers were tested with all 4 enzymes

at different concentration levels (0.1% wt/vol for milks and 5% wt/wt solutions, 0.1% wt/vol and 0.2% wt/vol for 10% wt/wt solutions) at 4°C for 12 and 24 h. Five percent (5% wt/wt) and 10% (wt/wt) solids rehydrated spray-dried permeate solutions prepared from 5 different suppliers, and a fresh liquid permeate produced in the pilot plant as described in Li et al. (2012) were also tested (0.1% wt/vol for 5% wt/wt solutions, 0.1% wt/vol and 0.2% wt/vol for liquid permeate and 10% wt/wt solutions) analogously.

Percentages of lactose hydrolysis were determined through HPLC using the method adapted from Upreti et al. (2006). One hundred microliters of each milk or solution was extracted in 900 μ L of mobile phase 0.013 *N* H₂SO₄ (2*N*, VWR International, West Chester, PA), vortexed, and centrifuged at 8,000 $\times g$ for 5 min. The top layer was filtered using 0.45- μ m nylon syringe filters (VWR International). Prepared samples were then placed on the HPLC (Waters 1525 Binary Pump, Waters, Milford, MA). Twenty microliters of the sample was injected (Waters 2707 Autosampler) onto the column (55°C, 0.5 mL/min, 300 $\times$ 7.8 ion exclusion, Bio-Rad Laboratories, Richmond, CA.). The injector temperature was at 4°C and a refractive index (RI) detector (Waters 2414 Refractive Index Detector, 30°C) was used. A 12-point standard curve for lactose (Sigma-Aldrich) was constructed over the range of 0.5 to 15,000 mg/kg in mobile phase. Lactose hydrolysis percentages were calculated based on the standard curve and dilution factors and comparison of hydrolyzed samples to unhydrolyzed control samples.

Descriptive Analysis of Hydrolyzed Chocolate Milk. The GL and HL enzymes were chosen from lactose hydrolysis results based on hydrolysis percentages. Bench-top processed skim chocolate milks (described previously) with 0, 2, 5, 7.5, or 10 g of added lactose (Hilmar 5000 Natural Lactose, Hilmar, CA) per 100 mL or spray-dried commercial powdered permeate (82.77% lactose) with 0, 2, 5, 7.5, or 10 g of lactose per 100 mL were hydrolyzed at 4°C for 24 h by GL and HL lactases with the goal to match the lowest acceptable sweetness level found in consumer tests. After hydrolysis, chocolate milks were heat treated at 85°C for 30 s, to deactivate the lactase, and cooled to 4°C. Milks were subjected to HPLC analysis to confirm lactose hydrolysis percentages (>90%). Before sensory evaluation, all milks were equilibrated to room temperature (20°C). A trained descriptive sensory analysis panel (*n* = 8, females 24 to 44 yr of age, not the same panelists as for sweet taste power functions) evaluated milk flavor attributes (aroma, cooked milk and chocolate flavor) and basic tastes (sweet, salty, and bitter) using the Spectrum descriptive analysis method (Meilgaard et al., 1999; Drake and Civille, 2003). Each panelist had more

than 150 h of experience with descriptive analysis of dairy products. Preliminary evaluations of commercial and experimental milks in blinded sessions confirmed that the panel and panelists were able to consistently differentiate ($P < 0.05$) products and attributes. Milks were presented in lidded 60-mL soufflé cups with 3-digit codes, and each sample was evaluated in duplicate by each panelist in separate sessions. Milks were presented to each panelist in a randomized monadic order with a 3-min enforced rest between samples. Panelists expectorated milks. Data were collected using Compusense Five (version 5.2, Compusense).

Young Adult Acceptance of Chocolate Milk Sweetened by Lactose Hydrolysis. Chocolate milks with sweetness levels 150 mM sucrose, 7.5 g/100 mL hydrolyzed lactose, and 10 g/100 mL hydrolyzed lactose were evaluated by young adult consumers (19–35 yr). The sucrose and hydrolyzed lactose concentrations were chosen from the results of the previous young adult sweetness reduction acceptance test and by descriptive analysis. Chocolate milks with sucrose and 2 different added lactose concentrations were manufactured by the North Carolina State University Dairy Education Unit, Raleigh, NC. The GL enzyme was added to lactose added chocolate milk at the concentrations of 0.3% (wt/vol) for 7.5 g/100 mL treatment and 0.4% (wt/vol) for 10 g/100 mL treatment to ensure >90% lactose hydrolysis within 24 h at 4°C. Hydrolyzed milk was heat treated with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 75°C for 16 s to deactivate the enzyme.

Before the consumer test, pilot plant-manufactured chocolate milk with sweetness levels 150 mM sucrose, 7.5 g/100 mL hydrolyzed lactose, and 10 g/100 mL hydrolyzed lactose were evaluated by descriptive analysis. Young adult consumers were recruited as previously described with the target number of consumers being 100. Sample presentations and questionnaire for consumers were the same as described in the previous section of young adult acceptance testing. All participants were compensated with a \$10 grocery gift card to a local shopping store. Compusense Five (version 5.2, Compusense) was used for data collection.

Statistical Analysis

Data analysis of power functions was performed using linear regression in XLSTAT version 2012.6.04 (Addinsoft, New York, NY). Analysis of variance was performed on young adult consumer liking data and hydrolysis percentage data using a generalized linear mixed model (PROC GLIMMIX) in SAS software (version 9.2, SAS Institute, Cary, NC). Analysis of variance with means separation (Fisher's least significant

difference) was performed on the liking data from children's test results and lactose hydrolyzed chocolate milk consumer test results (XLSTAT). Just-about-right scores were evaluated using chi-squared tests; statistical significance (superscript letters) for purchase intent (5-point scale) was obtained from Kruskal-Wallis non-parametric test; demographic questions were analyzed for frequency of choice (XLSTAT). All statistical analysis was performed at the 95% confidence interval.

RESULTS AND DISCUSSION

Experiment 1: Sugar Reduction in Skim Chocolate Milk

Thresholds and Power Functions of Sweetness in Different Media. The best-estimate detection threshold of sucrose in water was 7.77 mM. For skim milk, whole milk, and skim chocolate milk, the detection thresholds were 8.56, 11.39, and 7.81 mM, respectively. For the recognition threshold, sucrose in water was 12.87 mM, and for skim milk, whole milk, and skim chocolate milk, the threshold was 13.78, 15.63, 17.14 mM, respectively. Recognition thresholds established the starting points of sucrose concentrations for power functions in different matrices, to ensure that the sweetness intensity curve fell within the linear proportion of the power functions. The exponent for water (1.37) was higher than that of skim milk (1.22) and skim chocolate milk (1.12) ($P < 0.05$; Figure 1) and is consistent with Fujimaru et al. (2012). Lower power functions in milk compared with water make sense because milk, especially chocolate milk, is more complex than water. Similar results were observed for salty taste in dairy matrices by Drake et al. (2011), where cheese sauce had a lower exponent than soup and water. Iso-sweetness of a well-accepted commercial fat-free chocolate milk (17 g of added sucrose per 240 mL) was calculated as 205 mM sucrose using the power function of sucrose in skim chocolate milk, which provided the benchmark point for consumer tests.

Acceptance by Young Adults of Sweetness Reduction in Chocolate Milk. The sweetness levels for the young adult acceptance test were selected from the results of threshold and power functions where the lowest concentration was 17.14 mM (recognition threshold of sucrose in skim chocolate milk) and the highest concentration was 205 mM (benchmark point). The chocolate milk consumers ($n = 146$) were 49.3% male and 50.7% female, 19 to 35 yr of age, and were mostly Caucasian (65%) with annual incomes <\$55,000 (65%). Most of the consumers (>77%) did not have children. Sweetness liking scores increased with sweetness levels ($P < 0.05$). Sweetness levels of 205 and 142.2 mM were

Figure 1. Power functions of sucrose in water, skim milk, and skim chocolate milk.

not significantly different in overall liking, cocoa flavor liking, and thickness liking ($P > 0.05$), and they were both significantly higher compared with other sucrose levels ($P < 0.05$; Table 1). Most consumers (>70%) evaluated sweetness of 205 and 142.2 mM as JAR (Table 2). The results suggest that sugar reduction in chocolate milk is possible as long as it does not exceed 30% reduction (from 205 mM).

Sweetness is known to have a hedonic appeal. de Melo et al. (2009) found that sweetness was one of the drivers of liking for reduced-calorie chocolates. Similarly, Bayarri et al. (2011) found that sweetness was one of the drivers of liking for yogurt and yogurt-like products. Recently, Chollet et al. (2013) reported that consumers preferred yogurt with 10% added sugar, and that 30% sugar reduction from 10% added sugar in flavored yogurt was acceptable for consumers but 50% reduction would be too low. Thompson et al. (2004) reported that cocoa aroma was the driver of liking for chocolate milks in adult consumers, and this finding was subsequently confirmed by Thompson et al.

(2007). However, sugar or sweet taste reduction was not evaluated in these studies. Thompson et al. (2007) also reported that in focus groups, adult participants indicated that the commercial chocolate milks were often too sweet. Similarly, Li et al. (2014) reported that liking of coffee-flavored beverages was penalized by “too much” sweetness, but not as frequently as “too little” sweetness. These studies also suggest that the sugar content of most commercial chocolate milks could be reduced without affecting liking. In the current study, men reported higher liking scores for overall liking, flavor liking, and sweetness liking for chocolate milks, compared with women ($P < 0.05$). This is consistent with Mojet et al. (2005), in which men gave higher liking scores than women for chocolate drinks. Geiselman et al. (1998) reported that the liking of chocolate food for women was based on the perceived sweetness.

Acceptance by Children of Sweetness Reduction in Chocolate Milk. Hedonic scaling can be conducted with children over the age of 4 yr with proper scales, and older children (6–10 yr) can perform discrimina-

Table 1. Young adult liking scores for skim chocolate milk with varying sucrose concentrations¹ (n = 146)

Sucrose concentration		Overall liking	Overall flavor liking	Sweetness liking	Cocoa flavor liking	Thickness liking
g/240 mL	mM					
1.4	17.14	3.2 ^e	3.1 ^f	2.7 ^e	4.0 ^e	4.8 ^d
2.6	32.1	3.8 ^d	3.9 ^e	3.3 ^d	4.5 ^d	5.3 ^c
4.3	52.7	4.8 ^c	4.6 ^d	4.2 ^c	5.2 ^c	5.6 ^{bc}
7.1	86.6	5.8 ^b	5.7 ^c	5.3 ^b	5.8 ^b	5.9 ^b
11.7	142.2	6.8 ^a	6.8 ^b	6.6 ^a	6.7 ^a	6.5 ^a
16.8	205	7.2 ^a	7.2 ^a	6.9 ^a	7.0 ^a	6.8 ^a

^{a–f}Means of each attribute with different superscript letters in columns are significantly different ($P < 0.05$).

¹Data represent 146 consumers. Liking attributes were scored on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely.

Table 2. Just-about-right scores for skim chocolate milk with varying sucrose concentrations (n = 146, young adults)¹

Item ²	Sucrose concentration					
	17.14 mM (1.4 g/240 mL)	32.1 mM (2.6 g/240 mL)	52.7 mM (4.3 g/240 mL)	86.6 mM (7.1 g/240 mL)	142.2 mM (11.7 g/240 mL)	205 mM (16.8 g/240 mL)
Sweetness JAR (%)						
Not sweet enough	93.2 ^a	86.3 ^a	74.0 ^b	57.5 ^c	20.5 ^d	7.5 ^e
Just about right	5.5 ^d	11.0 ^d	24.7 ^c	39.0 ^b	71.2 ^a	74.0 ^a
Too sweet	1.4 ^c	2.7 ^c	1.4 ^c	3.4 ^{bc}	8.2 ^b	18.5 ^a
Cocoa JAR (%)						
Too mild	62.3 ^a	54.8 ^{ab}	47.9 ^{bc}	42.5 ^c	21.2 ^d	19.2 ^d
Just about right	22.6 ^c	30.1 ^d	41.8 ^c	53.1 ^b	74.0 ^a	74.0 ^a
Too strong	15.1 ^a	15.1 ^a	10.3 ^{ab}	4.5 ^b	4.8 ^b	6.8 ^b
Thickness JAR (%)						
Not thick enough	45.9 ^a	41.1 ^{ab}	37.7 ^{ab}	30.8 ^{bc}	24.0 ^c	21.9 ^c
Just about right	52.7 ^d	57.5 ^{cd}	61.0 ^{bcd}	64.0 ^{abc}	69.9 ^{ab}	73.3 ^a
Too thick	1.4 ^b	1.4 ^b	1.4 ^b	5.1 ^{ab}	6.2 ^a	4.8 ^{ab}

^{a-e}Means of each attribute with different superscript letters in rows are significantly different ($P < 0.05$).

¹Data represent 146 consumers.

²Just-about-right (JAR) scales were scored on a 5-point scale, where 1 or 2 = too little, 3 = just about right, and 4 or 5 = too much. The percentage of consumers that selected these options is presented.

tion tasks (Guinard, 2000). In the current study, for children 5 to 7 yr (n = 49), a higher percentage (55.1%) of children preferred the highest sugar concentration (205 mM) over the other 2 concentrations: 140.6 mM (30.6%) and 86.6 mM (14.3%; $P < 0.05$; results not shown). However, we detected no significant difference in overall liking scores between 205 and 140.6 mM sucrose (6.3 and 6.1 on a 7-point scale; $P > 0.05$), which were both significantly higher than 86.6 mM (5.2 on a 7-point scale; $P < 0.05$). Similar results were observed for children ages 8 to 10 yr (Table 3). A higher percentage (41.2%) of children preferred chocolate milk with 205 mM sucrose over the other 2 concentrations ($P < 0.05$). However, we observed no significant difference in overall liking scores for 205, 162, and 140.6 mM ($P > 0.05$), but 205 mM received significantly higher scores in sweetness liking and chocolate flavor liking compared with 140.6 mM ($P < 0.05$; Table 3).

The liking scores of all attributes showed a linear decrease with the decrease of sucrose concentrations below 140.6 mM ($P < 0.05$). For 11- to 13-yr-olds, differences

for overall liking, sweetness liking, and chocolate liking were observed between 205 and 162 mM sucrose (Table 4). A higher percentage (81.1%) of children perceived the chocolate milk as just about right in sweetness for 205 mM sucrose compared with 140.6 mM sucrose (64.2%; $P < 0.05$). However, we detected no difference in the percentages of children who thought the chocolate flavor was just about right for both concentrations ($P > 0.05$). A higher percentage (41.5%) of children preferred 205 mM over other sucrose concentrations ($P < 0.05$). The results of the current study showed that a clearer differentiation in liking scores with different sugar concentrations was observed with increasing age. Older children had more distinct preferences compared with younger children. It is also possible that the older children have better performance with lower noise because they can more effectively utilize the scale, which results in greater differences.

Guinard (2000) reported that children begin to develop reliable differentiation in the pleasantness of odors at the age of 5 yr, and another study showed that 6- to

Table 3. Acceptance scores for skim chocolate milk with varying sucrose concentrations (8–10 yr; n = 51)¹

Item ²	Sucrose concentration				
	205 mM (16.8 g/240 mL)	162 mM (13.3 g/240 mL)	140.6 mM (11.5 g/240 mL)	126 mM (10.3 g/240 mL)	86.6 mM (7.1 g/240 mL)
Overall liking	5.9 ^a	5.9 ^a	5.5 ^{ab}	5.2 ^b	4.1 ^c
Sweetness liking	5.7 ^a	5.6 ^{ab}	5.1 ^{bc}	4.7 ^c	3.7 ^d
Chocolate taste liking	6.1 ^a	6.0 ^{ab}	5.5 ^{bc}	5.1 ^c	4.1 ^d
Overall preference ³ (%)	41.2 ^a	13.7 ^b	15.7 ^b	17.6 ^b	11.8 ^b

^{a-d}Means of each attribute with different superscript letters in rows are significantly different ($P < 0.05$).

¹Data represent 51 consumers.

²Liking attributes were scored on a 7-point hedonic “smiley face” scale, where 1 = super bad and 7 = super good.

³Percentage of participants who preferred the sample.

Table 4. Acceptance scores for skim chocolate milk with varying sucrose concentrations¹ (11–13 yr; n = 53)

Item ²	Sucrose concentration				
	205 mM (16.8 g/240 mL)	162 mM (13.3 g/240 mL)	140.6 mM (11.5 g/240 mL)	126 mM (10.3 g/240 mL)	86.6 mM (7.1 g/240 mL)
Overall liking	6.0 ^a	5.0 ^b	4.9 ^b	4.9 ^b	3.3 ^c
Sweetness liking	5.5 ^a	4.9 ^b	4.7 ^b	4.5 ^b	3.2 ^c
Chocolate taste liking	5.7 ^a	5.0 ^b	4.7 ^b	4.6 ^b	3.4 ^c
Overall preference ³ (%)	41.5 ^a	20.8 ^b	13.2 ^b	17.0 ^b	7.5 ^b
Sweetness JAR (%)					
Not sweet enough	5.7 ^d	26.4 ^c	24.5 ^c	49.1 ^b	83 ^a
Just about right	81.1 ^a	62.3 ^{bc}	64.2 ^{ab}	43.4 ^c	15.1 ^d
Too sweet	13.2 ^a	11.3 ^{ab}	11.3 ^{ab}	7.5 ^{ab}	1.9 ^b
Chocolate JAR (%)					
Not enough chocolate	5.7 ^c	13.2 ^c	30.2 ^b	41.5 ^b	66 ^a
Just about right	79.2 ^a	73.6 ^{ab}	58.5 ^{bc}	54.7 ^c	26.4 ^d
Too much chocolate	15.1 ^a	13.2 ^{ab}	11.3 ^{ab}	3.8 ^b	7.5 ^{ab}

^{a–d}Means of each attribute with different superscript letters in rows are significantly different ($P < 0.05$).

¹Data represent 53 consumers.

²Liking attributes were scored on a 7-point hedonic “smiley face” scale, where 1 = super bad and 7 = super good. Just-about-right (JAR) scales were scored on a 3-point scale with smiley faces, where 1 = too little, 2 = just about right, and 3 = too much.

³Percentage of participants who preferred the sample.

12-yr-old children can scale the perceived intensity of sweet in orange-flavored beverages (Zandstra and de Graaf, 1998). Early studies of Desor et al. (1975) found that, compared with adults, children preferred higher concentrations of sucrose and lactose. However, in an ethnic group study, Thompson et al. (2007) reported that Hispanic children were less discerning than adults (Hispanic or Caucasian) in their preferences for chocolate milk sweetness and chocolate flavor. The chocolate milks in that study were all commercial products and had no sugar reduction (Thompson et al., 2007). In the current study, 11- to 13-yr-old children were more discerning on the sweetness of chocolate milk than were young adults. Young adults did not show differences on overall liking and sweetness liking between chocolate milks with 205 and 142.2 mM sucrose ($P > 0.05$), whereas 11- to 13-yr-old children showed differences between chocolate milks with 205 and 162 mM sucrose ($P < 0.05$). Monneuse et al. (1991) reported that boys preferred higher sweetness than girls, but in the current study, differences between boys and girls were not observed within each age category for children ($P > 0.05$). However, differences between male and female young adults were observed in the current study ($P < 0.05$), as previously described.

Because skim chocolate milk with 140.6 mM sucrose received an overall liking score of 4.9 on a 7-point scale for 11- to 13-yr-old children, where 5 equals good, and the overall liking score of 140.6 mM was not significantly different from that for skim chocolate milk with 205 mM sucrose for 5- to 10-yr-old children, we propose that the sucrose concentration in chocolate milk for both young adults and children can be reduced

to between 140.6 and 162 mM, which represents a 21 to 31% sugar reduction. Combined with young adult acceptance scores, 150 mM sucrose concentration in chocolate milk was chosen as the lowest acceptable sweetness level for subsequent experiments.

Experiment 2: Alternative Sweetening by Lactose Hydrolysis

Lactose Hydrolysis. Variability of lactose hydrolysis percentages (75–96%, 24 h) was observed among different milks (raw whole, pasteurized whole, and pasteurized skim), where LL resulted in the lowest percentage (75–84%) of hydrolysis compared with other enzymes (>95%; $P < 0.05$); no difference was detected among the other 3 enzymes for milks ($P > 0.05$). We also found no difference among different milks for each enzyme ($P > 0.05$; results not shown). Horner et al. (2011) also observed differences in lactose hydrolysis percentages among enzymes in refrigerated milks, and the hydrolysis was not affected by fat content or processing of milk. For spray-dried permeate, overall 0.2% (wt/vol) had higher percentages of hydrolysis for 10% (wt/wt) solutions compared with 0.1% (wt/vol) at both the 12- and 24-h time points ($P < 0.05$). Similar to milks, LL had the lowest percentages of hydrolysis for all permeates compared with the other 3 enzymes ($P < 0.05$). Variability was also observed among permeates from different suppliers (3.9–95%). However, total nitrogen and selected mineral analysis of potassium, calcium, magnesium, sodium, and chloride for all 5 permeates were not different ($P > 0.05$), which suggests that the differences in hydrolysis were due to other

component differences (Frankowski et al., 2014) or to differences in other minerals such as zinc, phosphate, or manganese (Kim et al., 1997; results not shown). Fresh liquid permeate had similar results to milks, where LL resulted in the lowest hydrolysis (>86%) compared with the other 3 enzymes (>96%; $P < 0.05$). Overall, 1 spray-dried permeate, 1 lactose, and the GL and HL enzymes were selected from lactose hydrolysis results because of consistent hydrolysis after 24 h at 4°C.

Descriptive Analysis of Hydrolyzed Chocolate Milk. Lactose hydrolysis by selected enzymes in chocolate milk with added lactose (0, 2, 5, 7.5, or 10 g/100 mL) and added spray-dried powdered permeate (0, 2, 5, 7.5, or 10 g of lactose content/100 mL) treatments were all >90%. Differences were not observed between GL and HL enzymes ($P > 0.05$; results not shown). Without added sugar, hydrolyzed skim chocolate milk had a sweetness score of 1.8 on an intensity scale from 0 to 15 compared with “not detected” (0) for chocolate milk with no hydrolyzed lactose (Figure 2). Lactose-free milk was confirmed to have higher sweet taste (Adhikari et al., 2010) compared with regular commercial milk. Hydrolyzed skim chocolate milk with 10 g of added lactose/100 mL matched the sweetness level of the benchmark chocolate milk (205 mM sucrose), and hydrolyzed skim chocolate milk with 7.5 g of added lactose/100 mL matched the sweetness level of the consumer-acceptable sucrose-reduced chocolate milk (150 mM sucrose; $P > 0.05$; Figure 2). For permeate treatments (Figures 3

and 4), hydrolyzed skim chocolate milk with permeate addition that contained 10 g of lactose matched the lowest acceptable sweetness of chocolate milk (150 mM sucrose; $P > 0.05$); however, the salty taste was obvious (7.6/15 intensity scale). As such, we determined that hydrolyzed chocolate milk with permeate addition was not suitable for a consumer test. Frankowski et al. (2014) reported that ingredients other than sodium, especially potassium and organic acids, in permeate contribute to salty taste. We detected no differences in aroma, cooked milk, or chocolate flavor of milks ($P > 0.05$). Bitterness was not detected in hydrolyzed chocolate milks with added lactose above 7.5 g/100 mL and added permeate with above 2 g of lactose content/100 mL. Studies have found that bitterness can be suppressed by salts (Keast et al., 2001; Lekrisompong et al., 2012), which explains why a higher content of lactose had to be added to milk compared with permeate added to milk to mask the bitterness of chocolate milk. The salty taste components of permeate may have contributed to reduced perception of bitter taste.

Young Adult Acceptance of Chocolate Milk Sweetened by Lactose Hydrolysis. Pilot plant-manufactured chocolate milk with 7.5 g/100 mL hydrolyzed lactose was lower in sweet taste than that of the 150 mM sucrose treatment, and the 10 g/100 mL lactose treatment was higher by descriptive analysis ($P < 0.05$). We detected no significant difference in other sensory attributes. One hundred three consumers

Figure 2. Trained panel sweet taste scores of skim chocolate milk with sucrose and lactose hydrolyzed skim chocolate milk with different levels of lactose addition (4°C for 24 h). Control = without hydrolysis; hydrolyzed = lactose hydrolyzed; ND = not detected. Different letters above bars signify significant differences ($P < 0.05$).

Figure 3. Trained panel sweet taste scores of skim chocolate milk with sucrose and lactose hydrolyzed skim chocolate milk with different levels of permeate addition (4°C for 24 h). Control = without hydrolysis; hydrolyzed = lactose hydrolyzed. Different letters above bars signify significant differences ($P < 0.05$).

participated in the consumer acceptance test (41.7% male and 58.3% female, 19–35 yr). The consumers were mostly Caucasian (58%) with annual incomes <\$55,000 (79%). Most consumers (>80%) did not have children. We detected no difference in appearance, overall liking, flavor, sweetness, cocoa flavor, thickness liking, or purchase intent between 10 g/100 mL hydrolyzed lactose milk and 150 mM sucrose milk (Table 5). The results are consistent with the previous young adult sweetness reduction acceptance test, which established that there was no difference in overall liking between benchmark

sweetness level (205 mM sucrose) and the lowest acceptable sweetness level (150 mM sucrose). Adhikari et al. (2010) found that other than higher sweetness intensity, ultrapasteurized lactose-free milk also has higher intensities of cooked and processed flavors compared with regular milk, and chalkiness is also present. Other flavor differences were not observed in the current study, perhaps because of the differences between plain milk and chocolate milk or due to processing and differences in age or storage among the commercial milks that were used in the previous study. The

Figure 4. Trained panel salty taste scores of skim chocolate milk with sucrose and lactose hydrolyzed skim chocolate milk with different levels of permeate addition (4°C for 24 h). Control = without hydrolysis; hydrolyzed = lactose hydrolyzed; ND = not detected. Different letters above bars signify significant differences ($P < 0.05$).

Table 5. Young adult acceptance scores for lactose hydrolyzed chocolate milks¹ (n = 103)

Item ²	Sucrose (150 mM)	Lactose high ³ (10 g/100 mL)	Lactose low ³ (7.5 g/100 mL)
Appearance liking	6.7 ^a	6.2 ^{ab}	6.1 ^b
Overall liking	6.8 ^a	6.6 ^a	6.0 ^b
Flavor liking	6.8 ^a	6.7 ^a	5.9 ^b
Sweetness liking	6.5 ^a	6.5 ^a	6.0 ^b
Sweetness JAR (%)			
Not sweet enough	24.3 ^b	16.5 ^b	39.8 ^a
Just about right	58.3 ^a	64.1 ^a	56.3 ^a
Too sweet	17.5 ^a	19.4 ^a	3.9 ^b
Cocoa flavor liking	6.9 ^a	6.6 ^a	5.9 ^b
Cocoa flavor JAR (%)			
Too mild	23.3 ^c	35.9 ^b	49.5 ^a
Just about right	70.9 ^a	60.2 ^{ab}	46.6 ^b
Too strong	5.8 ^a	3.9 ^a	3.9 ^a
Thickness liking	6.4 ^a	6.1 ^a	5.2 ^b
Thickness JAR (%)			
Too thin	32.0 ^b	40.8 ^{ab}	54.4 ^a
Just about right	63.1 ^a	58.3 ^a	41.7 ^b
Too thick	4.9 ^a	1.0 ^a	3.9 ^a
Purchase intent	3.5 ^a	3.4 ^a	2.9 ^b
Preference ⁴	40.8 ^a	43.7 ^a	15.5 ^b

^{a-c}Means of each attribute with different superscript letters in rows are significantly different ($P < 0.05$).

¹Data represent 103 consumers.

²Liking attributes were scored on a 9-point hedonic scale, where dislike extremely = 1 and like extremely = 9. Just-about-right (JAR) scales were scored on a 5-point scale, where too little = 1 or 2, just about right = 3, and too much = 4 or 5. Percentage of consumers that selected these options is presented. Purchase intent was scored on a 5-point scale, where would not buy = 1 or 2, may or may not = 3, and would buy = 4 or 5; for purchase intent, statistical letterings were obtained from Kruskal-Wallis nonparametric test.

³Lactose high is hydrolyzed lactose milk (GODO-YNL2 Yeast Neutral Lactase, 24 h at 4°C; Danisco A/S, Copenhagen, Denmark) with 10 g/100 mL added lactose; lactose low is hydrolyzed lactose milk (GODO-YNL2 Yeast Neutral Lactase, 24 h at 4°C) with 7.5 g/100 mL added lactose.

⁴Percentage of participants who preferred the sample.

amount of lactose required to achieve the same level of sweetness (>72 added kcal/240 mL) compared with regular sucrose sweetened chocolate milk (67.2 added kcal/240 mL) was too high to fulfill a calorie-reduced requirement. This is possibly because of the complexity of chocolate milk and the requirement to overpower the bitterness of cocoa. The viability of lactose hydrolysis as the sole sweetening source may be a possibility for other flavored milks such as vanilla that have no inherent bitter taste, and a lower level of sweetener and sweet taste may be acceptable. Future studies should address this application.

CONCLUSIONS

This study established the perception of sweetness intensity of sucrose in different dairy matrices and determined that sucrose reduction in chocolate milk for both children and young adults is possible as long as it does not exceed 30% reduction (from 205 mM). Sweetness of sucrose can be achieved by lactose hydrolysis of added lactose, and it is possible to use lactose hydrolysis of added lactose but not added permeate to sweeten chocolate milk. However, the additional

calories from added lactose indicate that this approach might be more suitable for other flavored milks with simpler characteristics, where the amount of added lactose can be reduced. Future studies should address the viability of lactose hydrolysis as a sweetening approach for other dairy beverages and investigate other alternative sweeteners.

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