Adapting blood glucose meter biosensors for the measurement of lactose in dairy ingredients

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ABSTRACT

Commonly used lactose assays [enzymatic spectrophotometric absorbance (EZA) and HPLC] for dairy ingredients are relatively expensive and time consuming. A blood glucose meter (BGM)-based method has successfully been documented as a rapid lactose assay in milk. However, the BGM-based method has not been evaluated in dairy ingredients. The objective of this study was to evaluate the BGM-based lactose analysis method in whey-derived (WD) and skim milk-derived (SMD) ingredients. The study was carried out in 4 phases. In phase 1, the effect of pH and lactose concentrations on the BGM reading was investigated using a factorial design with 2 factors: pH (6.02–7.50) and lactose (0.2 or 0.4%). We found that BGM readings were significantly affected by lower pH values at both lactose levels. In phase 2, the effect of total solids and ingredient type was investigated using a factorial design with 2 factors: ingredient type (WD or SMD) and total solids (0–8%). It was observed that the BGM reading was significantly affected by ingredient type and total solids. Phase 3 involved developing a linear relationship between the BGM reading and the EZA reference method to ascertain the accuracy of the proposed BGM method. Different ingredient types (WD or SMD) and non-lactose solids (0.5–27%) model ingredient dilutions prepared over a range of lactose contents (0.08–0.62%) were measured using the BGM and EZA methods. The average absolute percentage bias difference between the BGM method and EZA reference method results for these model dilutions was found to be between 2.2 and 7.3%. In phase 4, 15 samples procured from commercial sources ranging from 0.01 to 81.9% lactose were evaluated using the BGM method and EZA reference

method. The average absolute percentage bias difference for lactose results between the 2 methods ranged from 3.6 to 5.0% and 5.3 to 9.7% for well-performing and poorly performing meters, respectively. Overall, the BGM method is a promising tool for rapid and low-cost analysis of lactose in both high-lactose and low-lactose dairy ingredients.

Key words: lactose, dairy ingredient, biosensor, rapid method, blood glucose meter

INTRODUCTION

Lactose, a disaccharide sugar comprising glucose and galactose, is the principal carbohydrate present in dairy products (Huber and BeMiller, 2017). Lactose is a ubiquitous constituent of dried dairy ingredients, making up <2% in dry protein isolates and close to 100% in pure lactose powders (Huppertz and Gazi, 2016). Most common dairy ingredients are derived from either cheese whey or skim milk. The primary goal of protein isolate and concentrate manufacture is to remove lactose, fat, and minerals to fractionate the protein portion of the ingredient.

Routine analysis of lactose in dairy ingredients could lead to improved utilization of the ingredients as well as help correlate lactose concentration with the ingredient quality. For example, measuring the lactose content in permeate and retentate streams can be a valuable troubleshooting tool during the membrane concentration of dairy proteins (Olabi et al., 2015; Sluková et al., 2016). Likewise, incorporation of the correct amount of starting lactose can be a critical quality control tool for the optimization of whey fermentation (Sansone et al., 2010). Last, quality defects such as stickiness, caking, or browning in dairy powders are largely dependent on the amount and form of lactose present in dried ingredients (Olabi et al., 2015; Huppertz and Gazi, 2016; Gulzar and Jacquier, 2018).

Common methods of lactose analysis used by the dairy industry include HPLC (Upreti et al., 2006; Gabbanini et al., 2010) and enzymatic spectrophotometric

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absorbance assays (Lynch and Barbano, 2007). These established methods have a high level of sophistication and long analysis times that can be overcome by using biosensors, which are generally faster, simpler, and less expensive to use (Conzuelo et al., 2012; Glithero et al., 2013; Sharma and Leblanc, 2017). Few commercial biosensors for the measurement of lactose can be found in the current marketplace, largely due to the complexity of dairy product composition (Booth et al., 2017). Only recently, a commercial biosensor designed for lactose has been documented in the literature: the Biomilk 300 (Biolan Microbiosensores, Zamudio, Spain), which is tailored for the analysis of residual lactose in lactose-free dairy products (Churakova et al., 2019). Another lactose biosensor, Lactosens (Chr. Hansen Holding A/S, Hoersholm, Denmark), was also commercially launched with limited availability.

As a low-cost alternative to proprietary lactose biosensors, blood glucose meter (**BGM**) biosensors, usually associated with diabetes care, can be adapted to measure lactose in milk (Amamcharla and Metzger, 2011). First, lactose in a diluted sample is hydrolyzed using lactase enzyme, and the resulting glucose is measured using the BGM. Then, the BGM output is compared with a standard curve to measure the percentage of lactose in the original sample.

Any BGM is a possible candidate for measuring lactose as long as the enzymatic detection is based on glucose oxidase, glucose dehydrogenase–nicotinamide adenine dinucleotide, or glucose dehydrogenase–flavin adenine dinucleotide. Blood glucose meter sensors based on glucose dehydrogenase–pyrroloquinoline quinone cannot be used for lactose measurement because galactose can interfere with the glucose measurement (Schleis, 2007; Frias et al., 2010). Additionally, some sample matrix and environment-dependent interferences are known to cause bias in blood glucose measurements and may interfere in measurement of lactose in dairy ingredients. For example, a sample pH <6.8 was shown to erroneously provide lower BGM output for glucose measurements in blood (Dungan et al., 2007). Varying hematocrit levels (volume of red blood cells relative to plasma volume in blood) have been shown to bias the BGM output for glucose measurements in blood on a hematocrit-proportional basis (Ramljak et al., 2013). The BGM measurement accuracy can also be affected by temperature and, to a lesser extent, humidity (Erbach et al., 2016). To create a robust BGM-based lactose measurement method, this work explored strategies to compensate for potential sample matrix-dependent interferences such as pH and concentration of non-lactose solids in samples (similar to hematocrit effect on BGM output in blood).

MATERIALS AND METHODS

Experimental Design

The efficacy of the proposed BGM method was studied in 4 phases. In phase 1, the effect of pH and lactose concentration on BGM output bias was investigated. In phase 2, BGM measurements were performed on several dairy ingredient types and total solids combinations to evaluate the effect of these parameters on the BGM output. The findings from phases 1 and 2 were applied to phase 3, where a calibration procedure for quantification of lactose in model dairy ingredient dilutions was verified by analyzing the linear relationship of paired lactose results between the proposed BGM method and a reference method. In phase 4, the developed method was used to quantify the lactose content of commercial dairy ingredients to assess the robustness of the proposed method.

Buffer, Dairy Ingredients, and Solution Preparation

Phosphate-buffered saline was used as a diluent. A PBS buffer of 1× strength was prepared in 1-L batches from 10× PBS (Fisher Scientific, Pittsburgh, PA) and deionized water. Hydrochloric acid (1 N; Acros Organics, Pittsburgh, PA) was then used to adjust the pH (pH = 7 unless otherwise indicated). A calibrated Accumet XL150 pH meter (Fisher Scientific) was used for all pH measurements. Then, ACS-certified grade α -lactose monohydrate (Fisher Scientific) was used to adjust the lactose content of any model dilutions prepared. Whey-derived (**WD**) or skim milk-derived (**SMD**) ingredients described in this work were procured from Agropur Ingredients (Appleton, WI) and were obtained in dry form. All the powders were mixed with the titrated PBS diluent at room temperature using an overhead mixer equipped with a propeller-type spindle for 10 min, and the resulting dispersions were stored between 1 and 4°C for at least 24 h to ensure a complete rehydration and equilibrium of α - and β -lactose anomers before any further analysis.

Lactase Enzyme

Enzeco lactase NL β -galactosidase or lactase enzyme used in this work was a gift from the Enzyme Development Corporation (New York, NY). According to the material specification data, the enzyme is prepared from a dairy yeast belonging to the *Kluyveromyces* genus and has an activity level between 2,450 and 3,150 yeast lactase units/g, with an optimum temperature between 31 and 40°C and solution pH between 6 and 7.

BGM Measurements

The BGM units used in this work include the ReliOn Prime (Walmart, Bentonville AR), Nova Max Plus (Nova Biomedical Corp., Waltham, MA), OneTouch Verio Flex (LifeScan Europe, Zug, Switzerland), and FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK). All the units were capable of measuring at least 20 to 500 mg/dL (0.02–0.5%) glucose in blood. To ensure proper functioning, new test strip lots were tested using the manufacturer-recommended control glucose solutions before any measurements.

No special sample preparation except dilution in PBS buffer was required for BGM lactose analysis. The BGM lactose analysis proposed in the current research involved weighing 5.0 g of diluted sample into a test tube, thoroughly mixing the sample with 0.1 g of lactase enzyme (~245 yeast lactase units), incubating the mixed sample at 40°C for approximately 15 min, and then measuring the resulting glucose content by pipetting the incubated sample directly onto the capillary site of a BGM test strip. To maintain a consistent sample temperature, dilutions were removed from the incubation water bath just before the measurement and then promptly placed back into the water bath. All the BGM measurements were carried out in a temperature-controlled room (22–24°C) with relative humidity measured between <20 and 39%.

Enzymatic Spectrophotometric Absorbance Reference Method

An enzymatic spectrophotometric absorbance (EZA) assay was used as a reference method to quantify lactose in both model and commercial ingredient dilutions. Commercial lactose/D-galactose enzyme assay kits (R-Biopharm AG, Darmstadt, Germany) provided all necessary reagents to carry out the analysis. Instructions provided with the kits detailed the assay preparation and calculations, with minor adaptations as recommended by Lynch and Barbano (2007). A DU-650 UV-VIS spectrophotometer (Beckman Coulter, Brea, CA) was used to measure the absorbance of prepared assays at a wavelength of 340 nm. Disposable acrylic cuvettes with a 1-cm path length were used. Each sample was measured in duplicate, with all results reported as the average of duplicates.

All samples destined for EZA analysis were first diluted with deionized water to adjust the lactose concentration to approximately 0.05% (wt/wt). Solutions containing only lactose or low amounts of whey solids ($\leq 3\%$) were filtered through 0.45- μm nylon syringe filters and stored between 1 and 4°C for later use in the EZA assay. Samples containing caseins were

also prepared using a similar method, except these solutions were centrifuged at $2,097 \times g$ for 10 min at room temperature (Savant high-speed microcentrifuge HSC10K, Thermo Scientific, Waltham, MA) before filtering. Samples containing higher amounts of whey solids ($< 27\%$) were impossible to filter through syringe filters due to their viscosity; these solutions were first clarified via centrifugation at $8,410 \times g$ for 10 min and then filtered through 0.2- μm nylon centrifuge filters with a 2-mL capacity at $5,000 \times g$ for 15 min at room temperature (Legend XTR, Thermo Scientific).

Phase 1

The effect of solution pH and lactose concentration on BGM output bias was investigated in a 2×6 factorial design for 4 BGM brands. Model lactose solutions in PBS buffer with 0.2 and 0.4% (wt/wt) lactose concentrations were prepared at different pH levels (7.50, 7.00, 6.73, 6.53, 6.27, and 6.02) per the experimental design. The solutions were measured in a random order, in triplicate, using the proposed BGM method with 1 lot of test strips for each meter. The pH of each solution was measured again after assay preparation to ensure that great changes in buffered pH did not occur due to lactose and lactase addition. The BGM output measured for each sample was compared with the calculated glucose content of each lactose solution after hydrolysis (lactose %, wt/wt, divided by 2) to calculate a percentage bias difference (D_b) for each reading. The GLM procedure in SAS (Studio version, SAS Institute Inc., Cary, NC) was used for data analysis, treating pH and lactose concentration as class variables. Significant differences ($P < 0.05$) between experiment levels were detected using Tukey's honest significant difference test.

Phase 2

The effect of non-lactose solids amount and dairy ingredient type on BGM output was investigated using a 2×5 factorial experiment for 4 BGM brands. Model WD or SMD solutions at 5 total solids levels (0, 2, 4, 6, or 8%) were spiked with glucose monohydrate (Archer Daniels Midland, Chicago, IL) stock of known concentration to achieve 0.2% (wt/wt) glucose concentration directly measurable by the BGM biosensors. Whey protein isolate or micellar casein concentrate ($\geq 88\%$ db protein) were used to generate the WD or SMD solutions, respectively, due to their individual purities in the primary non-lactose component in dairy ingredients—namely, protein. Each solution was measured in a random order, in triplicate, using a slight modification of the proposed BGM method where 0.1 g of deionized

Table 1. One possible dilution scheme for lactose analysis of dairy ingredients via blood glucose meter, arranged by increasing dry-basis lactose content¹

Example ingredient	Estimated lactose (% dry basis)	Suggested dilution factor	Estimated post-hydrolysis glucose in dilution ² (mg/dL)	Approximate solids in dilution from dairy ingredient ³ (%)
Whey protein isolate	0.20	0.2857	30	26.9
Whey protein isolate	2.00	0.2857	280	26.9
Milk protein isolate 85	3.50	0.0327	60	3.08
Whey protein concentrate 80	9.00	0.0327	150	3.08
Milk protein concentrate 70	18.00	0.0327	300	3.08
Whey protein concentrate 55	31.00	0.0053	80	0.50
Nonfat dry milk	51.00	0.0053	130	0.50
Sweet whey	73.00	0.0053	190	0.50
Permeate	84.00	0.0053	220	0.50
Lactose	100.00	0.0053	260	0.50

¹The goal of any given dilution is to maintain a 0.03 to 0.3% (30–300 mg/dL) solution glucose level after lactose hydrolysis (assuming ~100% lactose hydrolysis), as this glucose range can be read by most meters.

²Calculated by dividing estimated diluted lactose percentage by 2 and multiplying by 1,000.

³Includes initial dilution, lactase addition, and assumed 4% moisture content of typical dried dairy ingredients.

water was added instead of 0.1 g of lactase enzyme such that the only analyte being measured was the glucose from the direct addition to the solutions (the dairy ingredients used as background solids are glucose free). The total solids content of each fully prepared dilution was verified using a Mark 3 LTE moisture analyzer (Sartorius AG, Goettingen, Germany). A percentage bias difference (D_s) for each reading was calculated between the BGM output values and known glucose concentration directly spiked into each solution. The GLM procedure in SAS was used for data analysis for each meter separately, treating ingredient type and added solids target as class variables.

Phase 3

Phase 3 was designed to assess the accuracy of the proposed calibration procedure for lactose analysis in common dairy ingredients and was carried out in 2 parts. Calibration equations were developed between the actual lactose concentration and corresponding BGM output measured for a series of model dairy ingredient dilutions. Subsequently, validation of these calibration parameters was performed in the second part.

Calibration Procedure. Eighteen WD model calibration standards were developed as follows: powdered whey protein isolate was mixed with PBS buffer to create 0.5, 3.0, or 27% total whey solids slurries that represented powder dilution factors of 0.0053, 0.0327, and 0.2857, respectively (Table 1 provides for further information on the selection of dilution schemes). The stock sample slurries were then partitioned into 6 samples each and spiked with lactose to achieve a final concentration between 0.08 and 0.62% (wt/wt) lactose. Similarly, 12 SMD model calibration standards were

developed: micellar casein was mixed with PBS buffer to create 0.5 or 3.0% total skim milk solid slurries (a 27% solids solution was not considered due to high viscosity). The SMD slurries were also divided into 6 samples each and spiked with lactose to achieve a final concentration between 0.10 and 0.58% lactose. Each calibration solution was measured with the proposed BGM assay in a random order and in duplicate using the proposed BGM method with 3 different strip lots each for the Nova Max Plus and FreeStyle Precision Neo brands. Each solution was also analyzed for lactose using the EZA reference method. The glucose results from the BGM method were paired with the lactose results from the EZA method (in mg/dL glucose and mg/dL lactose, respectively) to develop linear calibration curves that can be used to convert BGM signals to percentage lactose that are specific for each solids combination, BGM brand, and BGM test strip lot.

Verification Procedure. Eighteen WD and 12 SMD independent verification standards were prepared as described in the calibration procedure. The independent verification samples were then measured using the proposed BGM lactose assay alongside the EZA reference lactose assay, with the BGM results (in mg/dL glucose) being converted to percentage lactose using Equation 1 in conjunction with the linear parameters (slope and intercept) derived in the calibration procedure:

$$\% \text{ lactose} = \frac{\text{meter reading} - \text{intercept}}{\text{slope} \times F_i \times 1,000}, \quad [1]$$

where F_i is the product of dilution factors 1 to i . F_i is the product of all the dilutions performed through the measurement procedure.

The calibration-adjusted BGM results and the EZA reference results (both in percentage wt/wt lactose) were then paired together and method comparison statistics between the 2 methods were carried out using the REG procedure in SAS to determine confidence intervals ($\alpha = 0.05$) for the resulting linear slope and intercept parameters. Microsoft Excel (Microsoft Corp., Redmond, WA) was used to calculate average absolute percentage bias difference (**AD**) and average bias difference (**d**) between the paired calibration-adjusted BGM lactose result and EZA reference method lactose result. A coefficient of variation was also calculated between duplicate BGM measurements, with results expressed as an average result for all 3 test strip lots used.

Phase 4

Lactose in a sampling of commercial dairy ingredients was measured in duplicate using both the proposed BGM method and the EZA reference method. Two lots of test strips each were used for the Nova Max Plus and FreeStyle Precision Neo BGM brands. Ingredients were mixed with buffer at dilution factors of 0.0053 (0.5% total ingredient solids), 0.0327 (3.0% total ingredient solids), and 0.2857 (27% total ingredient solids) for 31 to 100, 3.5 to 18, and <2.0% lactose dry-basis ingredients, respectively (Table 1 provides further information on the selection of dilution schemes). The corresponding linear calibration curve parameters established in phase 3 were used to convert BGM outputs to percentage lactose in phase 4. Excel was used to calculate AD and d between each paired BGM-EZA lactose results, and a coefficient of variation was calculated between duplicate BGM measurements.

RESULTS AND DISCUSSION

Phase 1

Table 2 details the effect that pH and lactose concentration may have on BGM output bias, expressed as percentage bias difference (D_h) between the calculated post-glucose content of the lactose-hydrolyzed solutions and the BGM output. The addition of lactase and lactose did not greatly alter the buffer pH (≤ 0.08 pH unit shift after addition).

Effect of Lactose Concentration. At a given pH level, lactose concentration can have a significant effect on BGM output bias as observed by noncomparable ($P < 0.05$) D_h between the 2 lactose levels tested. The ReliOn Prime meter showed a strong positive bias associated with lactose concentration given that every result between the 0.2 and 0.4% lactose levels of the experiment was significantly ($P < 0.05$) different. On

Table 2. The effect of sample solution pH and lactose concentration on meter output percentage bias difference¹ (D_h) between the expected and measured glucose content for 4 blood glucose meter (BGM) brands²

pH	Nova Max Plus			FreeStyle Precision Neo			OneTouch Verio Flex			ReliOn Prime		
	0.2% Lactose	0.4% Lactose		0.2% Lactose	0.4% Lactose		0.2% Lactose	0.4% Lactose		0.2% Lactose	0.4% Lactose	
7.50	-17.54 ± 1.14 ^{a,x}	-16.34 ± 0.71 ^{a,x}		32.54 ± 0.79 ^{a,x}	39.88 ± 1.09 ^{a,x}		-1.35 ± 1.83 ^{a,x}	-3.85 ± 0.74 ^{a,x}		40.56 ± 1.83 ^{a,x}	78.43 ± 1.34 ^{a,y}	
7.00	-17.84 ± 1.21 ^{a,x}	-15.93 ± 4.18 ^{a,x}		34.95 ± 2.00 ^{a,x}	41.49 ± 3.51 ^{a,x}		-2.39 ± 0.70 ^{a,x}	-11.11 ± 0.73 ^{b,y}		39.08 ± 2.10 ^{a,x}	82.81 ± 1.36 ^{a,y}	
6.73	-17.77 ± 0.70 ^{a,x}	-17.78 ± 5.10 ^{a,x}		32.64 ± 2.37 ^{a,x}	40.51 ± 6.20 ^{a,x}		-5.32 ± 0.53 ^{b,x}	-18.16 ± 0.13 ^{c,y}		36.33 ± 4.13 ^{a,x}	80.07 ± 1.63 ^{a,y}	
6.53	-17.96 ± 1.41 ^{a,x}	-19.78 ± 1.38 ^{a,x}		37.14 ± 1.39 ^{a,x}	37.49 ± 2.85 ^{a,x}		-7.65 ± 1.22 ^{b,x}	-22.43 ± 0.69 ^{d,y}		34.06 ± 3.00 ^{a,x}	79.54 ± 1.36 ^{a,y}	
6.27	-22.94 ± 0.46 ^{a,x}	-28.82 ± 3.98 ^{b,x}		8.44 ± 2.40 ^{b,x}	22.60 ± 5.25 ^{b,y}		-25.24 ± 1.22 ^{c,x}	-34.12 ± 0.82 ^{e,y}		5.37 ± 1.16 ^{b,x}	50.02 ± 3.80 ^{b,y}	
6.02	-44.87 ± 1.16 ^{b,x}	-59.58 ± 0.27 ^{c,y}		-36.58 ± 1.62 ^{c,x}	-30.67 ± 1.20 ^{c,x}		-54.08 ± 0.53 ^{d,x}	-56.72 ± 1.34 ^{f,x}		-42.12 ± 0.96 ^{c,x}	-31.60 ± 1.20 ^{c,y}	

^{a-f} D_h values within a column with different superscripts differ significantly ($P < 0.05$).

^{x,y} D_h values within a row within a BGM type with different superscripts differ significantly ($P < 0.05$).

¹ $D_h = [(A - B)/B] \times 100\%$, where A is a given BGM reading and B is the calculated glucose content for a given solution after lactose hydrolysis. Values are means ± SD; n = 3.

²Nova Max Plus (Nova Biomedical Corp., Waltham, MA), FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK), OneTouch Verio Flex (LifeScan Europe, Zug, Switzerland), and ReliOn Prime (Walmart, Bentonville, AR).

the other hand, the FreeStyle Precision Neo meter has a similar bias, albeit nonstatistically for most pH levels tested. Conversely, the OneTouch Verio Flex appears to have a negative bias associated with lactose content, with the trend being significant ($P < 0.05$) at all pH levels tested except 7.50 and 6.02. The Nova Max Plus meter was unique, showing no discernable ($P > 0.05$) lactose-proportional bias at any of the pH levels tested except the lowest pH level of 6.02. Because the BGM output biases were nonzero and dependent on lactose concentration for most meters, any BGM will need to have its output calibrated over a range of lactose concentrations to make useful quantifications. The need for calibration of the meter output was thus necessary and found to be consistent with other literature where the BGM method has been used for lactose quantification (Amamcharla and Metzger, 2011; Heinzerling et al., 2012; Churakova et al., 2019). The nonzero bias apparent in all of the meter outputs was likely due to material differences between the background matrices measured as opposed to what the meters were originally developed for (i.e., blood).

Effect of pH. For all meters except the OneTouch Verio Flex, the BGM output biases were found to be comparable ($P > 0.05$) between the 7.50 and 6.53 pH levels within a lactose level and BGM brand, indicating that these meters should provide accurate measurement within this pH range once a calibration is created to correct for the constant apparent measurement bias. The OneTouch Verio Flex meter specifically appears to be very pH sensitive, with a strong positive bias associated with pH, indicating that this meter lacks measurement flexibility. Additionally, because the lowest pH level (6.02) always registered a significantly ($P < 0.05$) lower BGM output bias than any other pH level within a lactose level and BGM brand and the pH level of 6.27 showed a practical (albeit not always statistical) bias difference, it is recommended that measurements not be made at pH <6.53. These results are consistent

with reports showing unreliable BGM performance when measuring glucose in blood at pH <6.8 due to changes in the activity level for the mediator enzymes in a test strip (Dungan et al., 2007). In addition, a reduction in lactase activity during incubation step of the BGM method hinders the hydrolysis of lactose into glucose and galactose and lowers the amount of glucose in the solution. Per the lactase enzyme manufacturer's specifications, the lactase has a pH-dependent hydrolysis rate maximum between pH 6 and 7, and thus the finished dilution pH range for all further solution tested in this work was controlled to between 6.7 and 7.0 even though phase 1 results showed that no significant pH-dependent bias will be present in measurements utilizing some BGM sensors within the pH range of 6.53 to 7.50.

Phase 2

Table 3 details the effect that different amounts or types of dairy ingredients can have on a given BGM brand's output bias, with bias being expressed as the percentage bias difference (D_s) between the spiked glucose content (0.2% wt/wt) of each solution and the meter's output. Figure 1 illustrates the effect for each individual model dilution analyzed.

Effect of Ingredient Type. All meter output biases except for the ReliOn Prime meter were significantly ($P < 0.05$) affected by ingredient type. However, Figure 1 qualitatively indicates that the differences between similar-solids WD and SMD entries were relatively small on an individual basis, and it is worth noting that ingredient type contributed much less to the model error than did added solids amount (on average for all meters, 9.28 and 81.22% of the ANOVA model error were coming from solids type and amount, respectively). It is also impossible to ascertain whether the differences seen due to ingredient type are truly due to the differences in ingredient character or to slight dif-

Table 3. Statistical summary detailing the effect of skim milk- or whey-derived solids at different addition rates on percentage bias difference (D_s) between expected and measured glucose values for 4 blood glucose meter brands¹ at a fixed, spiked glucose concentration of 0.2%

Source of error	df	Nova Max Plus		FreeStyle Precision Neo		OneTouch Verio Flex		ReliOn Prime	
		MSS ²	P-value	MSS	P-value	MSS	P-value	MSS	P-value
Ingredient type	1	48.25	0.0317*	303.6	<0.0001*	68.39	0.0116*	40.68	0.2047
Added solids amount	4	255.9	0.0011*	1,066	<0.0001*	2,257	<0.0001*	1,956	<0.0001*
Type × amount	4	79.62	0.1056	161.1	0.0129*	34.68	0.4414	91.48	0.4477
Model error	9	388.5		1,530		2,361		2,088	
Total error	29	559.0		1,724		2,538		2,562	

¹Nova Max Plus (Nova Biomedical Corp., Waltham, MA), FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK), OneTouch Verio Flex (LifeScan Europe, Zug, Switzerland), and ReliOn Prime (Walmart, Bentonville, AR).

²Mean sum of squares.

*Statistically significant at $P < 0.05$.

ferences in actual total solids content measured between corresponding WD and SMD dilution series (data not shown). All the same, it may conservatively be best practice to account for solids type during calibration and subsequent quantifications.

Effect of Added Solids Amount. All of the meter output biases were significantly ($P < 0.05$) affected by added solids amount. As detailed in Figure 1, increasing levels of solids for either ingredient type tested resulted in signal depression (decreasing D_s) for the

Figure 1. Effect of skim milk-derived (SMD) or whey-derived (WD) solids at different TS levels on percentage bias difference (D_s) between expected and measured glucose values at a fixed, spiked glucose concentration of 0.2%. Blood glucose meters: (a) Nova Max Plus (Nova Biomedical Corp., Waltham, MA); (b) FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK); (c) OneTouch Verio Flex (LifeScan Europe, Zug, Switzerland); (d) ReliOn Prime (Walmart, Bentonville, AR). $D_s = (A - B)/B \times 100\%$, where A is a given blood glucose meter reading and B is the fixed, spiked glucose concentration known for each test solution. Values are means; error bars represent SD; $n = 3$.

FreeStyle Precision Neo meter and ReliOn Prime BGM brands or signal elevation (increasing D_s) for the Nova Max Plus and OneTouch Verio Flex BGM brands. This could be related to the analogous hematocrit effect in blood, where increasing levels of non-glucose solids may hinder glucose from getting to the enzyme test site. Intuitively, this explanation should universally translate into decreased BGM output; however, the Nova Max Plus and OneTouch Verio flex meters were both known to algorithmically adjust their outputs for hematocrit when measuring glucose in blood (Ramljak et al., 2013), so it is theorized that the positive relationship between D_s and solids level observed for these meters is a result of the correction algorithm overcompensating for the presence of dairy ingredients. These results imply that the solids level of an ingredient dilution must be accounted for during calibration. The most common commercial dairy ingredients were therefore partitioned into 3 dilution schemes that controlled the total solids into a dilution while simultaneously keeping glucose concentrations between 0.03 and 0.3% (30–300 mg/dL) post-lactose hydrolysis, well within the measurable range for most BGM brands. To model these dilution schemes, total solids levels of 0.5, 3.0, and 27.0% were chosen to represent measurement-ready dilutions for 31 to 100, 3.5 to 18, and <2.0% lactose dry-basis ingredients, respectively. Assuming a moisture content of 4% for most dairy powders, the 0.5, 3.0, and 27.0% total solids translate into dilution factors of 0.0053, 0.0327, and 0.2857, respectively.

Meters Selected for Further Testing. The Nova Max Plus and FreeStyle Precision Neo meters were selected for further testing in phases 3 and 4. The Nova Max Plus promises robust performance due to its low and relatively constant bias over the range of lactose and solids levels tested. The OneTouch Verio Flex and ReliOn Prime meters were not used for further testing because they showed strong output bias dependence on background pH and solids amounts, respectively.

Phase 3

The calibration data are not shown but were of high quality. For example, none of the calibration curves had a coefficient of determination (R^2) value less than 0.986, indicating good linearity. Also, a limit of detection (LOD) was calculated for each calibration curve and test strip lot used ($LOD = 3 \times RMSE/slope$, where RMSE is the root mean squared error for a particular model) and averaged 0.037% (0.024–0.060%) lactose for the Nova Max Plus meter or 0.051% (0.025–0.062%) lactose for the FreeStyle Precision Neo, well below the lactose levels measured in this work. Also, it was determined from the calibration data that test strip

lot-specific calibration parameters should be used for subsequent lactose quantifications as evidenced by non-comparable ($P < 0.05$) slope parameters between some of the test strip lots used in the higher solid's samples. The paired method statistics between the EZA reference method and calibration-adjusted BGM method lactose results are shown in Tables 4 and 5 for the independently prepared verification WD and SMD model dilutions, respectively.

WD Ingredient Dilutions. The BGM method precision was found to be acceptable with an average coefficient of variation $\leq 4.93\%$ for both of the meters studied. Accuracy in terms of agreement between the proposed BGM and EZA reference method was also good, with only 2 instances where regression slopes were significantly different from 1 (3.0% WD model dilutions, FreeStyle Precision Neo, strip lot 2; 27% WD model dilutions, Nova Max Plus, strip lot 2) and only 1 instance where intercepts were significantly different from 0 (27% WD model dilutions, Nova Max Plus, strip lot 2). A slope and intercept not statistically different ($P > 0.05$) from 1 or 0 indicated good agreement between the 2 methods. Average bias difference (d) in lactose results between the methods was relatively small, with the average value of $d \leq 0.02\%$ lactose in absolute value terms. Also, average bias difference did not trend high or low for any of the WD model dilutions, test strip lots, or meters tested, indicating that any differences observed between the test and reference methods were random. Examining the AD still invokes a favorable bias comparison between the BGM and EZA reference method given an average AD between 2.97 and 7.32%. However, examining the range of AD for each average listed in Table 4 reveals some relatively high values; the highest absolute percentage bias differences ($AD > 15\%$) are universally associated with the lowest lactose concentrations measured (<0.12% lactose). It is expected that there is a consistent amount of random error associated with the BGM measurement that is small in absolute terms but relatively high on a percentage difference basis when these errors occur at lower lactose concentrations.

SMD Ingredient Dilutions. Interpretations of the results for the SMD model dilutions were largely similar to those of the WD model dilutions. It can be concluded that the proposed dilution and ingredient type-based calibration schemes are likely to be successful in accurately predicting the lactose content of commercial dairy ingredient samples.

Phase 4

The as-is (wt/wt) lactose contents of 15 dry commercial dairy ingredients measured using the EZA

Table 4. Comparison between the blood glucose meter (BGM) method and enzymatic spectrophotometric absorbance (EZA) reference method for lactose measurement using BGM strip lot-specific calibration parameters for model whey-derived (WD) verification dilutions for 2 BGM brands¹

Dilution model and parameter	Nova Max Plus			FreeStyle Precision Neo		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
0.5% WD						
Slope	1.008	0.965	0.953	0.988	0.989	1.037
Intercept	0.002	0.018	0.025	0.003	0.009	0.000
R ²	0.990	0.978	0.986	0.977	0.990	0.990
95% CI slope	0.935 to 1.080	0.862 to 1.068	0.875 to 1.036	0.880 to 1.096	0.918 to 1.061	0.959 to 1.116
95% CI intercept	-0.025 to 0.028	-0.021 to 0.056	-0.006 to 0.055	-0.037 to 0.044	-0.018 to 0.036	-0.029 to 0.030
d ²	0.00 (-0.03 to 0.02)	0.01 (-0.03 to 0.04)	0.01 (-0.02 to 0.05)	0.00 (-0.03 to 0.04)	0.01 (-0.01 to 0.04)	0.01 (-0.01 to 0.05)
AD ³	5.69 (0.49 to 19.5)	6.23 (1.09 to 13.9)	6.87 (1.18 to 22.7)	5.45 (1.02 to 11.4)	4.60 (0.14 to 13.5)	4.64 (0.55 to 14.0)
Average CV ⁴ (%)		2.75 (0.35 to 6.60)			2.84 (0.00 to 7.08)	
3.0% WD						
Slope	1.029	1.050	1.006	1.032	1.082*	0.960
Intercept	-0.001	0.001	0.006	-0.007	-0.018	0.003
R ²	0.996	0.990	0.991	0.993	0.995	0.989
95% CI slope	0.983 to 1.075	0.973 to 1.126	0.937 to 1.076	0.973 to 1.092	1.025 to 1.139	0.890 to 1.030
95% CI intercept	-0.019 to 0.016	-0.027 to 0.030	-0.021 to 0.032	-0.030 to 0.015	-0.039 to 0.003	-0.023 to 0.030
d	0.01 (-0.01 to 0.02)	0.02 (-0.01 to 0.04)	0.01 (-0.02 to 0.03)	0.00 (-0.02 to 0.02)	0.01 (-0.02 to 0.03)	-0.01 (-0.04 to 0.02)
AD	3.29 (0.18 to 11.5)	6.16 (0.48 to 13.0)	5.76 (0.07 to 24.1)	3.68 (0.28 to 13.5)	5.87 (1.08 to 15.7)	5.10 (0.87 to 11.7)
Average CV (%)		3.00 (0.00 to 9.27)			2.66 (0.48 to 8.10)	
27% WD						
Slope	0.985	0.961*	1.035	1.045	1.019	0.984
Intercept	0.004	0.009*	-0.007	-0.007	0.000	-0.001
R ²	0.997	0.998	0.995	0.986	0.986	0.984
95% CI slope	0.946 to 1.023	0.931 to 0.992	0.983 to 1.087	0.958 to 1.131	0.931 to 1.106	0.887 to 1.064
95% CI intercept	-0.006 to 0.015	0.000 to 0.0172	-0.007 to 0.006	-0.030 to 0.017	-0.024 to 0.024	-0.025 to 0.023
d	0.00 (-0.01 to 0.01)	0.00 (-0.01 to 0.01)	0.00 (-0.02 to 0.02)	0.00 (-0.02 to 0.03)	0.00 (-0.02 to 0.04)	-0.01 (-0.04 to 0.02)
AD	3.84 (0.02 to 10.8)	2.97 (0.43 to 11.3)	5.46 (0.73 to 18.0)	5.16 (0.33 to 11.1)	4.47 (0.38 to 12.0)	7.32 (0.59 to 16.1)
Average CV (%)		2.01 (0.31 to 11.20)			4.93 (0.00 to 10.84)	

¹Nova Max Plus (Nova Biomedical Corp., Waltham, MA); FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK).

²Average bias difference. $d = A - B$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Numbers in parentheses are a range. Units are % lactose.

³Average absolute percentage bias difference. $AD = ((A - B)/B) \times 100\%$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Numbers in parentheses are a range.

⁴Calculated by averaging individual BGM CV % results for a given BGM and model dilution set. Numbers in parentheses indicate the range.

*Statistical difference ($P < 0.05$) from 1 or 0 for the slope or intercept parameters, respectively.

Table 5. Method comparison statistics between the blood glucose meter (BGM) method and enzymatic spectrophotometric absorbance (EZA) reference method for lactose measurement using BGM strip lot-specific calibration parameters for model skim milk-derived (SMD) verification dilutions for 2 BGM brands¹

Dilution model and parameter	Nova Max Plus			FreeStyle Precision Neo		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
0.5% SMD						
Slope	1.011	1.021	0.990	0.958	1.080*	1.030
Intercept	-0.002	-0.002	0.010	0.018	-0.024	-0.007
R ²	0.994	0.996	0.996	0.986	0.992	0.993
95% CI slope	0.956 to 1.067	0.976 to 1.065	0.945 to 1.035	0.878 to 1.039	1.009 to 1.151	0.971 to 1.089
95% CI intercept	-0.023 to 0.018	-0.018 to 0.015	-0.007 to 0.027	-0.012 to 0.048	-0.050 to 0.002	-0.029 to 0.015
d ²	0.00 (-0.02 to 0.03)	0.01 (-0.01 to 0.02)	0.01 (-0.01 to 0.02)	0.00 (-0.02 to 0.03)	0.00 (-0.02 to 0.05)	0.00 (-0.02 to 0.03)
AD ³	2.17 (0.09 to 8.23)	2.95 (0.07 to 6.89)	3.64 (0.01 to 10.23)	4.55 (0.72 to 9.95)	5.69 (1.18 to 14.7)	4.69 (0.40 to 12.9)
Average CV ⁴ (%)		2.45 (0.00 to 6.14)			2.75 (0.00 to 8.48)	
3.0% SMD						
Slope	0.926*	0.967	1.003	1.039*	0.961	1.033
Intercept	0.020*	0.013	0.009	-0.012	0.011	0.002
R ²	0.997	0.993	0.997	0.998	0.995	0.997
95% CI slope	0.890 to 0.963	0.909 to 1.025	0.965 to 1.040	1.006 to 1.072	0.911 to 1.012	0.993 to 1.073
95% CI intercept	0.007 to 0.034	-0.008 to 0.035	-0.005 to 0.022	-0.024 to 0.000	-0.007 to 0.030	-0.013 to 0.017
d	0.00 (-0.02 to 0.02)	0.00 (-0.03 to 0.02)	0.01 (0.00 to 0.02)	0.00 (-0.01 to 0.02)	0.01 (-0.01 to 0.02)	0.00 (-0.01 to 0.01)
AD	4.78 (0.15 to 21.7)	3.81 (0.30 to 10.9)	4.09 (0.03 to 19.7)	2.47 (0.42 to 7.47)	4.56 (0.10 to 8.90)	3.14 (0.77 to 9.73)
Average CV (%)		1.47 (0.00 to 4.68)			2.10 (0.30 to 9.71)	

¹Nova Max Plus (Nova Biomedical Corp., Waltham, MA); FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK).

²Average bias difference. $d = A - B$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Numbers in parentheses are a range. Units are % lactose.

³Average absolute percentage bias difference. $AD = (|A - B|/B) \times 100\%$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Numbers in parentheses are a range.

⁴Calculated by averaging individual BGM CV % results for a given BGM and model dilution set. Numbers in parentheses are a range.

*Statistical difference ($P < 0.05$) from 1 or 0 for the slope or intercept parameters, respectively.

Table 6. Comparison of lactose concentration (%) measured using the blood glucose meter (BGM) method and enzymatic spectrophotometric absorbance (EZA) reference method in commercial dairy ingredient samples using BGM strip lot-specific calibration parameters for the Nova Max Plus (Nova Biomedical Corp., Waltham, MA)

Ingredient	Lactose concentration (%)								
	EZA	BGM		CV ¹ (%)		d ² (%)		AD ³ (%)	
		Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Milk permeate	81.9	81.1	83.3	1.75	0.42	−0.78	1.41	0.96	1.72
NDM (low heat)	51.4	49.2	51.3	6.46	2.72	−2.20	−0.09	4.29	0.18
NDM (high heat)	49.6	49.6	50.7	7.14	4.83	−0.01	1.12	0.01	2.26
Buttermilk	47.2	44.7	45.4	0.79	1.54	−2.55	−1.86	5.39	3.93
Milk protein concentrate 70	12.9	12.7	13.6	0.78	9.81	−0.16	0.70	1.23	5.46
Milk protein isolate 85	5.78	6.18	5.91	0.79	3.91	0.40	0.13	6.89	2.33
Micellar casein	4.37	5.14	4.83	2.87	1.40	0.77	0.46	17.6	10.6
Whey permeate	76.3	71.2	71.1	1.91	1.82	−5.18	−5.28	6.78	6.91
Sweet whey	68.8	69.6	69.3	1.46	2.79	0.84	0.57	1.22	0.83
Whey protein concentrate 34	49.1	45.0	47.2	1.50	0.68	−4.17	−1.93	8.48	3.92
Whey protein concentrate 80	5.90	5.59	5.99	0.00	2.58	−0.31	0.09	5.22	1.59
Whey protein isolate 1	1.47	1.41	1.44	3.65	3.57	−0.05	−0.04	3.65	3.02
Whey protein isolate 2	0.03	<0.04	<0.05	—	—	—	—	—	—
Whey protein isolate 3	0.01	<0.04	<0.05	—	—	—	—	—	—
Whey protein isolate 4	0.70	0.68	0.67	4.21	0.70	−0.02	−0.02	3.05	3.54
Averages				2.56	2.83	−1.03	−0.36	4.98	3.56

¹Calculated between duplicate BGM measurements for each test strip lot. $n = 2$.

²Average bias difference. $d = A - B$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Units are % lactose.

³Average absolute percentage bias difference. $AD = (|A - B|/B) \times 100\%$, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result.

reference method and proposed BGM method with the modified calibration procedure are shown in Tables 6 and 7 for the Nova Max Plus and FreeStyle Precision Neo meter, respectively. The BGM method's precision was acceptable for both the Nova Max Plus and FreeStyle Precision Neo meters, with average coefficient of variation <2.83% and <3.54%, respectively. Accuracy of the BGM method in terms of agreement between the BGM results and EZA results was found to depend on the meter used.

Nova Max Plus Accuracy. The Nova Max Plus BGM achieved relatively accurate results, with an average AD of 4.98 and 3.56% for test strip lots 1 and 2, respectively. One outlier was noted in the Nova Max Plus results when measuring lactose in micellar casein (AD = 17.58 and 10.60% for test strip lots 1 and 2, respectively). The high error in terms of AD for micellar casein is consistent with observations in phase 3 where random BGM measurement error is inflated on a percentage basis at lower lactose concentrations; micellar casein was relatively low in lactose for the 3.0% SMD dilution-specific calibration series, measuring 0.14% lactose via the EZA method before adjusting for dilution (calibration range was 0.10–0.58% lactose). Thus, accurately measuring low amounts of lactose for a given calibration series may be a limitation of the BGM method. An alternative hypothesis for the high AD associated with this particular micellar casein ingredient

could be the glycosylation of the κ -casein macropeptide portion of the protein. Glycosylated dairy proteins can be covalently linked to glucose, galactose (Recio et al., 2009), or lactose (Lillard et al., 2009), and the extent of glycosylation in κ -casein macropeptide in particular can be difficult to predict because it depends largely on a cow's lactation cycle (O'Riordan et al., 2014). Although it is unlikely that these protein-attached carbohydrates could cause measurement inaccuracies with the proposed BGM lactose analysis, it must be mentioned as a possibility because proving otherwise is beyond the scope of this work.

FreeStyle Precision Neo Accuracy. The FreeStyle Precision Neo results showed a lower level of accuracy relative to the Nova Max Plus, with AD = 5.32 and 9.73% for test strip lots 1 and 2, respectively. A plausible explanation for the large amount of seemingly random error associated with the FreeStyle Precision Neo may relate to observations in phase 2, where the FreeStyle Precision Neo was the only BGM with an output bias significantly ($P < 0.05$) affected by the interaction between the type and amount of added total solids (see Table 3). The FreeStyle Precision Neo showed acceptable accuracy in phase 3 when the SMD material used to create the calibration standards was the same as the independent verification samples measured, implying that poor performance of the FreeStyle Precision Neo with commercial samples may stem from

material discrepancies between the commercial samples and the SMD ingredient chosen as background during calibration; this issue appears to be greatly exacerbated as higher levels of solids are incorporated (see high AD values associated with whey protein isolates 1 and 4 in Table 7). Thus, the FreeStyle Precision Neo may not be flexible enough to measure lactose in many different kinds of materials using the same model calibration parameters, which limits its practical use.

Blood glucose meter biosensors can be successfully adapted to measuring lactose in dairy ingredients if the following key concepts are adhered to:

1. A 1× PBS buffer should be used in dilutions as needed to control the final dilution pH to between 6.7 and 7.0.
2. Dilution schemes and corresponding calibration standards specific for a select range of dairy ingredients should be used. The composition of the material used in the calibration standards should loosely resemble the ingredients ultimately being measured (i.e., SMD vs. WD ingredients). Total diluted solids presence of 0.5, 3.0, and 27% can represent 31 to 100, 3.5 to 18, and <2.0% expected dry-basis lactose ingredients, respectively (these solids levels represent dilution factors of 0.005, 0.0327, or 0.2857, respectively, for future commercial sample measurements). Pure lactose

can be used to adjust the lactose concentration of the calibration standards anywhere from 0.08 to 0.6% as-is lactose.

3. A practical application of the method will use a BGM that is accurate, precise, and flexible (the Nova Max Plus worked well here).

CONCLUSIONS

A BGM can be used to routinely measure lactose in dairy ingredients. The suggested dilution factors are relevant to most dried dairy ingredients, but different dilution factors can be adapted to other dairy ingredients outside the scope of this work (e.g., liquid retentate) as long as the same concepts are applied. Once calibration curves have been developed, the advantages of the BGM method include achieving results in about 20 min, requiring no hazardous chemicals or expensive instrumentation, and requiring no sample preparation outside of sample dilution. Also, the method is low cost enough for routine use given a single test cost of less than US\$0.50 for the test strip and a one-time cost of approximately US\$20 for the BGM. It is not recommended that the BGM method be used to verify lactose absence in foods because lactose is not quantified directly with the method; thus, this method is more useful for applications such as process optimization or design, ingredient standardization, product develop-

Table 7. Comparison of lactose concentration (%) measured using the blood glucose meter (BGM) method and enzymatic spectrophotometric absorbance (EZA) reference method in commercial dairy ingredient samples using BGM strip lot-specific calibration parameters for the FreeStyle Precision Neo (Abbott Diabetes Care Ltd., Witney, UK)

Ingredient	Lactose concentration (%)								
	EZA	BGM method		CV ¹ (%)		d ² (%)		AD ³ (%)	
		Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Milk permeate	81.9	87.4	95.8	4.25	5.56	5.56	13.89	6.79	17.0
NDM (low heat)	51.4	54.3	60.9	4.01	1.98	2.98	9.50	5.81	18.5
NDM (high heat)	49.6	52.0	54.7	1.68	4.42	2.47	5.15	4.98	10.4
Buttermilk	47.2	51.3	50.6	0.85	2.38	4.05	3.41	8.57	7.23
Milk protein concentrate 70	12.9	13.1	14.6	1.65	2.08	0.22	1.67	1.67	13.0
Milk protein isolate 85	5.78	5.84	6.24	2.46	8.46	0.06	0.46	1.05	7.91
Micellar casein	4.37	4.27	4.32	6.73	3.49	−0.10	−0.05	2.32	1.15
Whey permeate	76.3	74.9	78.6	2.47	0.82	−1.41	2.28	1.85	2.99
Sweet whey	68.8	65.7	67.8	3.74	3.78	−3.04	−0.97	4.42	1.42
Whey protein concentrate 34	49.1	49.1	49.2	2.08	1.30	−0.06	0.05	0.12	0.11
Whey protein concentrate 80	5.90	5.44	5.74	1.98	0.68	−0.32	−0.03	5.37	0.44
Whey protein isolate 1	1.47	1.34	1.68	1.60	1.28	−0.13	0.21	9.13	14.0
Whey protein isolate 2	0.03	<0.04	<0.05	—	—	—	—	—	—
Whey protein isolate 3	0.01	<0.04	<0.05	—	—	—	—	—	—
Whey protein isolate 4	0.70	0.82	0.92	1.75	9.80	0.12	0.23	17.1	32.3
Averages				2.71	3.54	0.80	2.75	5.32	9.73

¹Calculated between duplicate BGM measurements for each test strip lot. n = 2.

²Average bias difference. d = A − B, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result. Units are % lactose.

³Average absolute percentage bias difference. AD = (|A − B|/B) × 100%, where A is the average calibration-adjusted BGM method lactose result and B is the average EZA reference method lactose result.

ment, and routine lactose measurements for quality control purposes.

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