ASEAN Journal on Science and Technology for Development

Volume 41 | Number 3 Article 4

2024

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Recommended Citation

V. Fundador, Erwin Oliver; E. Rivera, Karyn Thea; Calumba, Kriza Faye A.; and V. Fundador, Noreen Grace (2024) "On-Site Estimation Of Total Sugars In Flavored Milk Using A Glucometer," *ASEAN Journal on Science and Technology for Development*: Vol. 41: No. 3, Article 4.
DOI: https://doi.org/10.61931/2224-9028.1592
Available at: https://ajstd.ubd.edu.bn/journal/vol41/iss3/4

ARTICLE

On-site Estimation of Total Sugars in Flavored Milk Using a Glucometer

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Abstract

Sugar has been linked to obesity and chronic diseases. Unfortunately, some "healthy products," such as flavored milk, contain high sugar. In the Philippines, such an issue is made worse by local brands not putting nutritional labels on their products. Thus, a cheap, home-based method to measure the total sugars in flavored milk would be beneficial. In this paper, we have shown that after incubation with invertase and lactase, a glucometer can be used to estimate total sugars in flavored milk on-site. All experiments except the weighing of invertase and lactose were done in a home setting for actual proof of concept. The total sugar of four commercial milk products was determined, and the method's accuracy was estimated based on the nearness to the labeled claim. The estimated accuracy was 96.26, 111.41, 106.19, and 111.35 % for Nestlé Fresh Milk, Chuckie, Chocolait, and Nutriboost, respectively. These results would help health-conscious individuals who need at least a ballpark figure for their sugar intake. With glucometers costing only 7 USD and the test strips 0.2 USD a piece when purchased online, the study suggests that this simple method can be used for on-site estimation of total sugars.

Keywords: Glucometer, Sucrose, Lactose, Invertase, Lactase

1. Introduction

Consuming products high in sugar has been linked to a higher risk of obesity and chronic diseases (Mahato et al., 2020; Palma-Morales et al., 2023). These products include flavored milk, often advertised as a healthy option (Patel et al., 2018). Flavored milk may contain as much as two times the sugar as its unflavored counterparts (Coyle et al., 2019). In some countries like the Philippines, such an issue is even magnified due to local brands not placing nutritional labels on their products. Thus, a cheap on-site method to measure the total sugars would be beneficial.

Flavored milk contains lactose and added sugars in the form of sucrose or high fructose corn syrup (Mahato et al., 2020). In the Philippines, the added sugar is likely sucrose. Therefore, an on-site method to quantify both lactose and sucrose is needed.

Existing methods for quantifying lactose in milk products are laborious, costly, and require skilled technicians. These include high-performance liquid chromatography (Erich et al., 2012), an enzymatic method (Sánchez-Manzanares et al., 1993), polarimetry (Caprita, 2014), gravimetry (Amamcharla and Metzger 2011), and, recently, high-resolution ultrasonic spectroscopy (Lynch and Buckin 2023). Commercial kits are also available

Received 26 November 2023; revised 3 May 2024; accepted 7 May 2024.
Available online 14 June 2024

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https://doi.org/10.29037/2224-9028.1592

based on the enzymatic hydrolysis of lactose to glucose and galactose (Sigma Aldrich, 1998a). However, these kits require a 96-well plate reader, which is huge and expensive. Glucometers, on the other hand, can also measure lactose using the same principle (Amamcharla and Metzger, 2011; Wagner et al., 2020). These devices are not only cheap but also easily obtained.

Methods for quantifying sucrose in milk have limitations, are complicated, need more reagents, and are time-consuming. Previously, a quick way to determine sucrose in milk using Fourier transform infrared spectroscopy technique combined with multivariate analysis has been developed (Balan et al., 2020). Commercial kits can also be bought (Sigma Aldrich, 1998b). Both techniques require equipment that is not only bulky but expensive as well. However, sucrose has already been quantified in bread products using a cheap blood glucose meter (Fundador and Calumba, 2020). Therefore, quantifying sucrose and total sugars in milk products using a glucometer (i.e., blood glucose meter) is also possible.

Glucometers are designed to quantify the amount of glucose present in the blood, and the present study explores the extended function of glucometers for food analysis. Before detection, flavored milk must be incubated with lactase and invertase. Lactase degrades lactose to glucose and galactose, whereas invertase degrades sucrose to glucose and fructose. The glucose released after hydrolysis (Heinzerling et al., 2012) shall serve as the analytical signal representing the total sugars in flavored milk.

In this paper, we report a cheap, rapid, and easy glucometer-based method to estimate the amount of total sugars (i.e., lactose and sucrose) in flavored milk on-site. This was done by determining the percentage accuracy (%) of the method in determining the total sugars in four big brand names of milk products to assess the robustness of the method across a range of milk formulations. For actual proof of concept, all experiments except for weighing invertase and lactose standards were done on-site (i.e., in a home) using household reagents. The temperature and humidity were around 26.5 °C and 82% during the experiment, respectively.

It is worth noting that the word estimate was used instead of quantify because the percentage accuracy (%) was determined by comparing the measured value (for total sugars) against the labeled claim. The exact value (for total sugars) may deviate from the labeled claim for each production lot during manufacturing.

2. Materials and methods

2.1. Materials used

Glucometer. A blood glucose meter (GA-3, Sannuo Yizhun, China) with dimensions 100 × 58 × 23 mm was used. The apparatus came with test strips, but additional ones were also purchased separately.

Incubator. An egg incubator made from styrofoam, a heating element, and a thermostat was purchased online (Shopee, Philippines).

Reagents. ACS reagent grade lactose monohydrate (Dalkem, Quezon City, Philippines) was used as standard. β-galactosidase or lactase from dietary supplement lactase tablets was purchased from Webber Naturals-WN Pharmaceuticals ® Ltd (Coquitlam, BC, Canada). The invertase enzyme (I4504) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Milk Products. Commercial milk products were purchased from a local supermarket in Davao City, Philippines. These include Nestlé Fresh Milk (Nestlé Philippines Inc., Cabuyao, Laguna), Chuckie (Nestlé Philippines Inc, Cabuyao, Laguna), Chocolait (Magnolia Inc., Cavite), and Nutriboost Chocolate (Coca-Cola Beverages Philippines, Inc., Taguig).

2.2. Estimation of lactose and total sugars in milk products using a glucometer

Reagent Preparation. A homemade pH 5.5 acetate buffer solution was prepared using Nature's Spring alkaline (pH 9) drinking water from Philippine Spring Water Resources, Inc (Mandaue City, Cebu, Philippines), adjusted to approximately pH 5.5 using acetic acid obtained from commercially available distilled white vinegar from Kraft Heinz Food Company (Pittsburgh, PA, USA). The final pH was determined using a pH paper. It is worth noting that alkaline water was deliberately used so that more vinegar is needed to reach a pH of 5.5 and thus increase its buffering capacity. Lactase and invertase were dissolved in pH 5.5 acetate buffer to produce fresh solutions of 10 mg lactase tablet/mL and 20 mg invertase/mL, respectively. Each milligram of lactase tablet contains approximately 36 FCC (Food Chemical Codex) lactase units.

Sample Preparation for Lactose Determination. Each milk product (100 μL) was added to an Eppendorf tube containing both the fresh lactase (1.2 mL). Pipette mixing was done, and the mixture was incubated for 3 h at 37 °C using an egg incubator. The glucose released after hydrolysis represents the amount of lactose in the milk products. In this case, 100 μl of the sample was diluted to 1300 μl.

As seen in Equation (1), the dilution factor ($X_{lactose}$) was 13.

Sample Preparation for Total Sugars Determination. Each milk product (100 μL) was added to an Eppendorf tube containing both the fresh lactase (1.2 mL) and invertase solutions (20 μl). Pipette mixing was done, and the mixture was incubated for 3 h at 37 °C using an egg incubator. The glucose released after hydrolysis represents the total sugars of the milk products, specifically lactose plus sucrose. In this case, 100 μl of the sample was diluted to 1320 μl. As seen in Equation (1), the dilution factor ($X_{total\ sugar}$) was 13.2.

Estimating Lactose and Total Sugar (i.e., Lactose and Sucrose) in Milk Products. Glucometer readings were taken by putting the capillary site of the glucometer test strip in contact with a drop of the incubated sample preparation. The measured lactose (M_{lactose}) or total sugar ($M_{\text{total sugars}}$) per serving was calculated using Equation (1), while the % accuracy was obtained using Equation (2).

$$M = [G * L * X * S] / 1000000 \quad (1)$$

Where:

$$\text{Lactose} \xrightarrow{Lactase} \text{Galactose} + \text{Glucose}\ (\textit{moles glucose equal to moles lactose})$$

$$\text{Sucrose} \xrightarrow{Invertase} \text{Fructose} + \text{Glucose}\ (\textit{moles glucose equal to moles sucrose})$$

M = measured Lactose (M_{lactose}) or total sugar ($M_{\text{total sugars}}$) per serving (g)

G = glucometer reading raw (G_{raw}) or corrected (G_{corr}) (mmol/L)

L = molar mass of lactose or sucrose (342 g/mol)

X = dilution factor without ($X_{\text{lactose}} = 13$) or with ($X_{\text{total sugar}} = 13.2$) invertase

S = serving size of milk product (mL)

$$\%\ \text{Accuracy} = [(M / C) \times 100] \quad (2)$$

Where:

M = measured Lactose or total sugar per serving (g)

C = claimed Lactose or total sugar per serving (g)

2.3. Correcting the raw glucometer reading (G_{raw}) with the use of lactose standards

Standard Preparation for Lactose. Lactose solutions (85.0, 167, 254, and 346 mM) in pH 5.5 acetate buffer were prepared. Each lactose solution (100 μL) was mixed with 1.2 mL of fresh lactase (10 mg lactase tablet/mL) and 20 μL of invertase (20 mg invertase/mL) in an Eppendorf tube, resulting in standard solutions containing 6.44, 12.67, 19.23 and 26.20 mM lactose, respectively. The mixture was then incubated for 3 h at 37 °C.

Sample Preparation. Sample preparations for estimating lactose and total sugars were prepared as stated above.

Determination of the Calibration Factor at Each Glucometer Reading. Raw glucometer readings (G_{raw}) were taken by putting the capillary site of the glucometer test strip in contact with a drop of the incubated standard preparation. The calibration factor (CF) for each raw glucometer reading was then computed as shown in Equation (3).

$$\text{CF} = G_{actual} / G_{raw} \quad (3)$$

Where:

G_{actual} = Actual Concentration of Lactose (mM)

G_{raw} = Raw Glucometer Reading for Lactose (mM)

Estimation of Lactose and Total Sugar (i.e., Lactose and Sucrose) in Milk Products. The raw glucometer readings (G_{raw}) for the sample preparations were multiplied by the CF (corresponding to the nearest G_{raw}) to get the corrected glucometer reading (G_{corr}). G_{corr} was then plugged into Equation (1) and Equation (2).

3. Results and discussion

Lactose is the sugar found in milk. Flavored milk contains added sugar in the form of sucrose or high fructose corn syrup (HFCS) (Mahato et al., 2020). In the Philippines, the added sugar is probably sucrose. Lactase and invertase are needed to hydrolyze both disaccharides and release glucose for glucometer detection.

For flavored milk that contains HFCS, no invertase is needed for quantitation. HFCS comprises approximately a 50/50 mixture of glucose and fructose (Marcus, 2013) and is practically hydrolyzed sucrose. However, when only lactase was used, Table 1 shows measured sugar values close to the estimated lactose content. Therefore, the added sugar is probably sucrose, and lactose was the only sugar that was determined.

Since the lactose contents of Chocolait and Chuckie were not declared on the nutritional label, the values presented in Table 1 were calculated based on their fresh milk counterparts. For example, one serving of Magnolia Fresh Milk contains 1.33 g lactose per gram of milk protein. Since one serving of Chocolait includes 5 g of protein and no other

Table 1. Percentage accuracy (%) of the method using raw glucometer readings (G_{raw}).

Claimed Lactose per Serving (g)	G_{raw} after lactase treatment (mmol/L)	Measured lactose per serving (g)	% Accuracy for Lactose
Chuckie (6.67[a])	8.9 ± 0.29	7.1 ± 0.23	106.44 ± 3.44
Chocolait (6.67[a])	7.2 ± 0.32	8.0 ± 0.35	119.94 ± 5.25
Nutriboost (10)	5.8 ± 0.25	8.5 ± 0.37	85.00 ± 3.70
Claimed Total Sugar per Serving (g)	G_{raw} after lactase and invertase treatment (mmol/L)	Measured Total Sugar per serving (g)	% Accuracy for Total Sugars
Nestlé Fresh Milk (11.8)	9.8 ± 0.17	11.1 ± 0.19	93.7 ± 1.65
Chuckie (14.7)	16.2 ± 0.15	13.2 ± 0.12	89.9 ± 0.84
Chocolait (23)	17.4 ± 0.40	19.7 ± 0.45	85.7 ± 1.98
Nutriboost (33)	18.7 ± 0.52	27.8 ± 0.77	84.4 ± 2.34

Note: Results are means ± standard deviations of triplicate measurements.
Note: Serving sizes for each milk product are 250 mL for Nestlé Fresh Milk, 180 mL for Chuckie, 250 mL for Chocolait, and 330 mL for Nutriboost.
[a] Based on the Assumption that the amount of lactose is around 133% the labelled claim for protein (5 g per serving).

ingredient in the product contains protein, the lactose content can be calculated to be 6.67 g (i.e., 5 × 1.33). It is worth noting that Chocolait and Magnolia Fresh Milk are produced by the same company (i.e., Magnolia). In the case of Chuckie, the fresh milk counterpart was Nestlé Fresh Milk.

3.1. Quantitation of lactose and total sugar in milk products using the raw glucometer reading (G_{raw}) as basis

Quantitation of total sugars in milk products was done by incubating the sample with invertase and lactase. Table 1 shows that flavored milk products contain significantly more sugar than fresh milk. The percentage accuracy (%) based on the measured total sugar per serving of Nestlé Fresh Milk, Chuckie, Chocolait, and Nutriboost were 93.7, 89.9, 85.7, and 84.4, respectively. The results deviate from the labeled claim, especially for relatively high readings, such as in the case of Nutriboost with a glucometer reading of 18.7 mmol/L. Glucometer readings may differ from the actual value (Salacinski et al., 2014; Dickson et al., 2019). The US FDA regulation allows glucometers to deviate up to 20% of the actual value (Katz et al., 2020). While the blood sugar device may pose limitations when applied to food, calibration of the glucometers can to done to yield more accurate results.

The labeled claim does not necessarily represent the actual amount of total sugars. The exact amount may vary due to the manufacturing process; thus, deviations from 100% accuracy are expected. However, the % accuracy for total sugars in Nutriboost is too low (Table 1). We suspect that it might be related to the inaccuracy of the glucometer. As seen in Table 2, the higher the glucometer reading for the lactose standards, the higher the deviation from the actual value. The lactose standard with a raw glucometer reading (G_{raw}) of 19.87 mM had an actual amount of 26.20 mM. Thus, the reading is only 75.80% of the amount. The sample preparation of Nutriboost after invertase and lactase treatment had a glucometer reading (G_{raw}) of 18.7 mM, close to 19.87 mM. Thus, the percent accuracy of only 84.4% could be partly due to the inaccuracy of the glucometer, which can be fixed through calibration.

3.2. Quantitation of lactose and total sugar in milk products using lactose monohydrate to calibrate the glucometer

Complete hydrolysis of lactose will result in an equimolar amount of glucose. Therefore, glucometers can be calibrated using standard lactose solutions after enzymatic hydrolysis with lactase. As seen in Table 2, 6.43, 12.66, 19.23, and 26.19 mM of hydrolyzed lactose had raw glucometer readings

Table 2. Determination of the calibration factor (CF) of the glucometer.

Raw Glucometer Reading [G_{raw} (mM)]	Actual Lactose Concentration (mM)	% Actual Lactose Concentration	Calibration Factor (CF)[a]
6.96 ± 0.12	6.44	108.10 ± 1.86	0.93
12.33 ± 0.23	12.67	97.31 ± 1.87	1.03
15.53 ± 0.32	19.23	80.75 ± 1.67	1.24
19.87 ± 0.15	26.20	75.84 ± 0.57	1.32

Note: Results are means ± standard deviations of triplicate measurements.
[a] Calibration Factor (CF)= Actual Lactose Concentration/Raw Glucometer Reading (G_{raw}).

(G_{raw}) of 6.96, 12.33, 15.53, and 19.87 mM, respectively. In this research article, we used single-point instead of multiple-point calibration.

In multiple-point calibration, the best fit for all the points is drawn. However, the best-fit line for all the points is not necessarily the best for every point, especially if the relationship is not perfectly linear (i.e., $R^2 < 0.998$). As seen in Fig. 1, the coefficient of determination (R^2) was just 0.986, and some of the points at the lower lactose concentrations deviated from the calibration curve/line. Thus, doing multiple-point calibration for the analysis of lactose might be a problem in our case, and single-point calibration is a better option.

In single-point calibration, the calibration factor (CF) for the specific raw glucometer reading (G_{raw}) is determined (Table 2). As seen in Table 3, the raw glucometer reading (G_{raw}) of the sample preparation was multiplied by the appropriate CF to get the corrected glucometer reading (G_{corr}). The appropriate CF corresponds to the standard preparation with the nearest concentration to the sample preparation. Calibrating data points this way minimizes the errors associated with using calibration lines that may be the "best compromise fit" for all the points but not necessarily the best for a specific point, which is often observed when the data points are not perfectly linear (i.e., $R^2 < 0.998$) or when the data point falls outside the best-fit line.

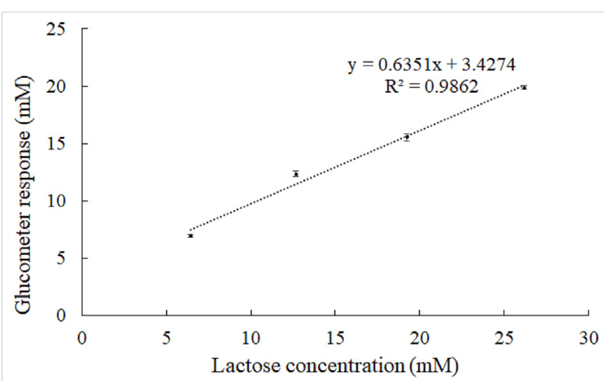

Fig. 1. Linearity of glucometer reading to sugar content.

The % accuracy for lactose and total sugars based on the corrected glucometer readings (G_{corr}) is presented in Table 4. Comparing the results before and after calibration, there was an improvement in the % accuracy, making it closer to 100%, after single-point calibration for "most" of the products.

In determining lactose content in Nutriboost, the % labeled claim decreased from 85% to 79.59% when calibrated. However, this does not necessarily mean the results became inaccurate after calibration. The lactose in milk products comes from the milk itself and is not added by the manufacturer, making it more difficult to hit specific targets, such as the labeled claim. The amount of lactose in milk can vary by up to 20% or more (Ibrahim et al., 2021). Moreover, some countries allow a ± 20% tolerance from the nutritional label (Curran, 2002). Thus, differences in the amount of lactose between production lots could be wider. Interestingly, the lactose content of Nutriboost (chocolate flavor) is reported as 10 g per 330 mL serving size and as 5 g per 200 mL serving size. Ten grams per 330 mL should equal 6 g per 200 mL.

The case is different for total sugars since the majority of the sugars is sucrose, which the manufacturer directly adds. Thus, tighter deviations from the labeled claim are more likely for total sugars. Interestingly, the total carbohydrates of Nutriboost are reported per 330 mL and 200 mL serving and are reported as 33 g and 19 g, respectively, which is proportionally correct.

In the future, the glucometer method can be run in parallel to High Pressure Liquid Chromatography-Refractive Index Detector (HPLC-RID) to get a clearer picture of the actual accuracy of the method. However, the results can already be

Table 3. Conversion of raw glucometer reading (G_{raw}) to corrected glucometer reading (G_{corr}) using the calibration factor (CF) corresponding to the nearest G_{raw}.

Milk product	G_{raw} after Lactase Treatment (mM)	CF corresponding to the nearest G_{raw} in Table 2	G_{corr} after Lactase Treatment (mM)
Chuckie	8.86 ± 0.29	0.93	8.25 ± 0.27
Chocolait	7.23 ± 0.32	0.93	6.73 ± 0.30
Nutriboost	5.83 ± 0.25	0.93	5.43 ± 0.23
Milk product	G_{raw} after Lactase and Invertase treatment (mM)	CF corresponding to the nearest G_{raw} in Table 2	G_{corr} after Lactase and Invertase treatment (mM)
Nestlé Fresh Milk	9.80 ± 0.17	1.03	10.06 ± 0.17
Chuckie	16.26 ± 0.15	1.24	20.15 ± 0.19
Chocolait	17.46 ± 0.40	1.24	21.64 ± 0.50
Nutriboost	18.70 ± 0.52	1.32	24.66 ± 0.68

Note: Results are means ± standard deviations of triplicate measurements.

Table 4. Percentage accuracy (%) of the method using the raw glucometer reading (G_{raw}) and the corrected glucometer reading (G_{corr}).

Claimed Lactose per Serving (g)	Measured Lactose per serving (g) Using G_{corr}	% Accuracy for Lactose Using G_{corr}	% Accuracy for Lactose Using Graw
Chuckie (6.67[a])	6.59 ± 0.21	98.93 ± 3.22	106.44 ± 3.44
Chocolait (6.67[a])	7.47 ± 0.33	112.09 ± 4.98	119.94 ± 5.25
Nutriboost (10)	7.95 ± 0.34	79.59 ± 3.43	85.00 ± 3.70
Claimed Total Sugar per Serving (g)	Measured total sugar per serving (g) Using G_{corr}	% Accuracy for Total Sugars Using G_{corr}	% Accuracy for Total Sugars Using Graw
Nestlé Fresh Milk (11.8)	11.35 ± 0.20	96.26 ± 1.70	93.7 ± 1.65
Chuckie (14.7)	16.37 ± 0.15	111.41 ± 1.04	89.9 ± 0.84
Chocolait (23)	24.42 ± 0.56	106.19 ± 2.45	85.7 ± 1.98
Nutriboost (33)	36.74 ± 1.02	111.35 ± 3.09	84.4 ± 2.34

Note: Results are means ± standard deviations of triplicate measurements.
Note: Serving sizes for each milk product are 250 mL for Nestlé Fresh Milk, 180 mL for Chuckie, 250 mL for Chocolait, and 330 mL for Nutriboost.
[a] Based on the Assumption that the amount of Lactose is around 133% the labelled claim for protein (5 g per serving).

sufficient for people who want to have a ballpark figure for their sugar intake.

4. Conclusion

This study describes a cheap, rapid, and easy glucometer-based method to estimate the amount of total sugars in flavored milk on-site. Based on the labeled claim, the estimated accuracy for the developed glucometer method was 96.26, 111.41, 106.19, and 111.35% for Nestlé fresh milk, Chuckie, Chocolait, and Nutriboost, respectively. The term “estimated accuracy” was used due to manufacturing variabilities as the labeled claim may not be the exact sugar content of the tested products. Therefore, better accuracy of the method is plausible. The method has to be run in parallel with HPLC-RID to confirm its true accuracy. Multiple glucometers can also studied for future research. However, the presented data can already be beneficial for people to use this method for ballpark estimates of their sugar intake.

Funding

There was no funding agency involved and all cost (minimal) were shouldered by the authors.

Conflict of interest

There are no conflicts of interest. All authors don't have any financial interest on this article.

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