Rapid measurement of the lactose content of cheese whey and process cheese using a commercially available blood glucose meter

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Project Objectives

Objective 1:

Development of a suitable buffering system for extending the applicability of the blood glucose meter method to cheese whey and process cheese.

Objective 2:

Evaluation of blood glucose meter method (BGMM) for determination of lactose content in cheese whey and process cheese, and compared the results with reference HPLC method.

Description of Work Performed

Provide a brief description of the work performed and their results.

Most of the available methods for the determination of lactose are time consuming, tedious, and require expensive instrumentation and skilled technicians. The dairy industry is in need of a rapid, inexpensive method that can be performed with minimum training to the operators. It is cost effective, if we adapt an existing technology of blood glucose meter for determination of lactose content of whey and process cheese. In this regard, a new generation ReliOn Confirm glucose meter was utilized for the determination lactose in cheese whey and process cheese. The blood glucose meter method was based on the hydrolysis of lactose into glucose and galactose in the presence of -galactosidase and water. This step is most common in any enzymatic determination of lactose. The reaction is as follows:

$$\text{Lactose} + H_2O \xrightarrow{\beta\text{-galactosidase}} \text{Glucose} + \text{Galactose}$$

The amount of glucose formed during the hydrolysis of lactose was proportional to the amount of lactose present in the sample. The concentration of glucose was measured using a blood glucose meter and correlated with the initial lactose concentration in the sample measured by the reference HPLC method.

In the experimental protocol, 1g of sample was added to 20g of 0.01M phosphate buffer (pH 7.4) and mixed vigorously at 60°C for process cheese or room temperature for whey. Five milliliters of the sample mixture was transferred to a test tube, and 0.01 ml of β-galactosidase was added. The solution was incubated at 40°C for 10 min to hydrolyze the lactose into glucose and galactose, and then analyzed for glucose in duplicate using the ReliOn Confirm glucose meter with four different lots of test strips. An individual calibration curve was developed for each test strip lot, and subsequently a universal calibration curve was also developed by pooling the data from all 4 test strip lots. The calibration curves were developed using known standards for both whey and process cheese. Simultaneously, the standards were also analyzed for lactose concentration using an HPLC-based reference method. The linear regression parameters were then obtained by using both blood glucose meter and HPLC results to establish the individual and universal calibration curves.

Individual calibration curves:

Process cheese:

An individual calibration curve was developed for each test strip lot by using process cheese with known lactose concentration ranges between 3.2 and 8.2%. The slopes and intercepts of individual calibration curves ranged from 1.23 to 1.37 and from -105 to -60, respectively.

Cheese Whey:

An individual calibration curve was also developed for each test strip lot using the model whey solutions that had a constant protein content of 0.8%, and different lactose concentrations ranging from 2 to 6%. These model whey solutions were standardized and prepared by mixing ultra-filtered whey retentate, whey permeate, lactose powder, and water in different ratios to obtained the final concentration (2%, 3%, 4%, 5%, and 6%) of lactose. The slopes and intercepts of individual calibration curves were between 0.945 to 1.009, and -54.96 to -38.73, respectively.

Universal calibration curve:

Process cheese:

A universal calibration curve was computed by pooling the data from the individual lots of test strips. The slope and intercept of the universal curve were 1.32 and -88, respectively.

Cheese Whey:

Similarly, a universal calibration curve was also developed by pooling the above data from all test strip lots used for the individual calibration models. The slope and intercept of universal calibration curve was 0.978 and -46.775, respectively.

Results:

The developed individual and universal calibration equations for cheese and whey were then used to determine the lactose concentration of respective process cheese and cheese whey samples, respectively. The results for each study are furnished below.

Process cheese:

Table 1. Percent lactose by HPLC and BGMM for all test strip lots using the individual calibration equations for process cheese.

Samples ID	Percent Lactose by HPLC Analysis	Percent Lactose by Glucose meter, Lot # 1	Percent Lactose by Glucose meter, Lot # 2	Percent Lactose by Glucose meter, Lot # 3	Percent Lactose by Glucose meter, Lot # 4	Bias Lot # 1	Bias Lot # 2	Bias Lot # 3	Bias Lot # 4
1	4.63	4.68	4.73	4.52	4.96	-0.05	-0.09	0.11	-0.33
2	4.69	4.58	4.50	4.72	4.85	0.11	0.20	-0.03	-0.16
3	4.75	4.55	4.84	4.64	4.82	0.20	-0.08	0.11	-0.07
4	4.96	4.69	4.75	4.73	4.97	0.26	0.20	0.23	-0.01
5	5.60	5.64	5.56	5.59	6.00	-0.03	0.04	0.01	-0.39
6	5.71	5.82	5.77	5.56	6.20	-0.11	-0.06	0.15	-0.49
7	6.55	6.77	6.64	6.34	7.23	-0.22	-0.10	0.20	-0.68
8	6.92	6.77	6.45	6.90	7.22	0.15	0.47	0.02	-0.31
9	6.94	7.04	6.72	6.70	7.52	-0.10	0.22	0.24	-0.58
10	7.23	6.89	6.90	7.23	7.36	0.34	0.33	0.00	-0.14
Min absolute bias						**0.03**	**0.04**	**0.00**	**0.01**
Max absolute bias						**0.34**	**0.47**	**0.24**	**0.68**
Mean absolute bias						**0.16**	**0.18**	**0.11**	**0.32**

The mean absolute bias was found to be between 0.11% to 0.32% for individual calibration equations of 10 different process cheeses samples.

Table 2. Percent lactose by HPLC and BGMM for all test strip lots using the universal calibration equation for process cheese.

Samples ID	Percent Lactose by HPLC Analysis	Percent Lactose by Glucose meter, Lot # 1	Percent Lactose by Glucose meter, Lot # 2	Percent Lactose by Glucose meter, Lot # 3	Percent Lactose by Glucose meter, Lot # 4	Bias Lot # 1	Bias Lot # 2	Bias Lot # 3	Bias Lot # 4
1	4.63	4.80	4.71	4.42	4.58	-0.17	-0.08	0.22	0.05
2	4.69	4.73	4.47	4.61	4.59	-0.04	0.23	0.08	0.10
3	4.75	4.69	4.82	4.54	4.61	0.06	-0.07	0.21	0.14
4	4.96	4.84	4.73	4.62	4.84	0.12	0.23	0.34	0.12
5	5.60	5.70	5.56	5.53	5.48	-0.10	0.04	0.07	0.12
6	5.71	5.88	5.77	5.49	5.32	-0.17	-0.06	0.22	0.40
7	6.55	6.74	6.67	6.32	6.41	-0.19	-0.12	0.23	0.14
8	6.92	6.74	6.47	6.90	6.65	0.18	0.45	0.02	0.27
9	6.94	7.00	6.75	6.69	6.47	-0.05	0.20	0.26	0.47
10	7.23	6.84	6.93	7.25	6.68	0.39	0.30	-0.02	0.55
Min absolute bias						**0.04**	**0.04**	**0.02**	**0.05**
Max absolute bias						**0.39**	**0.45**	**0.34**	**0.55**
Mean absolute bias						**0.15**	**0.18**	**0.17**	**0.24**

The mean absolute bias was found to be between 0.15% and 0.24% for the universal calibration equation. Moreover, we also used the above universal equation to measure the lactose content of 18 different process cheeses in a commercial process cheese manufacturing plant during production. This study was performed to validate the universal calibration curve equation for analysis of the lactose content of process cheeses during commercial manufacture. The results are illustrated in Table 3 below.

Table 3. Percent lactose by HPLC and BGMM for 2 test strip lots using the universal calibration equation for commercially manufactured process cheeses.

Samples ID	Samples Name	Lot # 1	Lot # 2	HPLC organic	Bias for Lot # 1	Bias for Lot # 2
1	Raw Blend Loaf	6.55	6.43	6.45	-0.10	0.02
2	Raw Blend Loaf	7.53	6.84	7.13	-0.40	0.29
3	Raw Blend Loaf	7.20	6.88	6.81	-0.39	-0.07
4	Raw Blend Loaf	6.77	6.75	7.15	0.38	0.41
5	Raw Blend Loaf	7.78	7.01	7.68	-0.10	0.67
6	Raw Blend Loaf	6.77	7.25	7.60	0.83	0.35
7	Raw Blend Slice	3.90	3.98	4.62	0.72	0.64
8	Raw Blend Slice	4.45	4.21	3.86	-0.58	-0.35
9	Raw Blend Slice	4.42	4.36	3.78	-0.65	-0.58
10	Finished Loaf	6.12	5.95	6.41	0.29	0.46
11	Finished Loaf	6.67	5.97	6.87	0.19	0.90
12	Finished Loaf	6.12	5.88	7.00	0.88	1.12
13	Finished Loaf	6.23	6.00	7.01	0.78	1.01
14	Finished Loaf	6.30	5.69	7.14	0.84	1.44
15	Finished Loaf	6.88	6.47	6.96	0.08	0.48
16	Finished Slice	4.46	4.07	4.35	-0.11	0.28
17	Finished Slice	4.25	4.26	4.35	0.10	0.09
18	Finished Slice	3.49	3.47	4.11	0.62	0.64
Min. absolute bias					**0.08**	**0.02**
Max. absolute bias					**0.88**	**1.44**
Mean absolute bias					**0.45**	**0.55**

The mean absolute bias was found to be between 0.45% and 0.55% for the 18 different commercially manufactured process cheeses.

Cheese whey:

Table 4. Percent lactose by HPLC and BGMM for all test strip lots using the individual calibration equations for cheese whey.

Samples ID	Percent Lactose by HPLC Analysis	Percent Lactose by Glucose meter, Lot # 1	Percent Lactose by Glucose meter, Lot # 2	Percent Lactose by Glucose meter, Lot # 3	Percent Lactose by Glucose meter, Lot # 4	Bias Lot # 1	Bias Lot # 2	Bias Lot # 3	Bias Lot # 4
1	4.46	4.51	4.44	4.41	4.34	-0.05	0.02	0.05	0.12
2	4.49	4.52	4.34	4.44	4.45	-0.03	0.15	0.05	0.04
3	4.44	4.38	4.57	4.35	4.38	0.06	-0.13	0.09	0.06
4	4.85	4.83	4.83	4.93	4.68	0.01	0.02	-0.08	0.17
5	4.94	4.95	4.65	4.58	4.79	-0.01	0.29	0.36	0.15
6	4.92	4.76	4.93	4.81	4.60	0.16	0.00	0.11	0.32
7	4.47	4.55	4.57	4.49	4.28	-0.08	-0.09	-0.01	0.19
8	4.71	4.72	4.52	4.53	4.39	-0.01	0.19	0.18	0.32
9	4.88	4.99	4.69	5.05	4.47	-0.11	0.19	-0.17	0.41
10	4.58	4.53	4.45	4.41	4.43	0.04	0.13	0.17	0.15
Min absolute bias						**0.01**	**0.00**	**0.01**	**0.04**
Max absolute bias						**0.16**	**0.29**	**0.36**	**0.41**
Mean absolute bias						**0.06**	**0.12**	**0.13**	**0.19**

The mean absolute bias was found to be between 0.06% and 0.19% for the individual calibration equations of 10 different cheese whey samples.

Table 5. Percent lactose by HPLC and BGMM for all test strip lots using the universal calibration equation for cheese whey.

Samples ID	Percent Lactose by HPLC Analysis	Percent Lactose by Glucose meter, Lot # 1	Percent Lactose by Glucose meter, Lot # 2	Percent Lactose by Glucose meter, Lot # 3	Percent Lactose by Glucose meter, Lot # 4	Bias Lot # 1	Bias Lot # 2	Bias Lot # 3	Bias Lot # 4
1	4.46	4.52	4.41	4.46	4.29	-0.06	0.05	0.00	0.17
2	4.49	4.54	4.29	4.50	4.41	-0.05	0.20	-0.01	0.08
3	4.44	4.40	4.54	4.40	4.33	0.04	-0.10	0.04	0.11
4	4.85	4.83	4.80	4.97	4.65	0.01	0.04	-0.12	0.20
5	4.94	4.95	4.62	4.63	4.76	-0.01	0.32	0.31	0.18
6	4.92	4.77	4.90	4.86	4.56	0.15	0.02	0.06	0.37
7	4.47	4.56	4.53	4.54	4.23	-0.09	-0.06	-0.07	0.24
8	4.71	4.73	4.48	4.58	4.34	-0.02	0.22	0.13	0.36
9	4.88	4.99	4.65	5.08	4.41	-0.11	0.23	-0.20	0.47
10	4.58	4.55	4.40	4.45	4.37	0.03	0.18	0.13	0.21
Min absolute bias						**0.01**	**0.02**	**0.00**	**0.08**
Max absolute bias						**0.15**	**0.32**	**0.31**	**0.47**
Mean absolute bias						**0.06**	**0.14**	**0.11**	**0.24**

The mean absolute bias was found to be between 0.06% and 0.24% for the universal calibration equation. Moreover, we also used the above universal equation to measure the lactose content of 10 different cheese whey samples during cheese manufacture. This study focused on validation of the universal calibration curve equation for analysis of whey with a range of lactose concentration typically observed during cheese manufacture. The results are illustrated in Table 6 below.

Table 6. Percent lactose by HPLC and BGMM for 2 test strip lots using the universal calibration equation of cheese whey during cheese manufacture.

Samples ID	Percent Lactose by HPLC Analysis	Percent Lactose by Glucose meter, Lot # 1	Percent Lactose by Glucose meter, Lot # 2	Bias Lot # 1	Bias Lot # 2
1	4.83	4.90	5.03	-0.07	-0.20
2	4.73	4.46	4.50	0.27	0.23
3	4.71	4.53	4.81	0.17	-0.10
4	4.56	4.55	4.64	0.01	-0.08
5	4.27	4.26	4.40	0.01	-0.13
6	4.10	4.16	4.02	-0.07	0.08
7	3.57	3.64	3.51	-0.07	0.06
8	3.01	3.25	2.94	-0.24	0.07
9	2.61	2.85	2.75	-0.23	-0.14
10	2.79	2.90	2.74	-0.11	0.04
Min absolute bias				**0.01**	**0.04**
Max absolute bias				**0.27**	**0.23**
Mean absolute bias				**0.13**	**0.11**

The mean absolute bias was found to be between 0.11% and 0.13% for the universal calibration equation of 10 different cheese whey samples during cheese manufacture.

Results of Technology or Process Assessed

This research demonstrated that the developed blood glucose meter method can be used to deliver a simple, rapid, and accurate method for routine measurement of lactose in cheese whey and process cheese at commercial dairy plant environment.

Benefit to Minnesota Economic Development

What agricultural material or commodity is impacted? Is higher value end product created by using agricultural materials or commodities? Is there a benefit to producers? Will the project create new or build upon existing jobs? What might be the expected growth for the first three years?

The BGMM for determination of the lactose content of whey and process cheeses will have an impact in the dairy industry at the commercial level. This rapid and simple technique for the quantification of lactose in cheese whey and process cheese can be used at-line during natural and process cheese manufacture or during whey processing. This test will allow manufactures to improve process control and product quality during natural and process cheese manufacture and during whey processing. Prior to the development of this test manufactures performed lactose testing using a spectrophotometric kit that takes several hours to perform and cost substantially more than the developed method.

Marketing

Describe your current marketing plan and any proposed changes. Will the company have adequate cash to achieve the marketing goals?

In order to facilitate adoption of the method by the dairy industry, a universal calibration equation for 3 to 4 test strip lots will be determine in our laboratory, and then given to commercial dairy plants to analyze their whey or cheese samples for lactose content during production. Simple technical training will also be given to the plant technicians regarding the experimental procedures for sample preparation and meter use.

Conclusion

The developed meter technology is promising and minimize the sample test time, and analysis cost for measurement of lactose in cheese whey and process cheese.

Future Needs/Plans

What has been discovered that might need to be addressed or developed to move the project to another level of success?

Promising results shown for the BGMM for rapid determination of lactose content in cheese whey and process cheese would be a great impact for the dairy industry in terms of saving money and time. In the continuation of this project, measurement of lactose content in whey based dairy products and cultured dairy products need to be address as future study for rapid measurement to determine the final quality, and to minimize cost. We also plan to work with the dairy industry to facilitate adoption and use of the method.