



November 1, 2011
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Re: Time interval analysis of lactose reduction/ glucose & galactose increase, and change in pH in Vitamin D milk whole milk before and after dosing with Lactase Drops™

Submitted by: Seeking Health, LLC
Received: 10/12/2011

LABORATORY REPORT

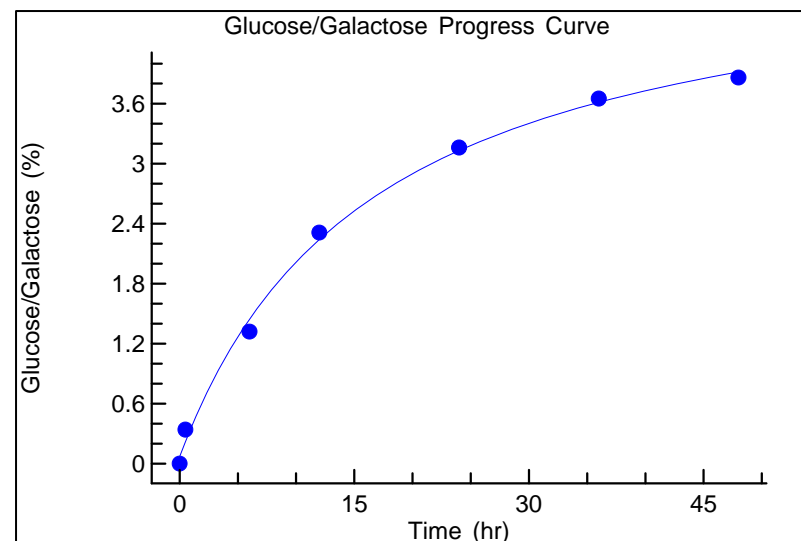
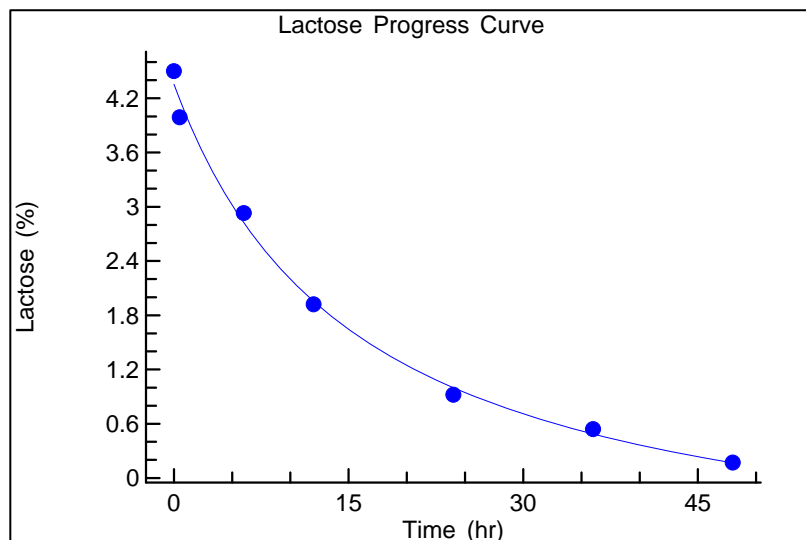
On October 12, 2011 Analytical Food Laboratories received a sample of item #023-05-001-12 Lactase drops. This product was sealed upon arrival and received in good condition by the laboratory. AFL purchased a half gallon of whole milk used for testing the enzymatic activity the lactase in Lactase Drops™ had towards the breakdown of lactose.

Methods:

The half gallon milk was held at 4°C throughout the study. The half gallon whole milk was tested for lactose and glucose+galactose initially before addition of Lactase Drops™. This represents a control, which should provide an initial value for sugars. After addition of 20drops of Lactase Drops™ from the sealed bottle to the half gallon of whole milk, the sugars were monitored by HPLC with RI detection at time 0, 6, 12, 24, 36 and 48 hours. At the respective time point, a 4g sample was weighed into a 25mL class A volumetric flask. Approximately 10mL ethanol was added, and solution sonicated for 10min. Next, samples were clarified using Carrez solutions and ethanol was again added to bring to volume. Samples were allowed to settle for 1 hour, filtered through C18 cartridge and an aliquot injected onto the HPLC following AOAC 977.20.

Results and Data Analysis:

Lactose gradually decreased from time 0 throughout the study showing the Lactase Drops™ do show enzymatic activity toward lactose. This can be seen by the lactose progress curve shown. The reaction catalyzed by lactase breaks down lactose into glucose and galactose; thus, these were monitored as well throughout the study. Glucose and galactose could not successfully be separated using the HPLC method used. Therefore, they were monitored together as one peak. These steadily increased over time after addition of Lactase Drops™ as shown in the glucose/galactose progress curve. These data suggest Lactase Drops™ are an effective removal system for lactose in whole milk providing approximately 34% reduction after 6 hr and 96% reduction after 48 hr incubation.



Progress curves fitted with a hyperbolic 3 parameter function.

Sample	smp wt (mg)	Initial dil (mL)	Dilution factor	fructose (mg/mL)	glucose and galactose (mg/mL)	sucrose (mg/mL)	maltose (mg/mL)	lactose (mg/mL)	%fructose	%glucose	%sucrose	%maltose	%lactose	total sugars(%)
Control*	4110.9	25	1	0.0000	0.0000	0.0000	0.0000	7.4028	0.00	0.00	0.00	0.00	4.50	4.50
Time 0 hr	4146.8	25	1	0.0000	0.5683	0.0000	0.0000	6.6154	0.00	0.34	0.00	0.00	3.99	4.33
Time 6 hr	4101.8	25	1	0.0000	2.1619	0.0000	0.0000	4.8100	0.00	1.32	0.00	0.00	2.93	4.25
Time 12 hr	4039.5	25	1	0.0000	3.7260	0.0000	0.0000	3.1067	0.00	2.31	0.00	0.00	1.92	4.23
Time 24 hr	4994.4	25	1	0.0000	6.3147	0.0000	0.0000	1.8337	0.00	3.16	0.00	0.00	0.92	4.08
Time 36 hr	6529.1	25	1	0.0000	9.5237	0.0000	0.0000	1.4077	0.00	3.65	0.00	0.00	0.54	4.19
Time 48 hr	8641.8	25	1	0.0000	13.3543	0.0000	0.0000	0.5902	0.00	3.86	0.00	0.00	0.17	4.03

*Control = Pre-lactase Drops dosing

Note: The initial time 0 after Lactase Drops™ addition showed some conversion of lactose, which is likely due to the small amount of time taken for preparing the sample for analysis. All other time points underwent the same procedure so all are normalized.

Sample Description	pH	Lactose (%)	Glucose (%)
Whole milk w/o lactase drops	6.63	4.50	ND
Whole milk with lactase drops Time = 0 hrs	6.76	3.99	0.34
Whole milk with lactase drops Time = 6 hours	6.81	2.93	1.32
Whole milk with lactase drops Time = 12 hours	6.68	1.92	2.31
Whole milk with lactase drops Time = 24 hours	6.75	0.92	3.16
Whole milk with lactase drops Time = 36 hours	6.69	0.54	3.65
Whole milk with lactase drops Time = 48 hours	6.79	0.17	3.86

Please feel free to contact me if there are any further questions.

Sincerely,



Cheri M. Turman, PhD.
Director of Chemistry