DeltaInstruments
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Application Note 202

The pH of milk by LactoScope FTIR

Application note:	ph in Milk
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Introduction

The pH of raw milk provides a good indicator for the bacteriological quality of milk [Baumgartner et al. 2003]. Normally the pH of milk is between 6.6 and 6.8. Bacterial growth, resulting from poor hygienic conditions during milking, storage time and temperature, causes souring of the milk.

Using FTIR, the pH can be estimated, simultaneously with other parameters like fat, protein, free fatty acid (FFA), non protein nitrogen (NPN) or urea content, etc., from the mid-infra-red spectrum of the milk sample.

A full spectrum PLS model for estimating the pH of milk on the LactoScope FTIR has been established on the basis of individual cow milk samples, collected at over 20 farms from all over the Netherlands and including spectra for both fresh and sour samples.

The results of the validation study presented in this report demonstrate the good applicability of the pH model towards the analysis of samples taken from practice, i.e. as routinely analyzed at a local milk control station. Results presented for pH series prepared by adding lactic acid to preserved raw milk show that such series can be used as a simple tool for calibration evaluation purposes.

Materials & Methods

Selected for the model validation study were 6 crates of individual cow milk samples (96 positions per crate, ±10 ml milk per tube) analyzed in the routine production of a local milk control station on a single day in June.

The sour samples were obtained through storage of the fresh samples (preserved with azide upon collection) at room temperature for a period of days up to a week. Excluded from the calibration set were sour samples with a pH less than 6, as to derive a model with special emphasis on spectral changes connected to the onset of souring.

All of the validation set samples were preserved with azide upon collection and were taken as to be representative for the average of an evening & morning milking

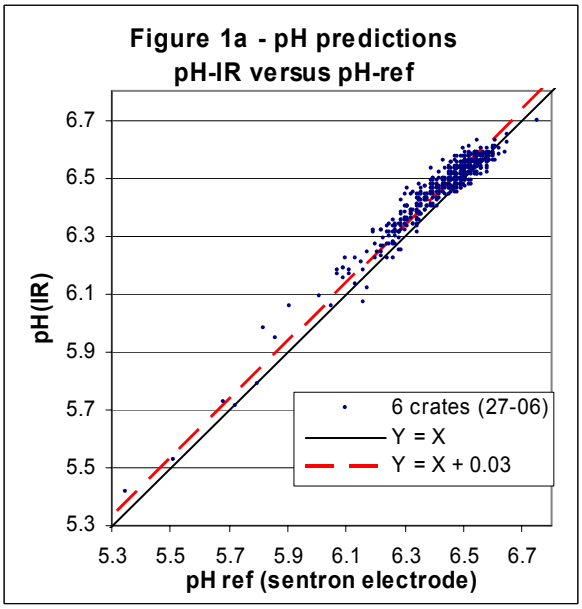
pH reference analysis
pH reference analyses were carried out using an ISFET pH electrode of Sentron. Samples of the validation were analyzed after routine measurement by LactoScope FTIR, at a temperature of 25±1°C in a water bath. pH reference analyses of the pH series were carried out upon preparation ("direct", milk at room temp.) and following analyses by FTIR ("after").

LactoScope FTIR measurements
Measurements by LactoScope FTIR were carried out according to standard protocol, on a system equipped with an XY-sampler for crates with 96 positions and running at a speed of 400 samples/hour, using the full spectrum PLS model for predicting the pH from the milk spectra.

Results & Discussion

Validation set

Results obtained for the validation set, encompassing the 6 crates of individual cow milk samples, are graphically represented in the figures 1 to 3.



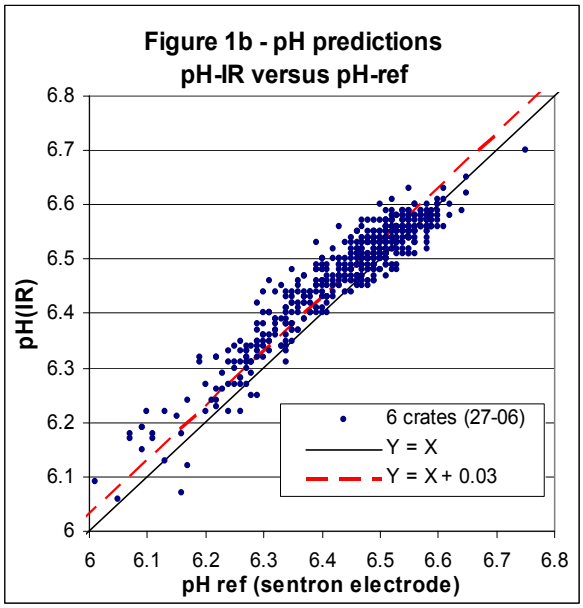
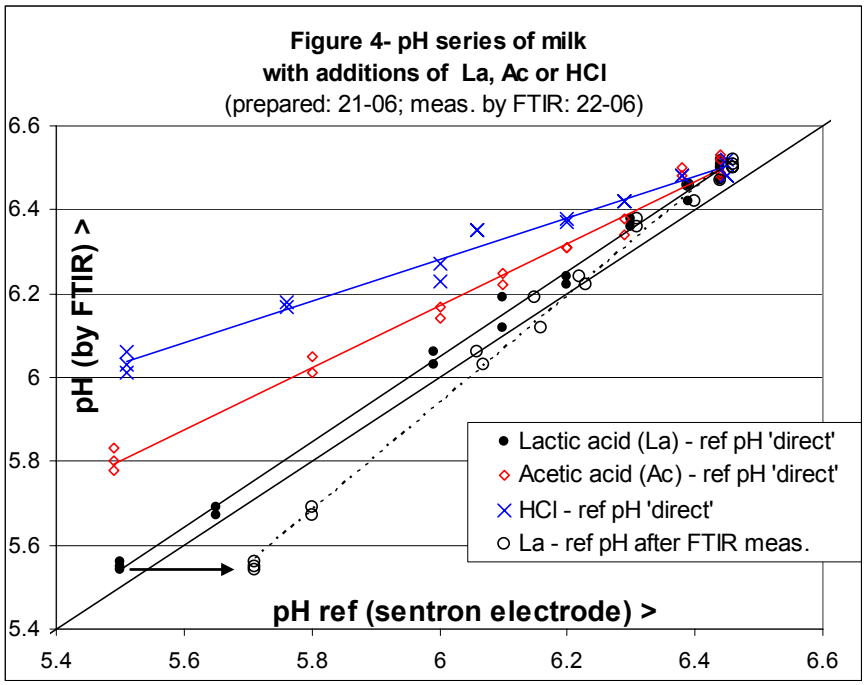


Figure 1 shows that a good correlation is obtained among predicted and reference values over the full range in pH for the validation set, i.e. from pH 6.7 down to 5.5, although the model was developed on the basis of samples higher in pH than 6 only. All results presented are uncorrected, i.e. these have not been corrected for (e.g.) a slope/bias mismatch relative to the reference values.

The model moreover was developed on a different instrument. From which it can be concluded that, as expected with the design of the Lactoscope FTIR, the model is directly transferable among instruments.

pH series of milk

As to test the ruggedness of the pH model developed, pH series of milk prepared through addition of lactic acid (La), acetic acid (Ac) and hydrochloric acid (HCl) were investigated. Lactic acid and acetic acid were chosen since the souring of milk is expected to result in an increase in the concentration(s) of primarily one or both of these two organic acids. HCl was considered in addition since this inorganic acid does not show an IR response of it self and thereby provides a measure of the pH dependent variation in IR response of the natural milk components (proteins, citrate, phosphate etc.).
As follows from the results presented in figure 4, pH reference values and predicted pH values were found to correlate well for each of the pH series individually.



- In contrast to the pH series of lactic acid, the decrease in pH over the series of acetic acid and of HCl was underestimated by 30% and 50% respectively by FTIR.
- pH ref values for acidified samples moreover, were found to drift/shift upward in time.

The upward shift in pH ref over time for acidified samples of the pH series of La has been visualized in figure 4 by plotting the FTIR results relative to the pH ref measurements carried out "direct" upon preparation (solid bullets) and "after" the FTIR determination (open circles). For the sample lowest in pH, the shift (indicated by an arrow in figure 4) exceeds 0.2 pH units. A similar drift in pH ref over time was observed for pH series of Ac and HCl (results not shown).
Clear is from the results for these pH series that errors in pH predictions by FTIR in principle can be quite appreciable and dependent on type of souring process and circumstances.

Results & Discussion

Table 4 - pH series lactic acid (La) - day to day reproducibility in pH

		pH ref (sentron electrode)			pH by FTIR								
	La(72%) added **in ul**	direct (21°C)	after 10 min (21°C)	after 1 week (25°C)	mean (1)	bias	SD (1)	mean (2)	SD(2)	29/jun	29/jun	03/jul	05/jul
a	0	6.53	6.53	6.52	6.47	-0.06	0.03	6.49	0.024	6.50	6.50	6.49	6.45
b	0							6.46	0.017	6.46	6.46	6.43	6.47
a	100	6.41	6.41	6.42	6.37	-0.04	0.02	6.36	0.028	6.35	6.39	6.38	6.33
b	100							6.38	0.024	6.35	6.40	6.39	6.36
a	300	6.17	6.18	6.23	6.15	-0.02	0.02	6.16	0.008	6.16	6.16	6.15	6.17
b	300							6.13	0.021	6.13	6.13	6.16	6.11
a	500	5.97	5.97	6.06	5.95	-0.02	0.01	5.95	0.013	5.97	5.95	5.95	5.94
b	500							5.95	0.006	5.95	5.95	5.94	5.94
a	1000	5.48	5.56	5.71	5.46	-0.02	0.02	5.48	0.017	5.48	5.45	5.48	5.49
b	1000							5.45	0.008	5.45	5.46	5.44	5.45
a	0	6.53	-	-	-	-	-	6.39	0.014	6.39	6.40	6.40	6.37
b	0							6.45	0.033	6.49	6.46	6.41	6.45
	Mean	-	-	-	-	-	-	-	0.019	6.14	6.14	6.14	6.13

Where:
direct: pH reference measurement upon the moment of preparation
after 10 minutes& 1 week: pH reference measurements after preparation (10 min & 1 week)
mean(1) & SD(1): mean and standard deviation over 8 measurements - with a & b combined
Bias: pH ref (direct) - mean(1)
mean(2) & SD(2): mean and standard deviation over 4 measurements - a & b treated separately
Mean (bottom row): the mean in the results for the corresponding column

For calibration evaluation purposes (preferably) the lactic acid series with reference pH values determined at the moment of preparation can be used. Since:

- it shows the closest correspondence among reference and FTIR measurements and
- results were found to show a good day to day reproducibility (table 4)

The preparation of the series is very simple, as it merely takes the direct addition of 0, 100, 300, 500 & 1000 µl of the concentrated lactic acid standard (72%) to 400 ml of a milk sample. With the tests reported here, the FTIR measurements were made in duplicate (a,b) in table 4), as to check for the influence of carry-over, which turned out to be small (table 4).
Useful to note in this context is that pH series are not needed for calibration adjustment. A bias only adjustment, based on the repeated measurement of a fresh preserved milk sample will normally do.

Conclusions

Although in theory (i.e. results for pH series with the different acids), pH measurements by FTIR may deviate appreciably from pH measurements by reference methods, it follows from results of the validation study that in practice the pH model derived for FTIR provides a useful tool in determining the bacteriological quality of milk. It looks that the spec regarding accuracy can be set to a standard error of prediction (SEP) of **<0.05 pH units**, where for fresh samples it is likely that an SEP on the order of 0.03 pH units can be normally achieved.

The spec for the short term repeatability standard deviation can be set to **<0.02 pH units**. The standard deviation for the long term repeatability or day to day reproducibility will be typically **< 0.03 pH units**.

References

References

Baumgartner C., Landgraf A., Buermeyer J., pH determination, (2003) Bulletin of IDF 383, page 23-28