J. of Dairy Science. 2006 Mar

ACIDITY IN MILK: COMPARISON OF METHODS

Determination of Acidity in Whole Raw Milk: Comparison of Results Obtained by Two Different Analytical Methods

Ms. A. Fabro, * H. V. Milanesio, *L. M. Robert,* J. L. Speranza, * M. Murphy, G Rodriguez, and R. Castañeda.

* División Rafaela, Centro de Investigación de Tecnología Láctea
Instituto Nacional de Tecnología Industrial, Rafaela, 2300, Argentina.
División Miguelete, Centro de Investigación de Tecnología Láctea,
Instituto Nacional de Tecnología Industrial, General Paz and Alvarellos, San Martín, Buenos Aires, Argentina.

Correspondence to:
M. Fabro; E-mail: mfabro@inti.gov.ar

ABSTRACT

In Argentina, one analytical method is usually carried out to determine acidity in whole raw milk: the IRAM 14005 standard, based in the Dornic method of French origin. In some international regulations the 947.05 AOAC Ed.16° is proposed as a method of analysis. As the basis of both methods is the same: titration with sodium hydroxide solution using phenolphthalein as an indicator, the results obtained by one method or another were considered always as equivalent.

The presence of some trends and discordant data, lead us to perform a statistical study to verify the equality in the obtained results.

As a result of the work done on more than 500 samples, the existence of significant differences between the results obtained by both methods was determined.

(**Key words:** acidity, milk)

Abbreviation key:

AOAC = American Organization Association Chemist; **IRAM** = Instituto de Racionalización de Materiales; **INTI** = Instituto Nacional de Tecnología Industrial

INTRODUCTION

What is usually known as milk acidity is the result of titration (Alais, 1965). Another author states that titrable acidity is the capacity of combination with a base (Goded and Mur, A., 1966).

The titration acidity is the sum of four reactions. The first three represent the milk natural acidity: acidity due to casein, acidity due to mineral substances, and acidity due to phosphates. The fourth one is called "developed" and is due to the lactic acid and other acids produced by lactose degradation because of microorganisms (Alais, 1965).

The measurement principle is unique and Van Slyke, L. L. and Bosworth, A. W. (1914) had already indicated it as common in a publication. It is based on adding to a given volume of milk, the necessary volume of alkaline solution (sodium hydroxide) of a perfectly known concentration, until reaching the neutralization point, which is determined by the turning of an indicator, generally phenolphthalein that turns from colorless to pink at pH 8.4.

Although the measurement principle is unique, there are different variations that differ in the concentration of the alkaline solution, the milk volume to titrate, the concentration of the solution of phenolphthalein, etc. In addition, there are different possible expressions of the obtained results.

Regarding the variations in volumes and concentrations, we can indicate, for example, that the method proposed by Van Slyke, L. L. and Bosworth, A. W. (1914), as the common one consisted of adding of a few drops of phenolphthalein solution as indicator to 100 cm^3 of milk and then a titration with NaOH N/10 solution until permanent pink coloration.

Nevertheless, in that work, authors proposed a new method so as to determine only the developed acidity: 100 cm^3 of milk are measured in a 200 cc Erlenmeyer, 50 cm^3 of distilled water are added, and 2 cm^3 of saturated solution of neutral potassium oxalate, after 2 minutes it is titrated with NaOH N/10 solution.

Continuing with the description of these variations, we can see that Goded and Mur, A. (1966) differentiate the official methods in some countries (at the time of the edition of this publication) according to:

Concentration of sodium hydroxide solution: Germany N/4, Argentina N/9 or N/10, Spain N/9, France N/9, Holland N/10, USA N/10.

Concentration of phenolphthalein: Germany 2%, Argentina 2%, Spain 1%, France 1%, Holland 2%, USA 1%.

Phenolphthalein solution volume: Germany 2 ml, Argentina 3 or 4 drops, Spain 0.5 ml, France 0.1ml, Holland: no indication, USA 2ml.

Milk volume to titrated: Germany 50 ml, Argentina 10 ml, Spain 10 ml, France 10 ml, and USA 20 ml.

Final color: pink in Argentina, Spain, USA, and France. However, in Germany and Holland it is contrasted with the color of a cobalt sulphate solution.

Regarding the ways of expression, the Dornic degree (°D), applied in France and Argentina, expresses the lactic acid content: the acidity expressed in Dornic degrees (°D) are the ml tenths of the N/9 sodium hydroxide solution used to titrate 10 ml of milk. As the molecular weight of lactic acid is 90 g/mol, 1°D is equivalent to 1 mg of lactic acid in 10 ml of milk or to 0.01% of lactic acid.

The Soxhlet-Henkel degree (S.H.) applied in Germany and Switzerland, does not use the lactic acid as reference. It is equivalent to 1 ml of N/4 sodium hydroxide solution used to titrate 100 ml of milk. Perhaps this concept is more appropriate since the titration does not only measure the lactic acid formed.

On the other hand, the acidity expressed in Thorner degrees are the milliliter tenths of N/10 sodium hydroxide solution used to titrate 10 ml of milk.

The variations in concentrations and volumes of milk, titration solution and indicator, can cause mistakes and possible incongruities in the results obtained by one or other technique in the same milk sample.

Alais (1965) states that, although the acidity measurement seems very easy to carry out, there are several causes of mistakes: the amount of indicator and end point of the titration. Goded and Mur, A. (1966) indicate as possible mistakes the indicator selected and its concentration, the alkaline solution selected and its concentration, the titration speed, the working temperature, and the milk dilution.

In the dairy laboratories of Argentina, the determination is usually made by means of the national method of IRAM 14005 since it is a national standard and it is a little bit faster than the alternative technique 947.05 AOAC Ed.16°.

Both methods have the same basis: acid-basic titration with sodium hydroxide solution using phenolphthalein as an indicator of the equivalent point.
There are differences in the volume and dilution of the test sample and in the titration solution concentration, as we indicated in all cases.
The results obtained by one standard or another were considered equivalent since they are very similar methods and share the same principle.
Nevertheless, when increasing the comparison of data among laboratories of the different companies using one standard or another, some discordant data and trends started to appear, mainly due to the definition of tolerated limits and methods to be applied in different regulations.
That fact led us to perform a statistical study to verify the equality in the obtained results applying one standard or another.

MATERIALS AND METHODS

Characteristics of the Samples:

We worked together with 8 dairy companies located in the main milk production areas of Argentina, so as to collect representative samples of the country production. Altogether 265 whole raw milk samples were selected, homogeneously distributed in time and in region during four months of sampling, which were analyzed in the laboratories of INTI.
The tested samples were taken from the tank truck in the milk reception area of the participating companies. All samples were obtained from dairy farms that refrigerate milk.
The sampling work was in charge of the companies participating in the study, which sent the samples to the dairy laboratories of the INTI in Rafaela and Miguelete, according to schedule.
The samples were identified with date and time of sampling and place of origin.
The samples were packaged in plastic bottles of 100 to 200 ml, containing milk up to the third part.
The delivery of the samples was carried out by the companies' own transport allowing the samples to be in the laboratories up to 16 hours after the sampling. No preservative at all

was used. The samples cooling temperature from the moment of the sampling to the moment of the analysis was always less than 10 ° C.
The samples were analyzed within 24 hours considered from the sampling time registered in the package with a difference not greater of 10 minutes by one technique or another.
In order to complete the study, the same sample was analyzed 36 times using one of the two methods alternatively: 18 times with one technique and 18 times with the other one. It was requested a greater volume of the selected sample to allow the analyses.

Sampling Areas:

The sampling was organized so as to obtain raw milk samples coming from the dairy basins of Santa Fe, Córdoba, La Pampa, Buenos Aires and Entre Ríos Provinces, according to the following description of the dairy basins:
Buenos Aires:
1- Mar y Sierras
2- Oeste
3- Abasto Sur
4- Abasto Norte
Entre Ríos:
5- "B"
6- "A"
Santa Fe:
7- Sur
8- Central
Córdoba:
9- Sur
10- Villa María
11- Noreste
La Pampa:
12- La Pampa

Summarized Procedures of the Applied Methods:

AOAC 947.05 Method summary (according to recommendations of JAOAC 30.130 and 34.239): Take 20 ml of sample, add 40 ml of boiled and cooled distilled water, and 2 ml of phenolphthalein (prepared at 1% in ethanol at 95%). Titrate with standardized NaOH 0.1 M until the first turn to pink color persists for 30 seconds. Add one drop more. Take the final volume consumed.

IRAM 14005 Method summary: Take 10 ml of sample, add 3 drops of phenolphthalein solution (prepared at 2 % in ethanol at 96 %). Titrate with standardized NaOH 0.111 M until first turn to pink color persists for 30 seconds. Add one drop more. Take the final volume consumed.

Formula Calculation:

The same formula was used for both methods to express the results in equivalent expressions or units:

$$\text{mg of lactic acid /100 ml of milk} = \frac{Vg \times N \times 90 \times 100}{Vm}$$

where

Vg = volume of the sodium hydroxide solution

N = concentration of sodium hydroxide standardized solution expressed in eq /L

90 = equivalent weight of lactic acid

Vm = volume of milk used for titration

RESULTS AND DISCUSSION

A total of 566 analytical results were obtained. From them, 530 belong to the subtotal of 265 in which one analytical method was applied and 265 in which the other analytical method was applied to each of the 265 samples. The remaining 36 analytical results belong

to the analysis of a same sample using alternatively one of the two methods: 18 times with one method and 18 times with the other one.

Two different tests were applied arriving in both cases to the same conclusions. The tests were: Pair Comparison Design for the 530 results from analysis of the 265 different samples, and Single-Factor Variance Analysis for the case of the 36 results on the same sample. For the statistical treatment of the data we consulted Montgomery, D. C. (1991).

Pair Comparison Design:

Condition 1: AOAC 947.05 Ed.16° Technique

Condition 2: IRAM 14005 Technique

Expression of the acidity values: mg of acid lactic/100 ml of milk

Number of Samples: 265

Observations: n1 + n2 = 530

Since 530 observations belong to 265 pairs of experimental material, it is possible to obtain interferences about the differences between the averages for both methods, if they are done regarding the average differences: μd.

To prove the Ho hypothesis: $\mu 1 = \mu 2$ is equivalent then to prove:

Ho: $\mu d = 0$

H1: $\mu d \neq 0$

the statistical test for those hypotheses is $\mathbf{t_o}$.

$$\mathbf{t_o} = \frac{\bar{d}}{S_d / \sqrt{n}}.$$

where $\bar{d}$ is the average of the differences = 32.083019

$$S_d = \sqrt{\frac{\Sigma (d_i - d)^2}{n - 1}}$$

if $t_o < t_{\alpha/2,n-1}$ the null hypothesis is fulfilled

In this case:

$$S_d = \sqrt{\frac{\Sigma (d_i - d)^2}{n - 1}} = \sqrt{340.55303} = 18.454079$$

$$t_o = \frac{\bar{d}}{S_d / \sqrt{n}} = \frac{32.083019}{18.454079 / \sqrt{265}} = 28.301261$$

$t_{\alpha/2,n-1} = t_{0.025.264} = 1.960$

$t_o > t_{\alpha/2,n-1}$, so the null hypothesis is not fulfilled: The average of the differences is not null.

Conclusion: For a 95 % confidence level ($\alpha = 0.05$), there is statistically significant differences between both methods.

Variance Analysis :

Table 1 shows the Variance Analysis results

Number of Samples: 1.Number of Observations: 36 (18 with each methods)

Treatment 1: Technique AOAC 947.05 .Treatment 2: Technique IRAM 14005

Table 1 .Analysis of variance.

Treatment	Acidity (mg of lactic acid/100 ml of milk)		Σy_i	Mean y_i
Treatment 1: Technique AOAC 947.05	111-112-110-110-110-112-110-110-111-112-111-112-111-112-112-110-110-111		1,997	110.94
Treatment 2: Technique IRAM 14005	149-152-158-153-153-153-151-149-149-146-141-154-151-141-148-143-159-143		2,693	149.61
			$\Sigma y_{i1} + \Sigma y_{i2}$ = 4,690	$(\Sigma y_{i1} + \Sigma y_{i2})/n$ = 130.27
Variation Source	Sum of Squares	Degrees of Freedom	Mean Square	F_o
Treatments	13, 456.002	1	13, 456.002	939
Experimental Mistakes	487.220	34	14.33	
Total	13, 943.222	35		

$F_{\alpha,a-1,n-1} = F_{0.05,1,34} = 4.12$

$F_o >>> F_{0.05,1,34}$

Conclusion: With a 95 % confidence level ($\alpha = 0.05$), it is possible to say that the use of one technique or another significantly influences the result of the acidity expressed in mg of lactic acid /100 ml of milk.

Other Tests:

For the case of the 530 data collected by applying one method or another for each one of the 265 samples, other statistical criteria can be applied, for example using the statistical Z_o or the statistical t_o.

For the case of the statistical Zo, both populations can be considered normal. Even in the case they are not, large enough sampling size (n = 265) makes the central theorem of the limit valid.

For the comparison of both methods the following hypotheses are then applied:

$H_o : \mu_1 = \mu_2$ (null hypothesis)

$H_1 : \mu_1 \neq \mu_2$

The null hypothesis can be proven applying the statistical one:

$$Z_o = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\sigma_1^2 / n_1 + \sigma_2^2 / n_2}}$$

if $Z_o < Z_{\alpha/2}$ the null hypothesis is fulfilled

In the case of study:

$\bar{y}_1 = 117.581132$ $\sigma_1 = 14.0266395$

$\bar{y}_2 = 149.664151$ $\sigma_2 = 11.4140363$

$Z_o = 28.8806168$

for an $\alpha = 0.05$ $Z_{\alpha/2} = 1.906$

$Z_o > Z_{\alpha/2}$, so the null hypothesis is not fulfilled

Conclusion: For a 95 % confidence level ($\alpha = 0.05$), there is statistically significant differences between both methods.

For the case of the statistical t_o, considering that the calculated standard deviations from the 265 observations are not the standard deviations of the population, we would use statistical t_o. In that case, the equality of variances hypothesis must be proven first and the equality of averages hypothesis could be proven afterwards to compare the methods object of study.

For the comparison of variances the following hypotheses are then applied:

$H_o : \sigma_1^2 = \sigma_2^2$ (null hypothesis)

$H_o : \sigma_1^2 \neq \sigma_2^2$

The null hypothesis can be proven applying the statistical:

$$F_o = \frac{S1^2}{S2^2}$$

if $F_o < F_{\alpha/2,n1,n2}$ the null hypothesis is fulfilled

In the case of study:

$$F_o = \frac{S1^2}{S2^2} = \frac{196.74660}{130.28022} = 1.51$$

$F_{\alpha/2,n1,n2} = F_{0.025,264,264} = 1.00$

$F_o > F_{\alpha/2,n1,n2}$, so the null hypothesis is not fulfilled: The variances are significantly different.

For comparison of averages, the following hypotheses are then applied:

$H_o : \mu_1 = \mu_2$ (null hypothesis)

$H_1 : \mu_1 \neq \mu_2$

Due to the proven difference of variances, the null hypothesis can be proven applying the statiticsal one:

$$t_o = \frac{\bar{y}1 - \bar{y}2}{\sqrt{\sigma_1^2 / n_1 + \sigma_2^2 / n_2}}.$$

if $t_o < t_{\alpha/2, \nu}$ the null hypothesis is fulfilled

In the case of study:

$\bar{y}1 = 117.581132$ $\sigma 1 = 14.0266395$

$\bar{y}2 = 149.664151$ $\sigma 2 = 11.4140363$

$t_o = 28.8806168$

for an $\alpha = 0.05$ y $\nu = 507$ $t_{\alpha, \nu,} = 1.645$

$t_o >> t_{\alpha, \nu,}$ so the null hypothesis is not fulfilled.

Conclusion: For a 95 % confidence level ($\alpha = 0.05$), there is statistically significant differences between both methods.

CONCLUSIONS

After applying different statistical tests, it is concluded in all the cases that there are statistically significant differences (with a 95 % confidence level) between the analytical results obtained by one method or another.

ACKNOWLEDGMENTS

The authors acknowledge the collaboration of the participating dairy industries and their personnel and of all the personnel of INTI Lácteos, especially Eduardo Storani for his collaboration with the translation.

REFERENCES

Alais, C. 1971. Ciencia de la leche. Principios de técnica lechera. Page 187 to 195.1st. ed. in Spanish. Compañía Editorial Continental. Barcelona.

Goded y Mur, A. 1966.Técnicas modernas aplicadas al análisis de la leche. Page 62 to 64. 1st. ed. Dossat, Madrid.

Luquet, F. M.1991. Leche y productos lácteos. Vol. 1. Page 344. Acribia, Zaragoza.

Montgomery, D. C. 1991.Diseño y análisis de experimentos. Grupo Editorial Iberoamérica, California.

Van Slyke, L. L. and Bosworth, A. W. 1914.The cause of acidity of fresh milk of cows and a method for the determination of acidity. J. Biol. Chem. 19: 73-76.