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

M. A. Fabro,*[1] H. V. Milanesio,* L. M. Robert,* J. L. Speranza,* M. Murphy,† G. Rodríguez,† and R. Castañeda†
*División Rafaela, Centro de Investigaciones Tecnológicas de la Industria Láctea, Instituto Nacional de Tecnología Industrial, Rafaela, 2300, Argentina
†División Miguelete, Centro de Investigaciones Tecnológicas de la Industria Láctea, Instituto Nacional de Tecnología Industrial, General Paz and Alvarellos, San Martín, Buenos Aires, Argentina

ABSTRACT

In Argentina, one analytical method is usually carried out to determine acidity in whole raw milk: the Instituto Nacional de Racionalización de Materiales standard (no. 14005), based on the Dornic method of French origin. In a national and international regulation, the Association of Official Analytical Chemists International method (no. 947.05) is proposed as the standard method of analysis. Although these methods have the same foundation, there is no evidence that results obtained using the 2 methods are equivalent. The presence of some trends and discordant data lead us to perform a statistical study to verify the equivalency of the obtained results. We analyzed 266 samples and the existence of significant differences between the results obtained by both methods was determined.
Key words: acidity, milk, methodology

INTRODUCTION

What is usually known as milk acidity is the result of titration (Alais, 1971). Titratable acidity is the capacity of combination with a base (Goded y Mur, 1966). The measurement principle is unique, and is based on adding, to a given volume of milk, the necessary volume of alkaline solution (sodium hydroxide) of an exact concentration until the neutralization point is reached, which is determined by the presence of an indicator, generally phenolphthalein, which turns from colorless to pink at pH 8.4.

Although the measurement principle is the same, there are variations among methods. Goded y Mur (1966) differentiated the official methods of several countries according to the concentration of the alkaline solution, the milk volume to titrate, and the concentration of phenolphthalein. In addition to methodological variations, there are different units in which to express the obtained results: Dornic degree (°D), Soxhlet-Henkel degrees (SH), and Thorner degrees.

These variations can cause possible incongruities in the results obtained by one or other technique in the same milk sample. Alais (1971) states that, although the acidity measurement is simple to conduct, there are several sources of error, including the amount of indicator and the determination of the end-point of the titration. Goded y Mur (1966) indicated as possible sources of error 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, determination of milk acidity is usually made using the method of Instituto Nacional de Racionalización de Materiales (**IRAM**) standard 14005 (IRAM, 1976) because it is the national standard method and it is somewhat faster to perform than the alternative technique, method 947.05 of the Association of Official Analytical Chemists (AOAC, 1990). Both methods are based upon the same principle but there are differences in the volume and dilution of the test sample and in the titration.

The AOAC method (no. 947.05; AOAC, 1990) was established as a methodology of analysis for acidity in a regulation: SGT No. 3/REC No. 40/94 of Código Alimentario Argentino Anexo Mercosur (Código Alimentario Argentino, 2003). According to this methodology, certain values of acidity were established as tolerated limits for normal milk. Many milk samples that were normal by the IRAM method were outside the tolerated limits when analyzed using the AOAC method. When comparing data among laboratories of different companies and different countries using one standard or another, some discordant data and trends began to appear. In the course of our research, we found out that these values of acidity were the normal limits fixed for milk under another standard, IRAM 14017, using the methodology of IRAM 14005 (IRAM, 1976).

Although the different methods described above have the same foundation, there is no evidence that the re-

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[1]Corresponding author: mfabro@inti.gov.ar

sults obtained are equivalent. This observation led us to perform a statistical study to verify the equivalency in the obtained results using different standards, which may help in the revision of the tolerated limits for each methodology.

MATERIALS AND METHODS

To determine if differences existed between the results obtained by both methodologies, 266 samples were analyzed by both techniques in the laboratories of the Instituto Nacional de Tecnología Industrial (Rafaela, Argentina) to enable a statistical analysis of the collected data.

Sample Characteristics

Our laboratory worked with 8 dairy companies located in the main milk production areas of Argentina to collect representative samples of Argentina's milk production. Whole raw milk samples were selected, homogeneously distributed by region and time over 4 mo of sampling.

Sampling Procedure

The sampling and delivery of the samples was carried out by the companies' own transportation allowing the samples to be in the laboratory within 16 h after sampling. No preservative was used. The samples were maintained from the time of sampling until the time of analysis at temperatures between 2 and 10°C. Each sample was analyzed using both methods within 10 min.

Analytical Methods

AOAC Method. To 20 mL of milk sample was added 40 mL of boiled and cooled distilled water, and 2 mL of phenolphthalein (prepared at 1% in 95% ethanol). The mixture was titrated with standardized 0.1 *M* NaOH until the first color change (to pink) persisted for 30 s. One more drop of 0.1 *M* NaOH was added and the final volume of 0.1 *M* NaOH added was noted.

IRAM 14005 Method. To 10 mL of sample was added 3 drops of phenolphthalein solution (prepared at 2% in 96% ethanol). The mixture was titrated with standardized 0.111 *N* NaOH until the first color change (to pink) persisted for 30 s. One more drop of 0.111 *N* NaOH was added and the final volume of 0.111 *N* NaOH added was noted.

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

Table 1. Results obtained for determination of titratable acidity in 256 milk samples using 2 methods. All values are expressed in milligrams of lactic acid /100 mL of milk

	Method[1]		
	AOAC	IRAM	Difference
Average	117.581132	149.664151	32.083019
Standard deviation	14.0266395	11.4140363	18.454079

[1]AOAC = Method 947.05, Association of Official Analytical Chemists (AOAC, 1990); IRAM = method 14005, Instituto de Racionalización de Materiales (IRAM, 1976).

$$\text{Lactic acid (mg)/100 mL of milk} = (Vg \times N \times 90 \times 100)/Vm$$

where Vg = volume of NaOH solution added, N = concentration of sodium hydroxide standardized solution expressed in Eq/L, 90 = equivalent weight of lactic acid, and Vm = volume of milk used for titration.

Statistical Methods

Two statistical tests to compare the methods were applied: pair comparison design and single-factor variance analysis (Montgomery, 1991).

RESULTS AND DISCUSSION

A total of 566 analytical results were obtained from 266 samples; 265 samples were analyzed by both methods and 1 sample was analyzed 18 times by both methods.

Statistical Tests

The pair comparison design was used to analyze the 530 results from 265 samples analyzed by both methods, and the single-factor variance test was used for the results from 1 sample analyzed 18 times by both methods.

Pair Comparison Design. To prove the Ho hypothesis: $\mu 1 = \mu 2$ is equivalent it was necessary to prove Ho: $\mu d = 0$. The statistical test for those hypotheses was t_o. In this case, $t_o = 28.301261$ and $t_{\alpha/2,n-1} = t_{0.025.264} = 1.960$. As $t_o > t_{\alpha/2,n-1}$, the average of the differences is not null; for a 95% confidence level ($\alpha = 0.05$), there is a statistically significant difference between both methods. Some parameters (in mg of lactic acid/100 mL of milk) of the obtained results from 256 samples analyzed using both methods are shown in Table 1.

Variance Analysis. Table 2 shows the variance analysis of the 36 results obtained from analysis of 1 sample multiple times using both methods. With a 95% confidence level ($\alpha = 0.05$), it is possible to say that the

Table 2. Variance analysis of 36 determinations of titratable acidity (18 for each method) in 1 milk sample using 2 methods

Method[1]	Acidity (y_i; mg of lactic acid/100 mL of milk)	Σy_i	Mean y_i
AOAC	111-112-110-110-110-112-110-110-111-112-111-112-111-112-112-110-110-111	1,997	110.94
IRAM	149-152-158-153-153-153-151-149-149-146-141-154-151-141-148-143-159-143	2,693	149.61

Source of variation	Sum of squares[2]	df	Mean square[3]	$F_0 = \frac{\text{Mean Square Treatments}}{\text{Mean Square Experimental}}$
Treatments[4]	13,456.002	1	13,456.002	939
Experimental[5]	487.220	34	14.330	
Error total[6]	13,943.222	35		

$F^{7}_{\alpha,a-1,n-1} = F_{0.05,\ 1,\ 34} = 4.12$
$F_o >>> F_{0.05,\ 1,\ 34}$

[1]AOAC = Method 947.05, Association of Official Analytical Chemists (AOAC, 1990); IRAM = method 14005, Instituto de Racionalización de Materiales (IRAM, 1976).

[2]Sum of square (Montgomery, 1991).

[3]Mean square = sum of square/degrees of freedom.

[4]Variation between values of both methodologies.

[5]Variation between values from the same methodology.

[6]Total variability between the all values (yi).

[7]Value of the distribution F for these test conditions and with 95% confidence.

use of one technique or another significantly influenced the result of the acidity expressed in milligrams of lactic acid per 100 mL of milk.

DISCUSSION

After applying 2 statistical tests, it was concluded that there are statistically significant differences (with a 95% confidence level) between the analytical results obtained by the 2 methods. The main causes of the differences could be the larger sample size and the sample dilution in the AOAC method. Both factors would allow better and earlier observation of the color change to pink, and thus addition of NaOH would stop earlier. The resultant acidity value would be lower. The difference in concentration of the alkaline solutions is very small and would not be an important factor.

CONCLUSIONS

The results of this study allow us to conclude that differences exist in the results obtained by both methodologies applied on the same milk sample. Results obtained using one method are not equivalent to those obtained by the other method, which correlates with anecdotal reports from dairy industry laboratories. The evidence obtained as a result of this work would allow us to avoid errors that could be present when results obtained by diverse methodologies are compared.

The results of this study are important for the Argentinean dairy industry. With this information, the industry can ask for a revision of the tolerated limit for acidity in the resolution SGT No. 3/REC No. 40/94 of Código Alimentario Argentino Anexo Mercosur.

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