

Marler J R & Boulet M. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. *J. Dairy Sci.* 41:1683-92, 1958.
[Div. Applied Biology, National Research Council, Ottawa, Canada]

To overcome problems associated with pre-existing procedures for citric acid analysis, we determined the optimum proportions of the various reagents required at different reaction temperatures. The recommended temperature of 32°C provides maximum sensitivity and stability of the color reaction in a simplified procedure. [The SCI® indicates that this paper has been cited in over 105 publications since 1961, making it the 11th most-cited paper published in this journal.]

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"This method was one of several analytical procedures developed while I was part of a food chemistry group conducting extensive studies on the chemistry of milk¹ at the biology division of Canada's National Research Council in Ottawa. During the mid-1950s, we were looking for reliable analytical methods that also embodied a convenient procedure that was suitable for everyday routine analysis. Quite often we had to develop (or modify) such procedures ourselves, and the method for citric acid is a case in point.

"There was a need for this particular method because the then available procedures for citric acid analysis had serious shortcomings, one of which was that they required relatively high temperatures and this made it difficult to control the volatility of the reaction. I remember how the rubber stoppers kept exploding from atop the reaction tubes, and how they would fly across the lab like unguided missiles. This, for understandable reasons, impaired the reliability of such procedures. Also, it meant that

one had to develop the agility of a center fielder in order to intercept the airborne rubber stoppers before they struck a colleague or other lab furnishings. Thus, it was in this setting that my colleague, Marcel Boulet, encouraged me to conduct a detailed study of the available methodologies, and this eventually led to the procedure that was published in 1958.

"Contrary to what had been advocated in previous procedures, I soon realized that the pyridine addition could precede that of acetic anhydride, and that both reagents could be added at room temperature. Thereafter, it was necessary to continue the reaction in a constant-temperature bath, so as to dissipate the excess heat generated by the exothermic reaction. But my main goal was to study the feasibility of using a relatively low reaction temperature, in hopes of eliminating the violent nature of the former techniques. This was achieved, but only after a considerable investment of time spent in learning exactly how different temperatures require different optimal concentrations of reagents (also water) to attain maximum intensity and stability of the color produced.

"One personal pitfall occurred when I required brief hospitalization after months of pipetting pyridine by mouth; thereafter, automated pipettes were used, and we ran the entire operation in a fume hood. We encountered only one subsequent problem, and this was caused by the hygroscopic nature of the citric acid monohydrate used as a reference standard; however, we solved this by recourse to trisodium citrate dihydrate.²

"It is gratifying to know that something you did 25 years ago continues to be useful and reliable.³ The frequency of the citations is, at least in part, attributable to the method's adaptability. For example, our procedure has not only found widespread use in agricultural research, but also in the pharmaceutical field."⁴

1. Emmons D B. Dairy research in Canada 1958-1964. *Dairy Sci. Abstr.* 28:389-406, 1966.
2. Marler J R & Boulet M. Preparation of a standard for citric acid analysis. *J. Dairy Sci.* 42:1885-6, 1959.
3. Bonatelli R, Jr. & de Azevedo J L. Improved reproducibility of citric acid production in *Aspergillus niger*. *Biotechnol. Lett.* 4:761-6, 1982.
4. Hartford C G. Rapid spectrophotometric method for the determination of itaconic, citric, aconitic, and fumaric acids. *Anal. Chem.* 34:426-8, 1962.