The influence of amyl alcohol in the Gerber method.

(Abstract of the lecture given by Mr TROSSAT of CECALAIT at CECALAIT's annual general meeting in June 1999.)

The Gerber acidobutyrometric method has been widely used as a routine method for the determination of fat in milk in most dairy laboratories and in most countries. Indeed, it is easier and cheaper than the reference Röse-Gottlieb method. However, the accuracy of the routine method has always been carefully examined by the standardization bodies, because it is well known that these two methods are not strictly equivalent. Moreover, some discrepancies between results coming from labs in different countries have been observed, depending on the origin of one reagent : amyl alcohol (see box at the end).

In France, CECALAIT, AFNOR, official laboratories (DGCCRF) and interprofessional laboratories worked together. Firstly, to solve the problem of differences, due to different amyl alcohols by a better definition of its composition. Secondly, the work was aimed at proposing an ideal reagent to ensure equivalent results between the Gerber and the Röse-Gottlieb methods, in a fat range as wide as possible.

↳ INFLUENCE OF ISOAMYL ALCOHOL

In the Gerber method, amyl alcohol should make easier fat separation in the butyrometer. However, a few years ago, some laboratories noticed differences in fat results depending on the origin of the alcohol. The differences were observed among french laboratories and also, more importantly, between french and foreign laboratories.

It was suggested that some alcohols might not comply with the specifications of the annex of V 04-210 french standard, describing the Gerber method (cf box). So, official laboratories analysed 7 usual alcohols, from 7 different suppliers and concluded that each of them complied with the standard. However, analysis by gas chromatography of the ratio of the two isomers present in the mixture showed that the proportion of 3-methyl-1-butanol varied from 70% to 100%.

Meanwhile, the examination of the results obtained from 1991 to 1996, in Cecalait's proficiency studies showed a discrepancy between Gerber and Röse-Gottlieb results. It was about –0,3 g at the time, for mean fat ranges (it is much smaller now), but was not constant for all fat levels.

So, in 1997, an interlaboratory study was organised to check the influence of the composition of amyl alcohol on the accuracy of the Gerber method.

It involved 10 laboratories analysing in duplicate, by the Gerber method, 10 samples with fat ranges from 15 to 50 g/l. They used 3 different alcohols with following isomer ratios :

- 3-methyl-1-butanol : 83% and 2-methyl-1-butanol : 17% (M3B1/M2B1 83/17)
- 3-methyl-1-butanol : 91% and 2-methyl-1-butanol : 9% (M3B1/M2B1 91/9)
- 100% 3-methyl-1-butanol (M3B1/M2B1 100/0)

The means of the results were compared to the results obtained at the same time on the same samples in an interlaboratory study using the Röse-Gottlieb reference method.

Table 5, page 11 in « La Lettre de CECALAIT » shows the results and the significative influence of the isomer ratio of amyl alcohol.

↳ TOWARDS AN « IDEAL » ISOMER RATIO …

Treating the results of this first interlaboratory study allowed the calculation of an « ideal » isomer ratio, for equivalent results between the Gerber and the Röse-Gottlieb method, when fat ranges from 15 to 50 g/l.

The 94/6 M3B1/M2B1 was thus calculated and validated through an interlaboratory trial.

Performed in 1998, it involved 28 interprofessional laboratories analysing, by the Gerber method with that type of alcohol, 10 samples with fat ranging from 15 to 50 g/l. The results were compared to those obtained at the same time by 21 laboratories analysing the same samples by the Röse-Gottlieb method : they showed a mean bias of about 0.05 g/l.

An assesment meeting in May 1999 highlighted the following points:

- amending the composition of amyl alcohol improves the adjustment of the two methods,
- Since 1996, the discrepancy between the two methods has reduced greatly, maybe because a change occured in the manufacture of the alcohol at the time. According to suppliers, the isomer ratio is generally about 95/5 of M3B1/M2B1 since the beginning of 1997.
- the French interprofession wishes the two methods to be equivalent in the mid fat ranges, between 30 and 50 g/l. This is indeed the most representative range for milk payment and trade, corresponding to the great majority of individual or herd french milks.

Therefore new calculations were necessary and finally it is the ratio **M3B1/M2B1 91/9** which has been proposed.

↳ TOWARDS THE STANDARD'S REVISION

Since then, AFNOR has almost completed a new version of V 04-210 standard, which will :

- specify the precise composition of the amyl alcohol, ie a mixture of the M3B1/M2B1 isomers in the proportion **91%/9%**, with an uncertainty of ± 2% for each part of the ratio. (This would have a maximum incidence of ± 0.06 g/l on the final result).
- specify the application domain for fat ranging from 30 to 50 g/l. In this common domain, both methods are equivalent. The standard will point out the differences which might be observed outside of this domain. In this case, other (internal) validation studies are highly desirable.

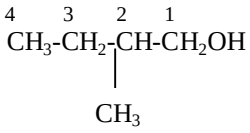
Suppliers should also be kept in touch in order to ensure that the manufactured alcohols comply with the isomer ratio specified in the standard. Eventually, this ratio will be put on the certificate of compliance of the reagent.

This new version of standard V 04-210 should be issued at the end of this year.

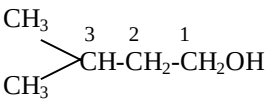
The amyl alcohol used in the Gerber method

This reagent, $C_5H_{12}O$, is actually, a mixture of two isomers.

2-methyl-1-butanol, or amyl alcohol
or *dl-sec*-butylcarbinol



3-methyl-1-butanol, or isoamyl alcohol
or isopentyl alcohol
or isobutylcarbinol



Some specifications of amyl alcohol, according to the annex of V 04-210 standard

- volumic mass between 0.808 and 0.818 g/ml
- colourless
- composed of at least 98%, in volume, of the mixture of following primary alcohols :
  - 3-methyl-1-butanol
  - 2-methyl-1-butanol
- without secondary alcohol.