CRITICAL POINTS OF KJELDAHL AND AMIDO BLACK METHODS : SUMMARY

Amido Black and Kjeldahl are based on different principles. But for historical or just convenient reasons laboratories may use either Kjeldahl or Amido Black method for routine analysis of proteins or for infra red calibration. As Amido Black is calibrated versus Kjeldahl, there should be no problem in comparing results of different laboratories. But both methods have critical points which should be carefully considered. If it is not the case, there might be differences in the results then.

- The critical points of the Kjeldahl method are :

  - during the digestion step : quality of reagents (acid, catalyst), relative amounts of test portion and reagents, temperature and duration of digestion. If these points are not well controlled, there might be insufficient digestion or overheating with vaporization or pyrolysis.

  - during the distillation step : excess of sodium hydroxide solution, sufficient volume of distillate, sufficient volume of boric acid, absence of leaks... must be well controlled points.

  - during the titration step : a right blank test and the accuracy of the titrating acid, which concentration might increase by evaporation in time, are the important points here.

Failures in controlling any of the above mentionned points will lead to lower results.

- The critical points of the Amido Black method are :

  - test portion systems : foaming of the calibrating samples is an important risk. The test portion is then too low and calibration will not be correct. Final errors may be as high ad +0.2-0.3 g/kg.

  - shaking : the dye-protein complex must be instantly and vigorously shaken in order that the dyestuff can bind to all binding sites. If it is not the case, it may lead to irregular errors and a poor repeatability.

  - separation of the dye-protein complex. The critical points are here :

    - centrifugation where time and acceleration must comply with IDF 98A:1985. If this is not well controlled, it may lead to a poor repeatability.

    - filtration : the complex may cross the filter. The results will be too high then, with errors that may reach +0.2-0.3 g/kg.

  - absorbance measure :

The chimical reaction is not totally stoechiometric so there is a small departure from a linear relationship. Therefore a three points calibration is necessary. If not, the errors may reach +0.1-0.2 g/kg at the 30 g/kg level.

At last, it is very important to be in a correct absorbance domain corresponding to the field of application ie :

- 24 to 40 g/l for cow milk
- 46-65 g/l for ewe milk

This is depending on test portion volume, pH and concentration of Amido Black solution, optical path. If the absorbance domain is uncorrect, the results will all be false, without any connection with the Kjeldahl method.

In conclusion, bad control of the Kjeldahl method will usually lead to underestimated results whereas bad control of the Amido Black method will lead to overestimated results.