MILK TESTING OF LIPOLYSIS AND PROTEIN MATTER

Automation of Milk Copper Soap Method and Amido Black Method with the 223 Sample Changer

TECHNICAL NOTE TN223

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INTRODUCTION

In laboratories specializing in milk analysis, there are two methods where Gilson could help with a semi- or full automation of the complete protocols. In comparison to the manual procedure, automation provides more robust and reproducible results and sample traceability.

The first method is the Copper Soap method for the calculation of the Lipolysis Index.

The second method is the Amido Black method for the calculation of protein content in milk.

WHAT IS THE LIPOLYSIS INDEX ANALYSIS FOR?

Lipolysis is the hydrolysis of lipids in milk, which produces free fatty acids (FFAs) that have both detrimental and desirable effects. The detrimental effects are due to the unpleasant flavours of short-chain fatty acids when present at high concentrations. However, under some circumstances and usually at lower concentrations, the short-chain FFAs impart desirable flavours to dairy products and other foods. For example, the characteristic flavour of some cheese varieties is due to their FFA content.

There are three distinct forms of lipolysis (Figure 1):

- **Natural Spontaneous Lipolysis**
  Lipases are secreted naturally by the udder and are therefore present in the milk at source, resulting in lipolysis.
- **Natural Induced Lipolysis**
  Lipolysis can be accentuated by mechanical and thermal shocks that the milk undergoes (especially during milking and cold storage). These shocks weaken the membrane of fat globules and thus promote the action of lipases.
- **Microbial Lipolysis**
  Microbial lipases are secreted by certain bacterial species (e.g., *Pseudomonas*), which are able to grow in cold conditions and especially in refrigerated milk. Note that these bacteria are prevalent in the environment (soil, plants, water, etc.) and their presence in the milk is due to contamination during milking or storage of milk. Indigenous milk lipase is destroyed by pasteurization, but the bacterial lipases are heat-stable and can remain active in processed milk and cause lipolysis during storage, even in the case of ultra-high temperature treated milk and dairy products.

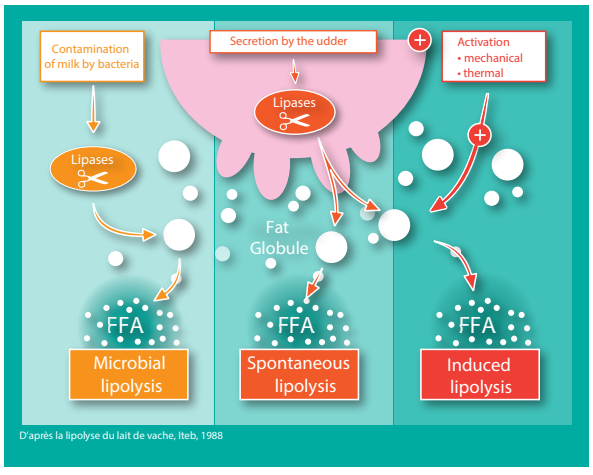

Figure 1
Mechanisms of lipolysis of milk

The level of lipolysis in milk and dairy products is usually measured by their FFA content. This measurement is called the "Lipolysis Index." It reflects the additive effects between these three types of lipolysis.

What is the Significance of the Lipolysis Index?

- These free fatty acids, which accumulate and oxidize, can cause flavour defects, unappreciated by consumers (rancid taste, bitterness, soap taste). The most sensitive products are butters, creams, and fat powders. Consumer milks or yogurts can also be affected.
- One of the criteria for judging the quality of milk is the Lipolysis Index, which gives information on the degree of alteration of the fat, estimating the intensity of lipolyzed flavour.
- The determination of the Lipolysis Index is used for the payment of milk in certain regions.

The Copper Soap Method for Measuring the Lipolysis Index

There are several methods to measure the Lipolysis Index. In some countries, these methods are validated and authorized by their Bureau of dairy industry (in France, they are published in the Bulletin de la Fédération Internationale de Laiterie (FIL) n° 265, in 1991). One of these validated methods is a chemical technique known as the "Copper Soap solvent extraction method."

- In this method, the free fatty acids are converted to copper soaps, extracted, and the copper reacted with a coloured reagent.
- The reagents used are: the copper solution ($Cu(NO_3)_2$), the extraction solvent CHM (Chloroform/Heptane/Methanol), the coloured reagent (sodium diethyl dithiocarbamate solution in n-butanol) and the optional solubilization reagent (EDTA)
- A UV-Vis spectrophotometer (440 nm) is used for quantification. Any spectrophotometer could be used to measure the colour. This spectrophotometer has the advantage that it can be combined with the analyses of Protein Matter (PM) using the Amido Black method, already used in the laboratories (see below).

WHAT IS THE PROTEIN MATTER ANALYSIS FOR?

Quantifying the Protein Matter of the milk is in direct relation to the quantification of the nitrogen matter of the milk.

A quick method for determining the nitrogen content of milk, sufficiently accurate and reproducible, is interesting from several points of view:

- Selection of dairy cattle producing high protein milk
- Price of milk paid to producers according to the nitrogen content, mainly in the cheese regions
- Experimentation on dairy cattle (influence on food, etc.)
- Measurement of the yield of milk cheese—in dairies, the cheese consisting essentially of most of the fat and nitrogenous matter of the milk

The Amido Black Method for Measuring Protein Matter

There are several methods to measure the Protein Matter content in milk. In some countries, those methods are validated and authorized by their Bureau of dairy industry. One of these validated methods is a chemical technique known as the "Amido Black method."

- The reagent used is the Amido Black 10B acidic solution.
- An excess of dye solution is reacted with the milk proteins in citrated phosphate buffer at pH 2.45. At this pH value, lower than the isoelectric point, the proteins are positively charged (basic groups of histidine, arginine, and lysine) and they combine with the negatively charged dye molecules; this results in the staining and precipitation of these proteins.
- Excess dye is determined by measuring the optical density of the supernatant. The larger the amount of protein, the lower the optical density and vice versa. Any UV-Vis spectrophotometer could be used to measure the colour. This spectrophotometer has the advantage that it can be combined with the testing of the Lipolysis Index using the Copper Soap Method already used in the laboratories (see above).

SYSTEM DESCRIPTION

Both of these methods, the “Copper Soap” method and the “Amido Black” method, require a similar system for automation: a liquid handler with syringe pump to prepare the sample (distribution of reagents) and a liquid handler with peristaltic pump to inject the sample into the spectrophotometer to read the absorbance of the prepared sample. This will give the result for the Lipolysis Index or Protein Matter.

Semi-Automated Protocol

For the first part of sample preparation, it is possible to use one VERITY® syringe pump as a stand-alone dispenser dilutor using the Special SPL-2542D-HDW (Figure 2).

The reading on the spectrophotometer is made manually.

Figure 2
VERITY® Dispenser Dilutor

Complete Automation of the Method (Figures 3, 4, and 5)

- 223 Sample Changer,
  - 123 mm or 183 mm Z-arm and standard or custom racks (depending on the customer’s sample tubes).
  - The 223 Sample Changer was chosen due to the low cost. GX-series liquid handlers could also be used.
- Dual syringe pump for sample preparation:
  - VERITY® 4220 Dual Syringe Pump with 5 mL syringes for the Copper Soap Method,
  - VERITY® 4120 Dual with Tee Syringe Pump with 25 mL and 1 mL syringes for the Amido Black method.
- MINIPULS® 3 Peristaltic Pump for injection towards the detector,
  - Optional: sometimes it is possible to use the pump included with the spectrometer
- TRILUTION® LH Software.
- Spectrophotometer (not sold by Gilson)
  - e.g., SAFAS with circulating cuvette

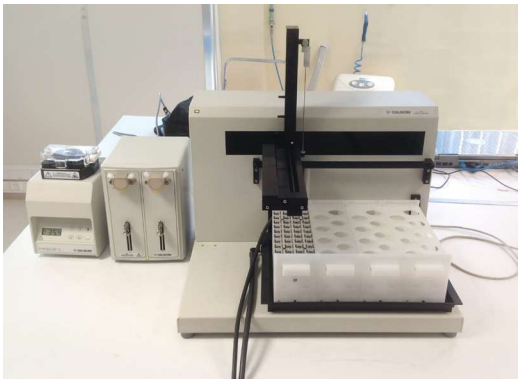

Figure 3
Gilson system before installation at customer site

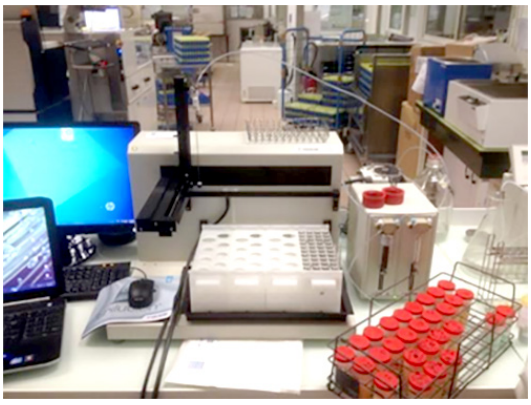

Figure 4
Gilson system at customer site

Figure 5
SAFAS spectrophotometer

TRILUTION LH Software for Automated Protocols (Figures 6, 7, 8, 9, and 10)

Example of a customer database:

- Four Applications
  - › Sample preparation
  - › Standard preparation
  - › Reading (for sample analysis and spectrometer calibration)
  - › Calibration of the syringes
- Methods
  - › Add reagents,
  - › Supernatant transfer,
  - › Reading,
  - › ...

With TRILUTION LH control, the system is simple for a lab user to run. Sample lists can be saved ready for a user to simply upload and run.

Figure 6
TRILUTION® LH Software configuration for sample preparation method

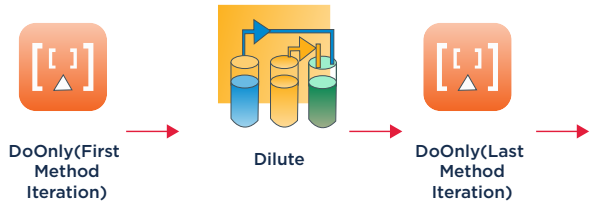

Figure 7
Example of tasks used for sample preparation method

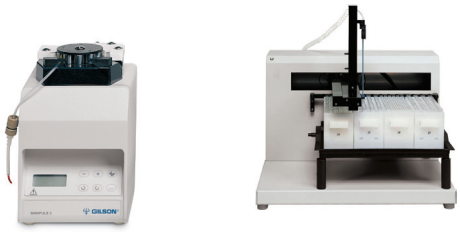

Figure 8
TRILUTION® LH Software configuration for UV-reading method

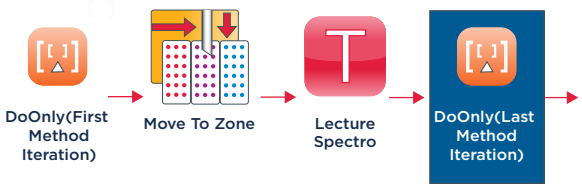

Figure 9
Example of tasks used for UV reading method (custom task could be created if needed to send the command to the spectrophotometer for the reading)

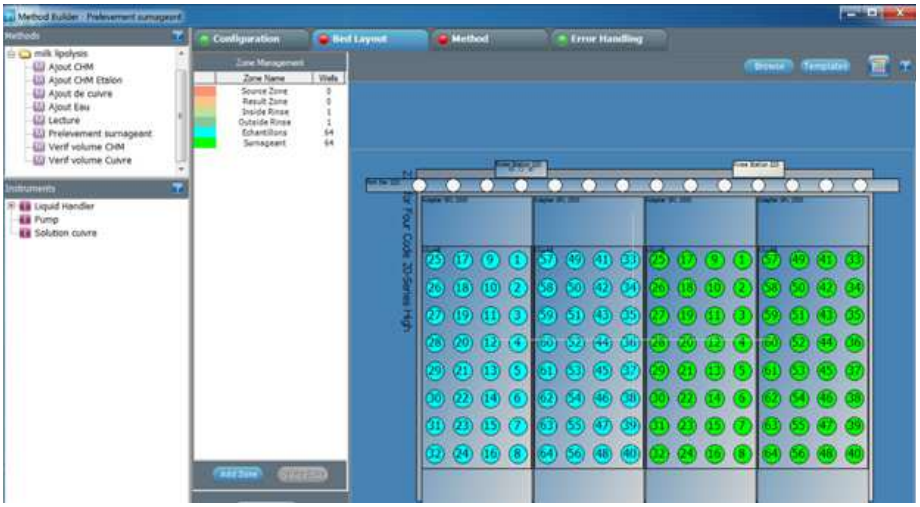

Figure 10
Example of TRILUTION® LH Software bed layout

EXAMPLE CONFIGURATION

PART NUMBER	DESCRIPTION	QUANTITY
191015	223 SAMPLE CHANGER (GILSON) DELUXE PKG	1
190610	Z DRIVE, 183MM	1
27067373	PROBE,221X1.5X1.1MM CON FL .45 ID TIP	2
19061040	PROBE HOLDER/GUIDE KIT, 1.5MM	1
27072001	COUPLING, NEEDLE/ TUBING	1
4701177592	TBG,VITON 4 X 6.5MM OD, 1 METER PIECE	5
2707251L	RINSE STATION, XL	1
36083122	SERIAL CABLE, 9-PIN/25-PIN	1
31130002	4120 DUAL SYRINGE PUMP W/TEE	1
25025343	SYRINGE, 1ML, 215/235	1
25025346	SYRINGE, 25ML, 215	1
49948392	TUBING,TRANSFER 2800UL,1000 X 3 X 2MM ID	1
SPL-2209-HDW	RACK, GX-271 SHORT 7-37X70MM BOTTLE	1
150498	RACK,CODE 22U,UNIV 10-18MM DIAX100-175MM	1
21063024	TRILUTION LH 4.0 LICENSE, LIFETIME	1
F155001	MP3 DRIVE UNIT 0.01 TO 48 RP, 110/220V	1
F117604	PUMP HEAD, R1 SINGLE CHANNEL SS	1
49942392	TUBING,TRANSFER 440UL, 1000X1.6X.8MM,FEP	1
F1410050	COUPLINGS, 200-16, 5/EA	1
F117938	TUBING, PVC, 1.02MM ID, WHT/WHT, 12/PKG	1
F1179941	CONNECTOR, PVDF 1-2 MM ID TUBING 10/PK	1
F117958	TUBING, PVC, 1.02 MM X 2.7 MM, 3 METERS	1
36078143	SHIELDED GSIOC CABLE, 30"	1
	PC AND POWER CORDS TO BE ADDED AS APPLICABLE	

CONCLUSION

The Gilson automated system for milk lipolysis and/or Protein Matter is ideal for milk producers/manufacturers, and for inter-professional analysis laboratories.

The determination of the milk Lipolysis Index is a mandatory parameter to fix the selling price of the milk.

Automation provides advantages compared to manual methods:

- Provides liquid handling tasks to prepare the samples
- Automates injections onto the detector for traceability
- Eliminates user errors
- Compliant with official standards

The system is easy to use with routine methods - just place the samples and reagents, and then press go!