CHARACTERISATION OF COAGULANTS: A TOOL FOR THE CHEESE INDUSTRY

Coagulants are technological auxiliaries indispensable to the cheese industry that can be of many origins:

- **<u>Animal</u>, obtained mainly from the maceration of ruminants' abomasa and generally containing a mixture of enzymes: chymosin and pepsin in various concentrations according to the age and the feed type of the young ruminant.**

- **<u>Microbial</u>, obtained by fermentation in controlled conditions. The three main ones available at this time are:**

    - **Acid proteinase native of *Cryphonectria parasitica* (formerly *Endothia parasitica*)**
    - **Acid proteinase native of *Rhizomucor pusillus* (formerly *Mucor pusillus*)**
    - **Acid proteinase from *Rhizomucor miehei* (formerly *Mucor miehei*).**

- **<u>Fermentative</u>, produced after cloning of the gene producing prochymosin *Aspergillus niger var awamori* and *Kluyveromycès lactis*.**

METHODS

Various methods exist for analysing the qualitative and quantitative characteristics of these coagulants as well as their coagulating activity:

◆ <u>Qualitative and quantitative determination</u>

- **The official French method, published in the OJFR of January 1981** for the determination of chymosin and bovine pepsin content, which is split into 2 parts:

    - *A qualitative method (Part A)* to identify the enzymes present and to ensure that only chymosin and bovine pepsin are present in the rennet extract (by an immunodiffusion method with specific antibodies).

    - *A quantitative method (Parts B and C)* to determine the active quantities of chymosin and bovine pepsin (in mg/L) in the rennet extract. This method includes the separation of the two enzymes using a chromatographic technique on an ion exchange column and the measurement of the coagulation time at 30°C, of a powder milk substrate reconstituted in the presence of calcium chloride 0.1M (pH 6.35).

    This method corresponds to version A of the 1987 IDF 110 method.

- **The current version of the IDF 110 method (B: 1997),** which presents the following modifications in relation to IDF 110A:

    - After chromatographic separation of the enzymes, a referral to ISO 11815 / IDF 157 for the measurement of the coagulating activity of each enzyme **at a coagulation temperature of 32°C (versus 30°C in the IDF 110A standard)**, which is performed on a substrate reconstituted from milk powder in the presence of calcium chloride **(presenting a pH of 6.5)**.

    - Results are expressed in % of chymosin and pepsin (from IMCU/g or ml values: International Milk Clotting Unit) with the aid of standard chymosin and bovin pepsin powders, in addition to the concentration in mg/L present in the previous version.

◆ Determination of the coagulating activity

- **ISO 11815 / IDF 157 standard** applied to bovine animal rennet and fermentative chymosin for the determination of the total coagulating activity in milk.

The measurements are performed on a substrate reconstituted from milk powder and calcium chloride presenting a pH of 6.5 at 32°C in comparison with chymosin and pepsin standards titrating 1000 IMCU/g.
Firstly, the coagulating activity is calculated in relation to the standards, and then the calculation of the total coagulation is executed by interpolation with a reference solution presenting the same composition (cf IDF 110 standard).

- **ISO 15174 / IDF 176 standard** applied to microbial coagulants for the determination of the total coagulating activity in milk.

The measurement is performed on a substrate reconstituted from milk powder and calcium chloride with a pH of 6.5 at 32°C compared to a microbial coagulant standard (Rhizomucor miehei) titrating 1000 IMCU/g.

SERVICES PROVIDED ON-SITE IN POLIGNY

- CECALAIT / ACTILAIT (contact X. Quervel : x.quervel@cecalait.com) proposes the following services concerning coagulants:

  - Quantitative determination of the chymosin and pepsin content in animal rennet according to the **OJFR of 1981** method or the **IDF 110B** method.

  - Determination of the coagulant activity of bovine rennet according to **ISO 11815 / IDF 157** standard and microbial coagulants according to **ISO 15174 / IDF 176** standard.

  - The supply of a standard milk powder to perform the quantitative and coagulating activity tests with the coefficients K_c and K_p at a pH of 6.3 and 6.5, and correspondences mg/L ↔ IMCU for the chymosin and bovine pepsin contents.

- INRA, Poligny (contact O. Rolet-Répécaud: rolet@poligny.inra.fr) proposes:

  - The qualitative method according to the **OJFR of 1981** or the **IDF 110B** standard.
  - The fermentative origin chymosin research.

The totality of these on-site competencies in Poligny enables a complete analytical offer to be proposed to the various actors in the field for the characterisation of coagulants.

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