SUMMARY OF LA LETTRE DE CECALAIT, N° 39 (4th quarter 2001)

(Translation : A. BAPTISTE, Correction : H. LAMPRELL)

Physico-chemical characterization of cheese : application to French Emmental

(summary of the lecture given by M SCHMITT, ITFF, at CECALAIT's Annual General Meeting 2001)

French Emmental is the largest french cheese : its weight can be over 75 kg, its production was over 240 000 t in 2000 *ie* more than 12% of the milk collection and more than 24% of the french production of ripened cheese. It is very frequently used in restaurants or the food industry and about 95% of consumers use it directly or for cooking. It represents 1% of the food sales in mass distribution, but with various brands and presentations. Therefore, there is a need for identification and characterization of the product. Its composition has thus been carefully examined in order to build a database.

The studies were launched a few years ago by the LARF, (laboratoire d'analyse alimentaire et de recherche fromagère de l'ITFF, the former ITG - *see abbreviations in La Lettre de CECALAIT n° 39)* and are still in progress. The work is divided into three parts :

- characterization of french Emmental among other hard cheeses,
- characterization of Emmental in processed cheese,
- definition of Emmental in the Codex.

Emmental and other hard cheeses

The aim of this work was to reveal the characteristics of Emmental among neigbouring cheeses, which could be mistaken for it, especially when grated. Many of these cheeses were thus characterized in order to build a database to give discriminating criteria.

➢ CHARACTERIZATION METHODS

Studies were carried out on samples taken from cheese plants, but also bought in retail stores. When possible, former work was also used. Characterization was done :

- on the one hand, by using "classical" physico-chemical methods in order to determine dry matter (DM), fat, nitrogen, calcium, salt…and also volatil fatty acids….
- on the other hand, by studying the proteic fraction

Indeed, proteolysis is very important in cheese technology. Two different types of analysis were chosen to follow this process : analysis of primary proteolysis and for further steps, the analysis of the water-soluble peptidic fraction.

- Primary proteolysis was followed by polyacrylamide gel electrophoresis of casein (PAGE), under different conditions. The degradation products of the caseins can be shown and quantified by densitometry and/or image analysis.
- the peptidic patterns of the water-soluble fraction of cheeses - representing only part of the proteolysis products- were obtained using a reverse phase high performance liquid chromatographic method (HPLC), allowing identification and quantification.

Firstly, a lot of French Emmentals were analysed in order to get an array of peptidic patterns, corresponding to different geographic origins, to different production methods, to the season…

Fig. 1 gives an example of such a peptidic pattern. (taken from the article by CHOPARD M.A., SCHMITT M., PERREARD E. et J.F CHAMBA : Aspect qualitatif de l'activité protéolytique des lactobacilles thermophiles utilisés en fabrication de fromages à pâte pressée cuite. Lait, 2001, V. 81, p. 183-194).

Fig 1 : Schematic representation of average peptidic patterns of water-soluble fraction of French Emmental (n=253) *see Lettre de CECALAIT n° 39, page 2*

➢ DISCRIMINATING CRITERIA

All the results obtained using the above methods (except HPLC) with numerous hard cheeses and French Emmentals were put together and examined.

Some criteria, already specified in the definition of Emmental, could indeed be considered as discriminating criteria. It is the case for the dry matter, one of the two basic analytical criteria for Emmental, *ie* :

dry matter ≥ 60% and "fat/dry" ≥ 45% .

Likewise, two of the important definition criteria of "French Emmental Selection" (as specified by professionals) revealed themselves as discriminating criteria. These are the calcium content and the ratio between α_{S1} and β caseins :

- calcium content > 850 mg/100 g of cheese.
- ratiot α_{S1}/β caseins > 0.6.

However, the important criteria of propionic fermentation did not need to be considered here.

Finally, hard cheeses that might be mistaken for Emmental could be split into several groups, according to the scheme described in Fig. 2. *see Lettre de CECALAIT n° 39, page 3*

Fig 2. shows that some cheeses are very close to Emmental and actually very difficult to identify. For these, all the available analytical criteria were used for a multiparametric statistical analysis : principal component analysis (PCA). So it was for French Emmentals. Finally, these could be clearly distinguished from the other products, even very close.

Moreover, it seems, from preliminary work, that peptidic patterns could also be used to distinguish French Emmental from other close hard cheeses, including Maasdams.

➢ CHARACTERIZATION OF EMMENTAL IN PROCESSED CHEESE

It is a special case linked to processed cheese trade. Indeed, GATT had specified lower import taxes on processed cheese made with Emmental. But, until now there was no method to check if Emmental had been used in the process or not. So, the LARF launched work which could later help the Customs laboratories in the development of a control method.

Experimental cheeses were manufactured with mixtures of cheddar and French Emmental, using a very denaturating process. The cheeses were then analysed by the characterization methods described above : physico-chemical methods, casein electrophoresis and HPLC of the water-soluble peptidic fraction.

Manufacturing conditions : STEPHAN pilot 24 kg, 300 to 3000 rpm, 118°C maximum. Experimental cheeses manufactured : simple processed cheeses, only made from raw cheese material, butter for fat standardization, processing salts and water, with expected dry matter around 50%. 4 types of raw cheese material were used :

- Emmental only : processed cheese named below, 100E,
- Cheddar only : processed cheese named below 100C,
- Emmental - Cheddar mixtures, with 75% Emmental, 25% Cheddar and inversely : processed cheese named below, 75E and 75C respectively.

Table 1, page 4 of La Lettre de CECALAIT n°39, gives the main compositional characteristics of the raw materials used (at the same ripening stage) and of the experimental processed cheeses made from them.

These results show that the main initial characteristics of Emmental and Cheddar, especially the ratio of caseins α_{S1}/β, are found again in the processed cheeses 100E and 100C.
But, in the processed cheeses made from mixtures, the obtained values are intermediate between the initial values of the raw materials.

Likewise, the HPLC patterns of water-soluble peptides "keep" specific peaks of the two raw materials in the processed cheeses. Thus, the same peaks, at the same retention times, are observed for Emmental as a raw material and for processed cheeses containing Emmental. Quantification of the initial proportions of the mixture can even be considered.

In conclusion, applying the same methods to more sophisticated process might be considered.

➢ DEFINITION OF FRENCH EMMENTAL

The need for characterization of cheese is also very important during Codex talks about standard C9 concerning the definition of Emmental. For example, to justify the importance of the specifications they defend, French (and other "traditional") Emmental manufacturers need a complete characterization of their products, but also of similar and/or competing products.

So, the ITFF launched new work to complete the results already obtained with French Emmental.

It aims at :

- characterizing Emmentals from other countries and comparing the results to those of French Emmentals.
- fully characterizing Maasdams, in order to determine the specifications for this type of cheese
- fully characterizing experimental cheeses where size and/or heating temperature of the curd are significantly different from the present French specifications.

Most of this work is still in progress. Some of the discriminating criteria determined for French Emmental may still work with other Emmentals, some might need a revision…Anyhow, the aim remains to propose an international "definition" of Emmental in the CODEX C9 talks, allowing in particular, to distinguish them clearly from Maasdam type cheeses.

In conclusion

The characterization of hard cheeses, including Emmentals, implies building databases with the detailed physico-chemical composition of these cheeses and using multiparametric statistical analysis in order to discriminate close, but different groups.

The list of abbreviations and bibliographic references are in « La Lettre de CECALAIT »

INTERESTING RECENT EU REGULATION

Council Directive 2001/114/EC of 20 december 2001 relating to certain partly or wholly dehydrated preserved milk for human consumption (JOEC L15 of 17 january 2002)

Commission Decision 2001/873/EC of 4 December 2001 correcting Directive 2001/22/EC laying down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs (JOEC L325 of 8 december 2001)

Council Regulation No 2375/2001 of 29 November 2001 amending Commission Regulation (EC) No 466/2001 setting

maximum levels for certain contaminants in foodstuffs (JOEC L321 of 6 december 2001)

Regulations 2162/2001, 2584/2001 & 77/2002, of respectively 7 november, 19 december 2001 & 17 january 2002, amending annexes I, II and/or III of regulation n° 2377/90 of the Council concerning maximum residue limits of veterinary drugs in foods of animal origin.(JOEC L291 of 8 november 2001, JOEC L345 of 19 december 2001, JOEC L16 of 18 january 2002

➢ also interesting

Council Directive 2001/102/EC of 27 November 2001 amending Directive 1999/29/EC on the undesirable substances and products in animal nutrition (JOEE L6 of 10 january 2002).

Commission Directive 2001/101/EC of 26 November 2001 amending Directive 2000/13/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs (JOEC of 28 november 2001)

Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products (JOEC of 28 november 2001)

↯ An updated list issued by DG 24 (on january 22nd 2001) concerning "Information on analytical methods for the detection of irradiated foods standardised by the European Committee for Standardisation" on http://europa.eu.int/comm/food/fs/sfp/fi07_en.html

Official Journals of the European Communities of the last 45 days may be consulted on the Internet http://europa.eu.int/eur-lex
Older texts may be searched by their date on http://europa.eu.int/eur-lex/en/search/search_lif.html *or consulted according to their topics on the Internet http://europa.eu.int/eur-lex/en/lif*

The accreditation : towards reference frame NF EN ISO CEI 17025

(Summary of the lecture given by M CHORIN – COFRAC - at CECALAIT's Annual General Meeting 2001)

According to regulation or customers wishes, there are continually more metrology and analysis laboratories, certification or inspection organizations seeking for accreditation. COFRAC is the French accreditation body. The reference frame they have used until now is decribed in standard NF EN 45001 (December 1989), which will be replaced by standard NF EN ISO CEI 17025 (May 2000) within a 2-year period (until January 2003). The new text is more complete and more precise on numerous points including metrology, uncertainty, sampling, interpretation and finally, the validation of methods.

The transition towards a new reference frame

Applying for accreditation at the COFRAC is a voluntary process which proceeds in several successive stages:

- official written request to the COFRAC
- reception of a file including an evaluation questionnaire, organization of the initial audit, carried out over 2 days by a quality control engineer and a technical expert.Before this step, there must have been :
  - proposal of team (*may be refused by the applicant)*.
  - schedule.
  - communication of the documents
- examination of the audit report by a Standing Committee of Accreditation and the permanent structure of theCOFRAC,
- decision.

The audit is based upon a reference frame. Until now, laboratories were accreditated to standard EN 45001, from December 1989. However, this text was replaced, in May 2000, by the standard EN ISO 17025, which :

- "extends its applicability to all laboratories,
- modifies the requirements relating to quality systems to put them in coherence with standards ISO 9001 and 9002. " (In AFNOR - standard NF EN ISO/CEI 17025, page 1)

The transition between the two reference frames took place from January to October 2001 as the laboratories had the possibility to be accreditated to either reference frame. From October 2001 to January 2002, the COFRAC began to use the new standard, except for some initial audits. As from January 2002, every COFRAC audit, *ie* initial or monitoring (conducted within 12 months after the initial audit) is conducted according to the new reference frame. All laboratories should be accredited to this text until January 2003. In the meanwhile, the monitoring audits will be carried out by a technical expert personnel and by a quality control engineer and not simply by a single expert.

The main differences between the two reference frames

➢ METROLOGY

In standard 17025, the requirements concerning metrology appear mostly in part 5: technical regulations, and in particular in paragraphs " 5.5 Equipment " and " 5.6 Traceability of measuring". To comply with these requirements, three stages are necessary :

- identification of any item of equipment likely to affect the accuracy or the validity of the test result, the calibration, the sampling,

- The set up of a programme of traceability for calibration standards and all relevant equipment to national standards. For this, the laboratories should specify in each case, the effective range and uncertainties of calibration and possibly, the conditions of use of the standard.

- Finally, ensurement of traceability.

The laboratory will later have to provide documentary evidence of the traceability to national standards by calibration or verification certificates issued by any assessed European laboratory accreditation body.

➢ UNCERTAINTY

In standard 17025, the requirements concerning uncertainty appear in part 5: technical regulations, and in particular in paragraph " 5.4.6 Estimating the uncertainty of measurement".

For the audits carried out according to the new standard, the assesment team will check that the laboratory started to identify and evaluate the various uncertainty components and to think of the calculations needed. Considering the volume of work necessary, the laboratories cannot be initially required to have calculated all uncertainties for all measurements ! However, the complete work carried out on uncertainties will be gathered from the audit reports and other local observations and examined by the Standing Committees of Accreditation, so that the COFRAC can draw doctrines from it. For example, it will specify if the calculations based on the reproducibility of the methods are sufficient or if it is necessary to take into account the propagation laws, all the significant factors... etc. Besides, the doctrines of the COFRAC are likely to evolve as additional information will be obtained from laboratory observations and calculations. Later, the assesment teams will have to verify how the laboratories set up the application of these doctrines.

Standard 17025 also specifies that the test report (paragraph 5.10.3) must include information on uncertainty in measurements in following cases:

- at the customer's request,
- when there is a declaration of conformity to some specification, if uncertainty affects its limits,
- when the method mentions limiting tolerances or thresholds to be reached,
- when the method gives a list of the components having an influence on the test results.

➢ SAMPLING

Sampling is discussed in paragraph 5.7 of the standard. But, it is only relative to the laboratories which deal, for themselves or for their customers, with sampling of substances or materials for further tests or calibrations. The sample must be representative of whole substance or material.

Then, the laboratories, that wish to include the procedure of sampling in the accreditation, must have a planning and a procedure of sampling, also available where sampling is done.

Planning must be based on adequate statistical methods. The procedures must take into account any factor to be controlled, so that the results of the tests and calibrations are valid. The most important information on planning and the procedure of sampling must be reported in the test report or the certificate of calibration.

However, most laboratories are not concerned, because their customers give them only a single sample. In this case, no sampling procedure can be set up and the laboratory cannot be accredited on this point. The test report will specify that the result is only valid for the analysed sample and cannot be extended to the whole batch. The laboratory cannot be held responsible when the sample is not representative. It remains that the laboratory can suggest sampling improvements to its customer.

➢ OPINION AND INTERPRETATIONS

The test reports (part 5.10) can contain opinions or interpretations, supplementing an analysis or calibration results. They must however be clearly identified. They may concern :

- Declaration of conformity or non conformity of the results compared to regulations,
- Respect of the contractual requirements,
- Recommendations on the use of results
- Recommendations to be followed for improvements.

In the same report, there may be opinions on tests that are accreditated or not, but they must be clearly separated. Opinions or interpretations can be communicated orally. However, it is necessary to keep a written formulation of them, in particular to specify on what they are based. In practice, it may be difficult to express any opinion or interpretation because the laboratory must not act as a consultant or an expert.

➢ VALIDATION OF METHODS

The validation of sampling, test or analysis methods is something new in this standard (part 5.4.5). However, over the last few years, the COFRAC has already included this requirement of validation of the internal methods in its reference frame for the accreditation of laboratories.

For the customer, this is a very important guarantee. For the laboratory, the validation will demonstrate the competence of those who :

- will have used non standardised methods,
- will have extended the domain of application of standardised methods,
- will have conceived or developed new methods.

In conclusion

The new reference frame NF EN ISO CEI 17025 is much more complete than the old standard EN 45001. The principal differences between the two texts relate to metrology, uncertainty, sampling, opinions or interpretations and validation of methods. The new reference frame thus covers the totality of the service, from sample taking to interpretations and is always directed towards the satisfaction of customers.

The list of abbreviations and bibliographic references are in « La Lettre de CECALAIT », page 10.

Interesting reading

Besides the list of bibliographic references, other interesting reading is pointed out below.

• MARTH E. H., STEELE J.H. (Eds). Applied dairy microbiology, 2nd edition. Marcel Dekker Inc. : New York, 2001, 736 pages, ISBN 0-8247-0536-X.

• a special issue on PLS methods of the journal Chemometrics and intelligent laboratory systems, 2001, V. 58 n° 2.

• an article on lipids and flavour of dairy products in http://www.johnlibby-eurotext.fr/articles/ocl/4/4/301-11/index.htm

.• the proceedings of the symposium held in May 2001, in Brussels (Belgium) « Exopolysaccharides from lactic acid bacteria», issued in International Dairy Journal, 2001, V. 11, n° 9.

AFNOR VALIDATION

AFNOR (French standardisation body) recently validated the following alternative method :

CHROMAGAR LISTERIA™, validated on december 13th 2001, (*n° of attestation CHR-21/1-12/01*), a medium for the quick detection of *Listeria* in foodsstuffs and environmental samples.

Validations run for a 4 year period. Afterwards, if the manufacturer wishes a renewal, further studies are necessary.

↳ RENEWALS

The validation of following methods was renewed :

- 3M Petrifilm for the quick enumeration of total flora, and respectively of *Enterobacteriaceae* (*n° of attestations 3M-01/1-09/89* and *3M-01/6-09/97*), until september 10th 2005, for all human foodstuffs.
- some Petrifilm P2000 for quick enumeration of coliforms *ie* :
  - ♦ reading at 14 h (*n° of attestation 3M-01/5-03/97*),
  - ♦ reading at 24 h of gas-producing and non-producing colonies (*n° of attestation 3M-01/5-03/97B),*
  - ♦ reading at 24 h of gas-producing coliforms (*n° of attestation 3M-01/5-03/97C)* .

They were renewed until march 18th 2005, for all human foodstuffs.

↳ END OF VALIDATION

The manufacturers did not ask for a renewal of :

- 3M Petrifim ***E. coli*** and coliforms for the quick enumeration of *E. coli* β-glucuronidase positive.
- TRANSIA Plate ***Salmonella***.

INTERESTING NEW STANDARDS

ISO AND/OR EUROPEAN STANDARDS

➢ concerning EN ISO 8261, already pointed out in the latest issue:

EN ISO 1211 (*ICS 67.100.10 Milk*) MILK. Determination of fat content. *Gravimetric method*
It is equivalent to ISO 1211:1999.

ISO 5725-3/TC1:2001 october 2001 (*ICS 03.120.30 : application of statistical methods*) Technical corrigendum 1 to ISO 5725-3:1994 (Accuracy (trueness and precision) of measurement methods and results - part 3 : intermediate measures of the precision of a standard measurement method)

ISO 5725-6/TC1:2001 october 2001 (*ICS 03.120.30 : application of statistical methods*) Technical corrigendum 1 to ISO 5725-6:1994 ((Accuracy (trueness and precision) of measurement methods and results - part 6 : use in practice of accuracy values)

EN ISO 8261 (*ICS 07.100.30 : Food microbiology 67.100.01 : Milk and milk products in general)* Milk and milk products -- General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination
This is a joint ISO/IDF/AOACI standard which supersedes IDF 122C:1996 standard.

ISO 14156:2001 MILK AND MILK PRODUCTS. Extraction methods for lipids and liposoluble compounds

ISO 14673-1/-2/-3:2001 MILK AND MILK PRODUCTS. -- Determination of nitrate and nitrite contents
Part 1: Method using cadmium reduction and spectrometry
Part 2: Method using segmented flow analysis (Routine method)
Part 3: Method using cadmium reduction and flow injection analysis with in-line dialysis (Routine method)

ISO 15161:2001 Guidelines on the application of ISO 9001:2000 for the food and drink industry

ISO DRAFT STANDARDS

EN ISO/DIS 6887-2 /-3 / -4 MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

Part 2 : specific rules for the preparation of meat and meat products.
Part 3 : specific rules for the preparation of fish and fishery products.
Part 4 : specific rules for the preparation of products other than milk and milk products, .meat and meat products and fish and fishery products.
NB : for milk products, the specific rules are described in EN ISO 8261, see above.

ISO/DIS 7932/A1. MICROBIOLOGY General guidance for the enumeration of *Bacillus cereus*. Colony count technique at 30°C. Amendment 1. Inclusion of precision data and limitation of confirmatory tests.

ISO/DIS 10273 MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*.

ISO/DIS 7251. MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS Horizontal method for the enumeration of presumptive *Escherichia coli*. Most probable number technique .

ISO/DIS 13302 Sensory analysis -- Methods for assessing modifications to the flavours of foodstuffs due to packaging

ISO/DIS 4121 Sensory analysis -- Guidelines for use of quantitative response scales

➢ also interesting

AFNOR, the French standardization body just issued a new standard concerning the acido-butyrometric method for the determination of the fat content of cheese. This new version describes the Van Gulik method, as the former one, but also describes the Heiss method. (*AFNOR standard V 04-287, february 2002*)