PROJECT CONTRACT NUMBER NP/43/033 and NP/01/034

INTRINSIC INDICATORS FOR PROCESSED MILK AUTHENTICITY

March 1998 – February 2001

OVERVIEW REPORT

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1) Title and summary

title of the project: " Intrinsic indicators for processed milk authenticity"
contract number: NP/43/033 and NP/01/034
March 1998 – February 2001

partners:

- Ministry of Small Enterprises, Traders and Agriculture
  Agricultural Research Centre – Ghent
  Department of Animal product quality and transformation technology
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activities and progresses :

The following activities were/are carried out:

- An inventarisation of the current situation in the Belgian consumption milk industry is made by the organisation of inquiries.
- A detailed kinetic study is executed on intrinsic indicators of milk as a basis for the development of indicators for processing authenticity and the development of a mathematical model.

- A mathematical model, based on kinetical data, that allows formulating the process conditions which cause the desired microbial inactivation with minimal fouling of the heat exchangers is developed for different types of heat treatment systems.
- Methods for the determination of potential intrinsic indicators are optimised and developed.

results :

The following results were obtained:

- The organisation of 2 inquiries among the most important Belgian consumption milk producing plants provided an inventory of the current situation in the Belgian dairy industry.
- Improved methods were developed for the determination of lactoperoxidase, hydroxymethylfurfural, and alkaline phosphatase (quantitative).
- Mathematical models for different heat treatment systems were formulated.
- The kinetics of physico-chemical changes of milk as a basis for processed milk authenticity indicators or relevant for fouling of heat exchangers were experimentally determined.

Individual results are given in the scientific reports submitted earlier and in the **enclosures**

2) Finalities

General objectives

There are two general objectives:

The first objective is to support legal authorities in their aim at guaranteeing the nutritional and hygienic quality of consumption milk and the second objective is to increase the production efficiency and to reduce the energy costs by rationalisation of the heat treatments.

Milk is a basic ingredient in our diet, especially for children, patients and elderly. From a nutritional point of view, it is an important source of high value proteins, vitamins and minerals. To increase the shelf life of consumption milk and to guarantee its microbiological safety, the product is heat-treated. The heat treatment is the final and correcting step in the production process of consumption milk. To avoid that this step becomes a corrective action to hide problems associated with former steps in the production line and to prevent overprocessing of the product forcing producers to apply the HACCP principle to all steps in the production process, the European Union is currently in a process of defining criteria for consumption milk. Conformity with such criteria (product conformity in terms of processing as indicated by labelling) will guarantee product quality. In addition, the environment will benefit from the application of mild heat treatments and production efficiency will increase because of reduced energy consumption and reduced fouling of heat exchangers during processing (fouling results in product loss, needs for cleaning and decrease of production efficiency).

This project is in line with the current program on pre-normative research for the food sector in the context of sustainable development and is directly related to two of its major objectives: (i) the development of control methods (analytical tools) to evaluate the authenticity of food products and (ii) the identification of criteria for the evaluation and control of production associated environmental impact in the context of new product criteria (norms) in relation to sustainable development.

Target of the project

The topic of authenticity is focused on the development of analytical procedures (intrinsic time temperature integrators) that will allow to evaluate the conformity of the product with its labelling in terms of the heat treatment that has been carried out to extend the shelf life of the product. The topic of production associated environmental impact is focused on aspects related to fouling of the production equipment as a consequence of protein denaturation taking place during the heat treatment. These two objectives are both aimed at in this project because progress on both aspects heavily relies on the same scientific principles namely kinetics (evolution as a function of time and temperature during the heat treatment) of intrinsic characteristics of milk.

Due to inherent limitations of in situ methods (e.g. problems associated with detection limits), it is current practice to quantify the effect (integrated impact) of a heat treatment on foods by the use of physical mathematical approaches or the use of time temperature integrators. In physical mathematical approaches, the impact of the process (e.g. in terms of microbiological safety) is obtained by calculation based on physical measurements of processing conditions (e.g. time temperature) and a mathematical model (kinetics of the target index). Typical examples are the calculation of pasteurisation and sterilisation values (processing values) to quantify the microbiological safety of heat-treated foods. The second approach is the use of time temperature integrators. Time temperature integrators are specific indicators/sensors (intrinsic or extrinsic to the product) that allow to directly measure quantitatively the impact of the process. In the context of the above-mentioned problem of authenticity, only intrinsic indicators are of interest. The innovative character of this project is clearly associated with this aspect. It aims at the identification of a number of intrinsic (product related) indicators that will allow to quantify the thermal impact in terms of food safety in such a way that they can be used to evaluate the conformity of the processed product with the processing terminology (thermisation, pasteurisation, UHT, sterilisation). For thermal processing of foods, it has already clearly been shown in literature that such indicators (intrinsic or extrinsic) must fulfil a number of conditions to quantify the impact of the process under different processing conditions (Hendrickx et al., 1995; Van Loey et al., 1996). The most important aspect is the kinetic requirement stating that both indicator and target index must have the same temperature sensitivity constant (equal z-value or activation energy). This means that in the context of this project, kinetic studies will be indispensable in identifying possible intrinsic indicators for processed milk authenticity.

Quality management at all levels of the production process must not only result in high product quality and authenticity, but should in addition lead to a more rational use of energy and optimal use of all resources in the production process. A number of aspects are associated to this problem. Minimal processing can lead to less energy consumption and by consequence to a better protection of the environment. In addition, minimal (thermal) processing will lead to less protein denaturation. Protein denaturation has been identified as being closely related to fouling of heat exchangers. Fouling due to protein denaturation (and other constituents such as minerals) has major economic (processing costs due to cleaning) and environmental impacts. Fouling will result in decreased heat transfer in the system resulting in higher energy requirements and associated costs to obtain the same result. Fouling also will result in production losses because of production interference due to cleaning and disinfecting procedures. In addition product is lost during the process of fouling (on the heat exchanger walls) and during shut down and restart of production operations (before and after cleaning). Also the use of water, detergents (cleaning agents) and disinfectants will have an associated negative environmental impact and energy cost. The repeated shut down of production equipment will lower production efficiency and shorten the lifetime of the equipment. In the context of this problem a quantitative approach (kinetics of all processes involved) is necessary to formulate optimal processes.

Aims of the project

Both problems discussed above namely (i) the authenticity of milk in terms of processing and (ii) environment associated problems with fouling of heat exchangers during processing can only be solved through quantitative systematic studies on reaction kinetics during milk processing. Therefore, this project will focus on a detailed kinetic study of intrinsic indicators of processed milk that will allow to identify the authenticity of heat treated consumption milk (compliance with possible European future directives) and to optimise all resources used in the process (the production of a high quality microbiologically safe product with minimal fouling of the production equipment).

The first aim of the project will consist in an inventory of the current situation in Belgium. Existing production lines in the Belgian dairy industry (production lines including thermisation, pasteurisation, high pasteurisation, UHT and sterilisation) will be characterised in detail. This part will include thermisation and pasteurisation lines of dairy products other than consumption milk (e.g. processing of milk prior to cheese manufacturing). This first part will consist in (a) an inquiry among the Belgian dairy industry (milk producing plants) on the type of production units being used and the time temperature conditions in use, (b) a detailed literature review on the following aspects (i) potential intrinsic indicators for evaluation of thermisation and heat treatment of milk, (ii) criteria for microbiological safety of milk (target micro-organisms, target levels and kinetics on thermostability of these micro-organisms) and (iii) fouling of heat exchangers during milk production and potential indicators for fouling, (c) the determination of levels of potential intrinsic indicators for thermal processing as well as other relevant physical-chemical parameters for milk produced under the identified processing conditions in order to evaluate current practices and the conformity with future European directives and (d) the determination of heat stability of milk produced under the identified processing conditions as this parameter is linked to the fouling occurring in that specific production line; in addition, protein degradation will be determined as this information can indicate the quality of the raw materials before processing (analysis of current practices in the whole production chain). Protein degradation will negatively influence heat stability.

The second aim of the project will be a detailed kinetic study of intrinsic time-temperature indicators (TTIs) of milk as a basis for (i) the development of indicators for processing authenticity and (ii) the development of a mathematical model that will allow to calculate optimal processing conditions in terms of consumer safety (high quality microbiologically safe product) and minimal fouling. These specific TTIs are heat sensible components present or formed in the product during heat processing, which show a time- and temperature dependent, easily measurable and irreversible change that mimics the changes of a target attribute (a safety or quality parameter) undergoing the same process. To be used as a TTI, a system must fulfil some practical, thermophysical and kinetic requirements. The temperature sensitivity of the TTI and the target attribute should be the same, and the reaction rate of the TTI must guarantee a measurable response after heating. Consequently, if a system is to be evaluated as a TTI, extensive kinetic studies are required. Kinetics describe the evolution of a reaction (inactivation, denaturation, and production) as a function of time. *How* the reaction proceeds (lineair, log-lineair,…) is expressed by the mathematical form of the kinetic model. The *rate* of the reaction is represented by kinetic parameters, which are obtained by fitting a model on the experimental data. This part of the project will consist of (a) the experimental determination of kinetics of physical-chemical changes of milk as a basis for processed milk authenticity indicators, (b) the experimental determination of kinetics of physical-chemical changes of milk relevant for fouling of heat exchangers, (c) the selection of indicators for authenticity and analysis of accuracy with which such indicators can be used and (d) the formulation of a mathematical model including kinetics for microbiological safety, kinetics of relevant time temperature integrators and kinetics of fouling.

The third aim of the project will be the implementation of the indicators and the optimisation of thermal processing of consumption milk. This part will consider (a) the validation of the selected indicators for determination of the intensity of heat treated samples at pilot scale, (b) the validation of the mathematical model at pilot scale and (c) the implementation and demonstration of a model based approach for optimising the heat treatment of milk (consumer safety with minimal equipment fouling)

In a final step (towards the end of the project) it is the aim to disseminate the results of the project by organising a workshop where the Belgian dairy industry will be informed on the outcome of the project and an analysis of the current Belgian situation in light of future European directives. The results should indicate how the current situation could be improved towards increased production efficiency with decreased energy cost and environmental impact.

3) activities and results

3.1) activities

launched activities

Two inquiries were held among the Belgian consumption milk industry: during the first year of the project, an inquiry was held among 8 different Belgian dairy factories. The participating factories supplied milk samples of different types of production units and, when possible, gave detailed information on the applied heat treatment. Since the first phase of the project consists of an inventarisation of the current situation and only one inquiry is a rather random indication, a second inquiry was organised. Due to the dioxin crisis, only 7 dairy factories took part in the second inquiry.

In addition to the existing know-how of both partners, a profound literature review on potential intrinsic indicators, the availability of kinetic information of the thermal inactivation, criteria for microbiological safety of milk and fouling of heat exchangers during milk production and potential indicators for fouling was carried out at the start of the project. During the project, this literature study was complemented when necessary. For each selected component, analytical methods were implemented and checked for their accuracy and repeatability (for an overview of the selected methods: see below). For the following parameters, other methods than those normally used in the laboratory were developed or optimised:

- A quantitative method for determination of *alkaline phosphatase* was tested (method IDF: FIL – IDF 82A: 1987). Alkaline phosphatase is an endogenous milk enzyme that is inactivated during heat treatment of milk. The determination of alkaline phosphatase allows differentiation between raw and pasteurised milk.
- A quantitative method for determination of *lactoperoxidase* was tested ("Hernández M. et al. (1990). *Isolation and properties of lactoperoxidase from bovine milk*. Neth. Milk Dairy J., 44, 213-231 " with some minor modifications). Lactoperoxidase is also an endogenous milk enzyme that is inactivated during heat treatment of milk. The determination of it allows differentiation between pasteurised and high-pasteurised milk.
- A precise, quantitative method for determination of hydroxymethylfurfural (HMF) was developed. HMF is formed in different ways when milk is heat treated in such a way that it can be used as a heat treatment parameter. For the determination of HMF in milk samples, only a colorimetric method was available. The major drawback of this method is the lack of specificity of the reagent applied and the instability of the formed coloured complex. Moreover, due to the sample preparation applied, additional HMF is formed. Therefore a more specific chromatographic method was developed and a gel filtration step for removing lactose was added to the sample preparation to avoid the formation of additional HMF during hydrolysis.

The levels of potential intrinsic indicators for thermal processing such as lactulose, furosine, β-lactoglobulin, alkaline phosphatase and lactoperoxidase as well as other relevant physico-chemical parameters such as heat stability and pH were determined in the samples obtained from the two inquiries. This made it possible to evaluate current practices and the conformity with possible future European directives. The results of both inquiries were described in two reports (see **enclosures 1 and 2**). Each participating company received such a confidential report with the data of the heating parameters for the samples they provided, the position of each sample in the entire research and the conformity of their products with possible future European directives. The participating dairy factories were informed about the results of the screening during a first workshop. On their request, they could get further explanation or pass their comment during an individual conversation.

The heat stability of milk produced under different identified processing conditions was determined. The heat stability is the residence time of the milk in a glass tube in an oil bath at 140°C before coagulation starts. The heat stability was determined in the samples of both inquiries.

Since preliminary research showed the presence in raw milk of a papain inhibitor which was inactivated by heating the milk at temperatures between 60 and 65°C and since actually no convenient endogenous indicator for thermisation exists, the potential application of this inhibitor as indicator for thermisation must be verified. A method was tested to determine the papain activity.

With the UHT - pilot installation available at DVK, raw milk was treated applying 16 different temperature time combinations using the indirect system. The resulting milk samples were analysed and heat stability, pH, protein degradation, lactulose content, furosine content, β-lactoglobulin content, hydroxymethylfurfural content and the protein – and fat content were determined.

The kinetics of the selected milk components were characterised, in order to use them as indicators for determining the authenticity of heat-treated milk and for fouling of the heat exchangers. First isothermal experiments (experiments at a constant temperature) were performed in order to define the kinetic model describing inactivation, denaturation or formation of the indicators and to assess their thermostability and kinetic model parameters (reaction rate constant k, decimal reduction time D, activation energy E_a and z-value). Secondly, calculated kinetic parameters were validated for variability of (i) extrinsic (temperature) and (ii) intrinsic (raw material) factors. (a) Non-isothermal experiments (experiments under time variable temperature conditions) were conducted to simulate more realistic process conditions. (b) Isothermal experiments will be repeated for milk samples collected over one year in order to evaluate the seasonal variability that can be associated with the calculated parameters. Based on this kinetic information, global time-temperature tolerance diagrams were constructed, which combine the kinetic information of the indicators studied with the inactivation kinetics of milk microorganisms found in literature. Such diagrams allow to visualize the impact of a thermal process on milk and to formulate criteria for controlling milk authenticity as well as process conditions causing optimal microbiological inactivation with minimal quality losses. Throughout the course of the project, it has become clear that contradictions exist in literature regarding the 'optimal' experimental design and statistical analysis in kinetic parameter estimation. In this context, an additional activity has been taken up in comparing and evaluating different statistical procedures for experimental design and data processing (2-steps lineair vs. 1-steps non-lineair regression, individual confidence intervals vs. joint confidence regions,…)

A mathematical model including data concerning bacterial markers for different types of heat treatment, kinetics for microbiological safety, kinetics of relevant time temperature integrators and kinetics of fouling was formulated. The model provides an optimization algorithm, which allows the optimization (maximalisation or mininalisation) of a chosen goal function. The kinetics of microbial markers allow to formulate equivalent processes in terms of safety. The kinetics of fouling together with the optimization algorithm allow to identify the process conditions resulting in minimal fouling. The kinetics of temperature-time integrators for authenticity allow to verify if the processes, equivalent on basis of microbial markers also result to a same level of indicator. Different heat treatment installations of 4 different Belgian dairy factories were/are modelled: a direct and indirect UHT-system, a thermisation, pasteurisation and sterilisation system were chosen. Improvements concerning reduction of fouling of heat exchangers during processing and reduced energy consumption were suggested to the respective firms by the means of a report (see **enclosure 3**) and a personal visit to the firm.

Methods

After checking their acurracy and repeatability, the following methods were used for the determination of the possible intrinsic indicators:

(a) furosine : the formation of furosine was measured by RP-HPLC according to the method of Resmini *et al.* (1990)

(b) lactulose: concentrations of lactulose were determined using a D-glucose//D-fructose test combination of Boehringer Mannheim (1995).

(c) β-lactoglobulin : the acid-soluble β-lactoglobulin content was quantified by HPLC according to the method of the International Dairy Federation (IDF) (FIL-IDF 178 A, 1999)

(d) alkaline phosphatase: the activity of alkaline phosphatase was determined spectrophotometrically at the absorption maximum using p-nitrophenyl disodiumphosphate as substrate following the procedure of the DF (FIL-IDF 82A, 1987).

(e) lactoperoxidase: the lactoperoxidase activity was determined on the basis of an adopted procedure of Hernández *et al*. (1990). Before measuring the activity spectrophotometrically at 412nm with ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) and H_2O_2 as substrate, milk proteins were precipitated by adding 1.75M acetic acid and 1M sodium acetate.

(f) protein degradation: protein degradation was measured spectophotometrically using the method of Polychrondiadou (1988) with 2,4,6 trinitrobenzeensulfonic acid as substrate.

(g) hydroxymethylfurfural: the total HMF content was quantified spectrophotometrically at 443nm using thiobarbituric acid as substrate following the method described by Keeney & Bassette (1959).

A more detailed description of these methods can be found in a previous report (period 1/9/1999 → 29/2/2000).

Material used and information sources

The samples for inventarizing the situation in the Belgian dairy industry were obtained from 8 different Belgian dairy factories. When possible, they gave as detailed as possible information on the heat treatment applied. Literature was obtained from the library of the DVK, the Universities of Ghent, Louvain and Antwerp. For the experiment with a pilot installation, the installation for UHT-treatment available at DVK was used. The raw milk used was obtained from a local dairy plant. In that way, the industrial situation could be approached as good as possible.

Remarkable elements, points of discussion etc. for the choices made

For the formulation of a mathematical model, 4 dairy factories were chosen out of the 8 participants. To make this choice, it was ensured that 4 different types of heat treatment could be modelled. The 16 different temperature-time combinations applied with the experiment with the pilot installation were chosen in such a way that a maximum of combinations could be applied on one day.

3.2) Results

interim results

As mentioned above, the results of both inquiries were presented in two reports. Both reports contained the results of the different analyses of all the samples, mean values per heat treatment and the dispersion for each parameter and relations between the different parameters. In the second report, also a comparison was made between the results of the first and second inquiry (see **enclosure 1 and 2**) .These results helped to orientate the research in an early stage in such a way that relevant indicators (for authenticity and fouling) could be traced especially for the Belgian situation. Furthermore, these results gave a first indication of the existing variability .

Studying the heat inactivation/denaturation of alkaline phosphatase, lactoperoxidase and β-lactoglobulin under isothermal conditions, first-order kinetics were observed, *i.e.* the enzyme activity or the undenatured protein concentration decreased log lineair as a function of time. Considering only industrially relevant time-temperature conditions, formation kinetics of hydroxymethylfurfural, lactulose and furosine could be simplified to a pseudo-zero order reaction, *i.e.* concentration of the chemical compounds increased lineair as a function of time. Kinetic parameters were estimated using a 2-step lineair regression and a 1-step non-lineair regression method. For the milk proteins studied, results obtained using both statistical techniques were comparable, but the 95% confidence interval for the predicted values were smaller when the 1-step non-lineair regression method was used. For the chemical compounds studied, only for furosine the global 1-step regression approach seemed to be superior to the individual 2-step regression approach. Next, the estimated kinetic parameters were evaluated under non-isothermal conditions. For inactivation of alkaline phosphatase and lactoperoxidase, it seemed that kinetic parameters computed from isothermal experiments could be used to predict thermal inactivation under non-isothermal conditions. However, for denaturation of β-lactoglobulin and for formation of hydroxymethylfurfural, lactulose and furosine, it was concluded that estimates from regression on the non-isothermal data were preferred to those from regression on the isothermal data, because the former bring about the best correlation between the predicted and the observed response under variable temperature conditions, while the latter seem to underestimate consistently the actual denaturation or formation. Finally, with regard to the kinetic parameter estimation procedure performed in a global fit on data obtained under isothermal and under non-isothermal conditions, the importance of taking into account the correlation between the simultaneously estimated kinetic parameters by construction of joint confidence regions was clearly demonstrated. Individual results can be found in the scientific report (01/09/1999 – 29/02/2000) and two articles submitted for publication (see below).

Kinetics seemed to be independent of the origin of raw milk and based on their kinetic information, the studied milk components seem to be good indicators for quantitatively measuring the impact of a thermal process on milk. However, if to be used as markers of industrial processes, calibration on an industrial level is necessary. Because kinetics only give pairs of kinetic parameters, and only residual activity or concentration can be measured and checked for possible variation. In addition, the effect of variability in milk composition on kinetics must be studied, and obtained kinetic data should be compared with microbial inactivation kinetics and with industrially applied or legally defined temperature-time conditions. Further experimental work will be undertaken and discussed in future.

final results

A mathematical model for fouling reduction for an UHT–installation of a Belgian dairy factory was developed. (see **enclosure 3**) and presented to the company in question.

enclosed results

As a result of the project, methods for determination of potential intrinsic indicators were tested. A quantitative method for the determination of the enzymes alkaline phosphatase and lactoperoxidase was validated. Profound research was carried out on a quantitative method for the determination of hydroxymethylfurfural. Apart from the project, both methods will be used also for other projects in the research group.

The 2 inquiries, the organisation of workshops and the modelling of different heat treatment systems resulted in improved and narrow contacts with the Belgian dairy industry. All the participating factories tried to give all the necessary information and milk samples.

time schedule of the project

Task	1998	1999	2000	2001
STAGE 1 :	March - …		… - February	
Task 1.1	March – August			
Task 1.2	March – October			
Task 1.3	April - …		… - February	
STAGE 2 :	August - …		… - September	
Task 2.1	August - …		… - September	
Task 2.2		July - …	… - September	
Task 2.3		July - …	… - September	
Task 2.4			March – September	
STAGE 3 :			June - …	… - February
Task 3.1			June - December	
Task 3.2			September - December	
Task 3.3				January - February

Stage 1: <u>Inventory stage</u>

- Task 1.1 : Inquiry towards the Belgian dairy plants
- Task 1.2 : Study of literature
- Task 1.3 : Screening of the existing situation with potential intrinsic heat indicators

Stage 2: <u>Kinetics of intrinsic indicators for authenticity and fouling</u>

- Task 2.1 : Kinetics of relevant physical - chemical parameters as a basis for authenticity determination
- Task 2.2 : Kinetics of physical – chemical parameters relevant for fouling of heat exchangers
- Task 2.3 : Selection of indicators for authenticity and determination of the accuracy of the analytical methods
- Task 2.4 : The formulation of a mathematical model for optimisation of the heat treatment of milk

Stage 3: <u>Implementation and demonstration stage</u>

- Task 3.1 : Validation of intrinsic indicators for authenticity of consumption milk
- Task 3.2 : Validation of the mathematical model : minimalisation of fouling
- Task 3.3 : Dissemination of the results : organisation of an information and discussion forum

elements allowing to value the obtained results

From industrial point of view, the development of TTI's (i) offers the possibility for optimalisation and a higher economical efficiency of dairy processes by reduction of energy costs and environmental pollution, (ii) responds to consumers demand for microbiological safe products of high quality, and (iii) meets the requirement of legally defined criteria for controlling product authenticity. On a scientific level, the benefit and new information of the project consists of the comparison of kinetic data gained under isothermal and non-isothermal conditions and the comparison of different statistical approaches, which can contribute to a wider use of *e.g.* non-lineair regression and joint confidence regions for data-analysis.

extent of the circulation and dissemination of the results

- Contact group 'Milk and Dairy products' : information meeting for the Belgian dairy industry and related institutes: annual presentation of the research and services of the DVK.
  18 December 1998
  CLO – Department of Animal product quality and transformation technology - Melle
  → presentation of the past and future work within the scope of the project

- Workshop about the interim results of the project
  21 April 1999
  CLO – Department of Animal product quality and transformation technology - Melle
  → presentation of the past and future work within the scope of the project
  The following people were invited to this workshop :
    - contact persons of the participating companies
    - Mrs. Mathieu, DWTC
    - Mr. Keymeulen, director of the Centre for Agricultural Research – Gent
    - Mr. Rijckaert, Inspector-general development, development of animal products

- Journée d'étude 'Recherche prénormative dans le secteur alimentaire'
  27 May 1999
  Palais de Congrès – Bruxelles
  → presentation of the past and future work within the scope of the project

- Thirteenth forum for applied biotechnology
  22-23 September 1999
  Het Pand – Gent
  → presentation of the work concerning modelling heat exchangers

'Modelling heat exchangers for the thermal treatment of milk'
K. Grijspeerdt, L. Mortier, J. De Block and R. Van Renterghem
Med. Fac. Landbouw – Univ. Gent , 64/5b, 1999 three-monthly periodical ISSN 0368-9697

- Journée d'étude 'Approches analytiques en matière d'authentification des produits agroalimentaires'
  20 October 1999
  Centre de reserches agronomiques de Gembloux – Département qualité des productions agricoles
  → presentation of the work concerning authenticity

'Intrinsic indicators for monitoring heat damage of consumption milk'
Jan De Block, Leen Mortier and Roland Van Renterghem

- The participating firms received a personal report with the results of the analyses of the samples that they supplied on the occasion of both inquiries. The reports were send in January 1999, April 1999 and January 2000. The reports also include information about their products as compared to the global Belgian production. (see **enclosure 1 and 2**)

- scientific publications

'Inactivation kinetics of alkaline phosphatase and lactoperoxidase, and denaturation kinetics of β-lactoglobulin in raw milk under isothermal and dynamic temperature conditions'
Claeys W., Ludikhuyze L., Van Loey A. & Hendrickx M.
Journal of Dairy Research, submitted for publication (2000)

'Formation kinetics of HMF, lactulose and furosine in raw milk under thermal and dynamic temperature conditions'
Claeys W., Ludikhuyze L. & Hendrickx M.
Journal of Dairy Research, submitted for publication (2000)

- The companies of which a heat treatment system was modelled, received (or will receive) a detailed report with the results of the modelling, with suggestions for possible improvements concerning reduction of fouling and energy consumption (see **enclosure 3**). The reports were (or will be) presented during a visit at the company in question.

4) Actors of the research and collaboration

4.1) Presentation of the teams

4.1.1) Laboratory for Authenticity Research

Size and composition of the research group

- Lic. R. Van Renterghem: Head of the division and from 01-10-2000, head of the department.
- Dr. J. De Block: Head of the laboratory, senior research assistant
- Dr. K. Coudyzer: Research assistant (IWT-project)
- Lic. D. Cartuyvels: Research assistant (IWT-project)
- Lic. A. Braekman: Research assistant (IWT-project)
- Lic. L. Mortier: Research assistant (DWTC-project)

- M. Merchiers: Industrial engineer
- C. Roels: Laboratory technician
- K. Brossé: Laboratory technician

The research group is also assisted by dr. engineer K. Grijspeerdt for the mathematical modelling

Institutional description

Ministry of Small Enterprises, Traders and Agriculture (DG6)

Agricultural Research Centre-Ghent (CLO)

Department of Animal Product Quality and Transformation Technology (DVK)

Laboratory for Authenticity Research

The most prominent research activities of the DVK are consumer directed research to improve the market position of animal products, research to improve the quality and the hygiene of animal products, implementation of total quality management along the food chain from farmer to consumer and research on the transformation of animal products.

Resources of the research group

The financial resources of the research group are from the Ministry of Small Enterprises, Traders and Agriculture (overhead costs (± 1000000 BEF/year)), 3 IWT- Projects (Flemish Government) (collaboration with 2 dairy plants and one food factory ± 9000000 BEF/year) and 1 DWTC-Project (this project): ± 3000000 BEF/year

Activities of the research group

The activities of the group concerning authenticity can be divided into (a) authenticity research in relation to the production methods used. This implies the development of methods to evaluate the heating conditions used and to verify correspondence with the label, (b) authenticity research in relation to the composition of the product. This implies the development of methods to verify if the composition of the product is in agreement with the label or claim of the product, (c) authenticity research in relation to the origin of the food in terms of animal species. The interest of this research is the support of legal authorities.

Apart from the authenticity research, the group is involved in bilateral IWT-projects with industrial partners. These projects are more or less confidential and concern the improvement of dairy products and production methods. The interest of this research is to improve the competitiveness of the Flemish food industry.

Production of the research group

An overview of the most recent publications is given in **enclosure 7**. The results are annually presented at an information meeting for the Belgian dairy industry and related institutes (contactgroup Milk and Dairy products). The results are also reported in the annual report of the DVK and the final reports of the IWT-projects (confidential). The laboratorium for authenticity also executes analyses and expertises for the Belgian food industry. Members of the research group are regularly invited to give lectures.

Scientific position

(a) On a European scale: participation of the meetings (8/year) of the Chemist Milk Experts Group of DG VI of the European Union; this results in multiple collaboration with other research groups. (b) On a worldwide scale: active in several Joint Action Teams of the International Dairy Federation (IDF). (c) On a national scale: firm contacts with the dairy industry and part of the food associated industry. Regular contacts with the universities of Louvain and Ghent.

Strategy

(a) With respect to research: to improve the know how of the group by the implementation of most recent research and development of new research methods. This implies for instance in the evaluation of new methods by participation in ring tests. Much of this research is not possible without the financial support from different research projects. They supply the necessary funds for the recruitment of new collaborators and the purchase of modern scientific equipment.

(b) With respect to education and training: participation in symposia, workshops and congresses; knowledge of the recent scientific literature in the field; exchange of know how and methods with other institutes.

(c) With respect to the industry: there is an active and a firm and confidential contact with the Belgian dairy industry and several other firms of the food associated industry.

(d) With respect to the society: authenticity research allows the legal authorities to dispose of objective and neutral information for a good policy and improves the consumers confidence in food products.

Experience with forms of collaboration and networks

The research group is involved in bilateral projects with the industry (IWT-projects) and is collaborating with the Laboratory of Food Technology of the KUL (University of Louvain) for the present project. The most recent projects are given in **enclosure 5**.

Significant elements from the history, perspectives and projects of the research group

Based on a new principle, a new method for the differentiation of proteins was developed and published as first. Future new projects are the study of parameters for the evaluation of a new treatment for liquid food and the study to improve the stability of food products with long shelf life.

In the near future the platform of the activities of the group will be broadened from dairy products to animal products in general (this means that also meat and eggs will be subjects of research). Also more attention will be paid to research on species authenticity.

4.1.2) Laboratory of Food Technology, Katholieke Universiteit Leuven

Size and composition of the research group

The research group consists currently of:

- 1 academic responsible: Prof.Dr.ir. M. Hendrickx
- 4 post-doctoral co-workers: Van Loey A., Ludikhuyze L., Smout C. and Indrawati,
- 7 PhD students (researchers): Denys S., Van de Velde M., Verachtert B., Shiferaw N., Guiavarch Y., Fachin D., Claeys W., (+ 4 vacancies which are being filled at this moment),
- 4 technicians: Terclavers J., Roba H., Neyens L. and Mampaey I.,
- 2 administrative co-workers: van Cuyck L. and Donné C.,

and a variable number of students conducting thesis research (9 in the academic year 1999-2000).
The academic responsible, one technician and one administrative co-worker are permanent, all other co-workers are temporary.

Institutional description

The Laboratory of Food Technology is a research unit within the Department of Food and Microbial Technology, a subdivision of the Faculty of Agricultural and Applied Biological Sciences, located at Katholieke Universiteit Leuven (KULeuven). KULeuven today is hosting 27.000 students, has 280 research units and is employing 3400 researchers over all disciplines.

Resources of the research group

The financial resources of the Laboratory of Food Technology are from the KULeuven (Research Council), from regional (VLIR, IWT, FWO), national (DWTC) and international (EU) funds, and from direct cooperation with the industry. Types of funding comprise research projects (including budgets for equipment, consumables and personnel), and post-doctoral and doctoral scholarships. A description of the research facilities is given in **enclosure 4**. A list of partnerships and networks in which the Laboratory of Food Technology participates, is given in **enclosure 6**.

Activities of the research group

The Laboratory of Food Technology has focused its research activities on the development of scientifically based methods to quantitatively evaluate the impact of physical preservation and/or processing techniques of foods in terms of quality, safety and functionality, and the use of these methods in design, evaluation and optimisation of such processes. In this field, kinetics and quantitative modelling play a key role.
With regard to processing technologies, research on physical preservation/processing techniques includes existing technologies - such as thermal processing (sterilisation, pasteurisation, blanching), cooling and freezing – as well as new technologies such as high pressure processing and pulsed electric fields.

In the area of thermal processing, research is mainly focused on heat penetration and distribution studies, enzyme based time temperature integrators for thermal processing of foods (*e.g.* of milk), low temperature preservation of foods, kinetics of quality evolution as a function of temperature and gas composition (MAP, CAP) and temperature conditions and residence time in the distribution chain. The research on novel processing techniques involves kinetics of food quality related enzymes (including polyphenoloxidase, pectin-methylesterase, lipoxygenase, myrosinase, polygalacturonase, lactoperoxidase, alkaline phosphatase) and of other quality attributes of foods (e.g. colour, nutrients) as influenced by temperature, high pressure, high electric field pulses, or sequential treatment by high pressure/thermal/high electric field pulses.
For these purposes, the research group possesses the necessary software and know-how for data-analysis, mathematical modelling and engineering aspects (kinetic models, calculation of high pressure freezing and thawing time, *etc*…).

Production of the research group

The research unit is publishing on a regular basis its research results in peer review journals and disseminates through participation in and organization of conferences. The output of the research unit is summarised in the next table. In addition, a list with 15 examples of peer review journal papers and a list of conferences organised by the unit are given in **enclosure 8 and 9** respectively. A full list can be provided on request.

Table: Publication output of Laboratory of Food Technology (1995-1999)

	1995	1996	1997	1998	1999	Total '95 –'99
Papers in peer review journals	5	17	11	15	14	62
Conference proceedings with review	1	6	13	13	5	38
Conference abstracts	10	9	15	4	10	48
Books	/	1	2	/	1	4
Totals	16	33	41	32	30	152

The research unit is also strongly involved in national and international collaborative work, exchanging know how and research results. As an indication of this collaborative work, a representative list of multi-partner research projects (last 5 years) is given in **enclosure 6**.

Scientific position

Next to regular fellowship, awards and travelling awards, the work of the group has been recognised on an international level by the 'Institute of Food Technologists' (Samual Cate Prescott Award for excellence in food research), the 'Koninklijke Academie van België' (Octaaf Callebaut price) and the 'Institute of Thermal Processing Specialists' (Student Award). The involvement in the different projects listed in **enclosure 6** illustrates the national and international position of the research unit between different centres of excellence.

The research quality of the group is recognised in honouring research proposals (especially at EU-level). The unit has been (is) recognised as a centre of excellence in the European Research training programmes (EU-HCM, international Fellowship Programme 1993-1996 and recently 'Marie Curie Training Site' 2000-2004).

Strategy

The Laboratory of Food Technology organises an International Course (ICP) leading to the degree in 'Msc. in Postharvest and Food Preservation Engineering'. The purpose of this program is to train undergraduates and graduates in the field of food technology and food engineering with special emphasis on the fundamentals and the engineering aspects of postharvest and food preservation technology. Next, a continuing education in food technology, Better Process Control School, is offered.

Scientific consulting is offered to the industry on problems related to the key research areas of the group. A first type of services consists in design, evaluation and optimisation of thermal processes through heat penetration and heat distribution studies on industrial scale equipment. A second type of services involves shelf-life studies of food products during refrigerated storage.

Experience with forms of collaboration and networks

The Laboratory has a large experience with cooperation on a national as well as on a European level, on a scientific as well as on an industrial level. In a number of cases our research unit has operated (is operating) as the coordinator of the project or is carrying out a key role player in the project. Examples of projects are given in **enclosure 6**.

Significant elements from the history, perspectives and projects of the research group

The growing demand of consumers and industry for foods that are convenient to store and use and yet have a high quality or an improved/unique product functionality (*e.g.* texture, health benefits), has stimulated research on modified and novel processing technologies. In this context both improved existing thermal technologies and innovative non-thermal technologies (high hydrostatic pressure, high electric field pulses) are being investigated by the research group, what might contribute to a sustainable development in the food sector.

For the moment, the research group cooperates in different national and international projects concerning characterisation and optimisation of existing thermal processes (sterilization, pasteurisation, blanching, cooling, freezing), combined processes and novel processing techniques, and is or has been playing a key role on the topics "kinetics" and "development of product history integrators for evaluation of impact of processes" in funded research projects.

4.2) Position and Significance of the Project

4.2.1) Laboratory for Authenticity Research (DVK-CLO)

Scientists related to the project

- Lic. R. Van Renterghem (licentiate chemistry; head of the division and from 01-10-2000, head of the department): general supervision
- Dr. J. De Block (doctor in Biochemistry; senior assistant and head of the research group): supervision of the project
- Lic. L. Mortier (licentiate chemistry): realisation of the project and reporting with respect to the work on the intrinsic indicators and analysis of the consumption milk.
- Engineer M. Merchiers (industrial engineer): automation of enzymatic methods.
- Dr. engineer K. Grijspeerdt (civil engineer): realisation of the project and reporting with respect to the modelling of heat exchangers.

Connection of the project with other activities of the research group

This project allowed us to realise a vision on the heat treatment of the Belgian consumption milk and how energy consumption and fouling could be reduced. Since research on intrinsic indicators for heating is one of the core activities of the group, the validation and development of methods was of great importance and the experience and knowledge obtained in this project is actually used in the other projects of the group,. This experience will also be beneficient for obtaining projects in the future and resulted in better contacts with the industry. Also the automation of enzymatic methods for the determination of for milk important parameters could be realised.

Contribution of the project to the research group:

This project supplied the research group the necessary funds for the recruitment of a new collaborator, consumables and the acquisition of scientific and technical equipment such as an instrument for the automation of enzymatic determination methods. Also this project allowed the increase of our knowledge on intrinsic indicators for the evaluation of the heat treatment of milk products and how these indicators could be used for the development of mathematical models for the evaluation of the Belgian production lines for consumption milk in relation to heating and fouling by deposit formation. This research gave the group the opportunity to upgrade his research to an international level.

4.2.2) Laboratory of Food Technology, Katholieke Universiteit Leuven

Scientists related to the project

The research group has an extensive expertise in the domain of kinetics, time temperature integrators and thermal processing, and possesses consequently indispensable know how for development of intrinsic indicators for processed milk authenticity. Researchers working on the project are prof.dr.ir. M. Hendrickx, dr.ir. L. Ludikhuyze and ir. W. Claeys.

Connection of the project with other activities of the research group

The present project agrees completely with our research topics concerning time temperature integrators for evaluation of thermal processes. The knowledge acquired during the project can be used for or extended to novel technologies since quantitative representation of their impact is necessary for industrial implementation and application.

Contribution of the project to the research group

In the context of the project, one additional person (ir. W. Claeys) was recruited. Also, new analytical methodologies and one new apparatus (HPLC) were implemented. With this project, the specific expertise of the research unit in the area of kinetics and time temperature integrators is linked with the specific know how of the "Departement Kwaliteit Dierlijke Producten, Centrum voor Landbouwkundig Onderzoek" in dairy science and technology.

4.3) Network of scientific collaboration

4.3.1) The "official partners":

Participating research groups:

1) Laboratory for Authenticity Research
   Department of Animal Product Quality and Transformation Technology (DVK)
   Agricultural Research Centre-Ghent (CLO)
   Ministry of Small Enterprises, Traders and Agriculture (DG6)
   Brusselsesteenweg 370, B-9090 Melle, Belgium

The group exists of eight collaborators and is specialised in authenticity research and research for the improvement of dairy products and production methods. The group has specific expertise in the area of intrinsic indicators for the heat treatment of dairy products.

2) Laboratory of Food Technology
   Department of Food and Microbial Technology

Katholieke Universiteit Leuven
Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium

The group exists of about 35 collaborators and is internationally recognised as a centre of excellence. The group has specific expertise in the area of kinetics, time temperature integrators and thermal processing of foods

The Laboratory of Food Technology of the KUL was invited by the Laboratory for authenticity research. The experience of both institutes is complementary and linking the specific expertise in the area of kinetics with the specific know how of a specialist in dairy science and technology allows research to develop intrinsic indicators for thermal processed milk authenticity. The work on the project is divided on a fifty-fifty base. The project consists of clearly described tasks and for each task the implementation and input by both research group is described in detail. The division of the research tasks was established at the start of the project. During the course of the project it was not necessary to make changes in the division of the tasks.

4.3.2) The "unofficial partners" and potential users of the results

Participating dairy companies:

The industrial "partners" of the project produce more than 90% of the Belgian consumption milk. They have good knowledge and practical experience with heat exchangers and thermal processing of milk.

All recognised Belgian producers of consumption milk were visited and were invited to co-operate in the project. They were also asked to identify contact persons for the co-operation. This co-operation includes the delivery of milk samples of all process lines and the acquisition of information about these process lines. Four companies were invited to co-operate with the development of a mathematical model for reduction of fouling and energy consumption. The technologies chosen were thermisation, pasteurisation, indirect UHT-heating, direct UHT-heating and sterilisation. The choice of the invited firms was based on their experience with one of these technologies, their affiliation with one of the prominent dairy concerns in Belgium. The co-operation of these companies consisted of detailed and confidential information of their process units.

The following dairy companies took part :

dairy company	delivery of milk samples and information about the process line	development of an mathematical model
Belgomilk	X	X
Campina	X	X
Inex	X	
Interlac	X	X
Inza	X	X
Madibic	X	
Molkerei Walhorn	X	
Olympia	X	

4.3.3) The character of the relations and exchanges

For the co-operation with the research institute one contact person in each company was responsible.

4.3.4) Intermediary objects and exchange carriers

Each company received a detailed confidential report concerning the consumption milk they produced and its conformity with the expected European legislation. From this report they were also able to position their products in the entire Belgian consumption milk production (see **enclosure 1 and 2**).
Each company co-operating in the development of one of the mathematical models was (or will be) visited for the presentation of the mathematical model concerning their production unit which was subject of the study and received (or will receive) an extensive and detailed report with suggestions to correct or improve the actual situation (see **enclosure 3**).

4.3.5) Mechanisms regulating the network

A report will be drawn in which the results of the research will be presented. This report will be presented on a workshop for the Belgian dairy industry and related institutes. The actual situation will be evaluated and suggestions for improvement will be formulated.

4.3.6) Organisation

The DVK behaves as promoter of the network. The project has no management committee, but every three months a meeting between both partners is organised. On these occasions results are presented and discussed as well as the division of the tasks. Interim contacts are held by phone,fax and email.

5) Balance and perspectives

Balance of the project

A good picture of the processing of Belgian consumption milk was obtained and can be placed in a European context with regard to a possible future legislation. Each participating dairy company was informed about the position of their different consumption milk products, its position in the whole Belgian situation and its agreement with possible future European legislation. Furthermore, a close co-operation with four prominent dairy factories resulted in model systems for reduction of fouling in heat exchangers and reduction of energy costs for the most conventional heating techniques (such as pasteurisation, direct and indirect UHT-heating and sterilisation). This allowed these factories to take action, if necessary, to reduce their energy costs and production losses due to fouling which would lead to better economics and less pollution.

Some of the frequently used methods for evaluating heat damage were improved and their kinetic were studied profoundly. These results are, or will be published in international scientific journals.

Perspectives

Towards the end of the project, there will be a dissemination of the results: the results will be published and a seminary for the dissemination of the final results and conclusions will be organised. Dairy factories that are interested with regard to the processing and production of consumption milk will be supported in the future.

On the meetings of the Chemist Experts Group of DG VI of the European Union, much attention will be paid to the discussions concerning the possible legislation for the heat treatment of consumption milk and Belgian legal and controlling authorities will be informed. Newly presented methods will be integrated.

The most recent evolutions in the field of methods and parameters for the evaluation of heat processing of foods will be followed closely. Newly presented methods will be integrated in our research group and the group will participate in relevant ring tests.

On the basis of bilateral contacts with dairy factories, possibly new projects will be set up and finally, much attention will be paid to new technologies for processing of foods.

Balance of the PODO

This project was for the participating research groups of very great importance since it allowed following realisations: the recruitment and training of new collaborators and the improvement to an international level of our know how concerning the evaluation of heat processing during the production of consumption milk. This allows us to discuss on international forums and allows the legal and controlling authorities to rely on our expertise.