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Intrinsic indicators for monitoring heat damage of consumption milk

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The general principles of the most important available indices to assess heat treatments in the dairy field are discussed. Available indices related to two types of chemical reactions are described: (1) the degradation, denaturation and inactivation of heat labile components, and (2) the formation of "new" substances which are not present in the unprocessed product. Further, a screening of the Belgian consumption milk with respect to heat processing is presented.
Keywords. Consumption milk, heating parameters, intrinsic indicators, heat treatment, Belgium.

Indicateurs intrinsèques pour mesurer la dégradation thermique du lait de consommation. La première partie discute des principes généraux de la plupart des indices disponibles pour évaluer les traitements thermiques dans le secteur laitier. Deux types d'indices sont décrits. Ils sont basés sur les réactions chimiques suivantes : (1) la dégradation, la dénaturation et l'inactivation de composants thermolabiles intrinsèques, et (2) la formation de "nouvelles" substances absentes dans le produit non traité. La seconde partie décrit la situation en Belgique pour le lait de consommation.
Mots-clés. Lait de consommation, paramètres thermiques, indicateurs intrinsèques, traitement thermique, Belgique.

1. INTRODUCTION

In order to increase the shelf life of milk and milk products and to guarantee the microbiological safety, a heat treatment is applied. This heat treatment is the last and correcting step in the production of consumption milk. In order to avoid that such a correcting step would lead to "over processing" (due to a lack of care on preceding steps) and to oblige the producers to apply the HACCP- principles, an European legislation will be worked out with legal standards for consumption milk. Conformity of the product with the label will be a guarantee for the quality. Therefore, heat treatment conditions must be chosen in such a way that the desired results (hygienic safety and prolonged shelf life) can be achieved while undesirable changes (reduction of nutritional value, undesired reactions and changed organoleptic properties) are minimised. Therefore, heat treatment conditions must be defined by assuming a range with lower and upper limits. For the definition of heat treatment, as well as for the control of end products, indicators of heat treatment and suitable analytical methods are very important.

2. GENERAL PRINCIPLES

Evaluation of heat treatment is possible if irreversible changes are induced in the product. The most interesting are (bio)-chemical reactions.

Two types of chemical reactions may be used to assess heat treatments :

– the degradation, denaturation and inactivation of heat-labile components, e.g. whey proteins or enzymes ("Type 1"-indicators);
– the formation of "new" substances, e.g. lactulose or products of the Maillard reaction ("Type 2"-indicators).

However, these indicators have also many limitations. Upon heating of a dairy product heat damage depends on both duration as well as intensity (temperature) of heating. The assessment of only one indicator does not allow to distinguish between a prolonged heat treatment at relatively low temperature and a short heat treatment at a higher temperature. In most cases more than one heat indicator will be used.

Also pH as well as concentration of the constituents of the products will influence the chemical and bio-

chemical heating reactions. Moreover, the range of possible heat treatments, from pasteurisation to in bottle sterilisation is far to broad for the use of just one intrinsic indicator. Finally, a good intrinsic indicator requires a relative simple analytical method.

3. DESCRIPTION OF AVAILABLE INTRINSIC INDICATORS

3.1. Type 1-indicators

These are components that can be denatured or inactivated by heating. Two important categories are enzymes and whey proteins.

Enzymes are heat labile and loss of activity is in most cases easily to detect by a simple colour reaction. Alkaline phosphatase and lactoperoxidase are two important intrinsic indicators for monitoring heat damage of pasteurised milk.

Since alkaline phosphatase is stable to temperatures slightly higher than those required to destroy milk pathogens, the control of the activity of this enzyme is the most important indicator for evaluating the hygienic safety of pasteurized milk. This means that pasteurised milk must be negative for the phosphatase test.

Determination of the activity of lactoperoxidase, which is a rather stable endogenous enzyme, can be used as a simple test for the determination of the upper limit of pasteurization. Pasteurized milk must show a positive lactoperoxidase reaction and must be labelled as “highly pasteurized” when a negative result is obtained.

The individual whey proteins show distinct differences in thermal stability; the order of heat stability of the principal proteins is: α-lactalbumin > β-lactoglobulin > bovine serum albumin > immunoglobulins. The whole whey protein fraction, as well as its individual components, may be used as indicators of thermal treatment. There is no essential difference between the heat classification of skim milk powder and the evaluation of the heat treatment of liquid milk on the basis of whey protein denaturation; the heating of liquid milk is the reason for thermal denaturation in both cases.

The whole whey fraction is often used as an indicator for monitoring heat damage. Most important are the whey protein nitrogen index (WPNI) and the heat number. The WPNI is the amount of undenaturated whey protein N (soluble in saturated NaCl) expressed as milligrammes per gramme of milk powder or liquid milk and can be determined by a turbidimetric detection. The heat number is expressed as the percentage of nitrogen insoluble at pH 4.8.

Monitoring consumption milk in order to make the distinction between pasteurized milk and UHT milk is often carried out by determination of acid soluble β-lactoglobulin. Chromatographic techniques allow these determinations with high precision and accuracy but variations in the absolute and relative concentrations of β-lactoglobulin in the milk may be a drawback.

3.2. Type 2-indicators

In contrast to type 1 indicators which are based on the degradation, denaturation or inactivation of heat-labile components, type 2 indicators are based on the formation of “new” substances. During heat treatment of milk, lactose is involved both in the Maillard reaction and in isomerisation and subsequent degradation reactions.

Among the sugars derived from lactose, lactulose undoubtedly represents the most widely studied index for differentiating heated milks and for evaluating the heat load to which milk was subjected. Lactulose is a very interesting indicator for the study of heating of milk and milk products: the determination methods are accurate and precise (De Block *et al.*, 1996) and can be carried out by column chromatography, gas chromatography or by an enzymatic method. Determination of lactulose allows distinction between pasteurized milk, UHT milk and sterilized milk.

Also components formed by the Maillard reaction can be used as intrinsic indicators for monitoring heat damage. One of those products is hydroxymethylfurfural (HMF) which is suited as an indicator for severe heat treatments (UHT and sterilization). HMF can be formed by whatever reducing sugar and can also be used as heating indicator for sugar containing lactose free dairy products.

Furosine is a very interesting Maillard product and can be obtained by the acid hydrolysis of heated milk or milk products. Furosine can also be used for monitoring the heat treatment of consumption milk (Van Renterghem, De Block, 1996). Moreover, high concentrations of furosine are formed during the production of milk powder due to favourable reaction condition during this process. Therefore there is a considerable higher ratio of furosine to lactulose for milk powder than for consumption milk. Determination of this ratio allows to demonstrate improper additions of reconstituted milk powder in consumption milk (pasteurized milk and UHT milk). Addition of reconstituted milk powder during the production of consumption milk will lead to abnormally high furosine values for pasteurized milk and to an abnormally high ratio of furosine to lactulose for UHT milk. Finally, furosine determination can also be used for the detection of milk powder addition during cheese production. Cheeses normally produced from raw or pasteurized milk would have elevated furosine contents if milk powders were used during production.

4. WHICH TYPES OF HEAT TREATMENTS ARE DEFINED BY THE BELGIAN LEGISLATION AND WHICH EUROPEAN LEGISLATION CAN BE EXPECTED IN THE FUTURE?

4.1. Pasteurized and highly pasteurized milk

According to the Belgian legislation pasteurized milk must be obtained by a short heat treatment at high temperature (minimal at 71.7°C during 15s or a similar combination), or by a pasteurization process for which another temperature/time combination is used in order to obtain a similar effect. Pasteurized milk has to react negatively on the phosphatase test and positively on the peroxidase test. Production of peroxidase negative pasteurized milk is allowed on the condition that its label declares "highly pasteurized milk".

The European Union Milk Expert Group DG6 will use the phosphatase test for the lower heating limit and the lactoperoxidase test for the upper heating limit of pasteurized milk. For highly pasteurized milk the lactoperoxidase test has to be negative. In order to evaluate whether a peroxidase test is negative or not, possibly a quantitative test will be used in future. At the moment it is not yet clear whether or not other criteria will be used for the distinction between pasteurized milk and highly pasteurized milk and for the upper limit of highly pasteurized milk. Lactulose in pasteurized milk should be below detection limit and may not exceed 50 mg/l in highly-pasteurized milk. However, the latter criteria are under discussion due to limitations in sensibility of the detection methods.

Also proposals in which β-lactoglobulin concentrations of more than 2600 mg/l would be required for pasteurized milk and 2000 mg/l for highly-pasteurized milk are under discussion now due to variations in the β-lactoglobulin content of milk.

4.2. UHT milk

UHT milk is defined by the Belgian legislation as a continuous heating process for a short time at a high temperature (at least 135°C during at least 1s) in order to eliminate all micro-organisms and spores using aseptic air tight packaging material and with minimal changes in chemical, physical and organoleptic properties. As to the European legislation, the lower heating limits for UHT milk will probably correspond with the upper limits of highly-pasteurized milk. Further, for the upper heating limit of UHT milk the lactulose content will have to be below 600 mg/l and the acid soluble β-lactoglobulin concentration should be higher than 50 mg/l. However, the latter norm is still being discussed.

4.3. Sterilized milk

According to the Belgian legislation sterilized milk has to be heated and packaged in hermetically sealed bottles or bricks with sealing that remains intact. Further storage during 15 days at >30°C may not be harmful for the product. Sterilized milk usually is produced in two steps consisting of a continuous heating step at 130–140°C during several seconds and an in bottle sterilization at 110–120°C during 10–20 min. European norms for sterilized milk will be a lactulose concentration above 600 mg/l and a β-lactoglobulin concentration below 50 mg/l.

5. MATERIALS AND METHODS

5.1. Milk samples

All recognized Belgian consumption milk producers were invited to co-operate. This co-operation included the delivery of milk samples of all process lines and the acquisition of information about these process lines. This information related to the process type (as far as UHT-milk is concerned, distinction was made between direct and indirect heating systems) and to the temperature/time combinations used in heat treatments.

5.2. Methods

The formation of furosine was measured by reversed phase HPLC according to the method of Resmini *et al.* (1990). The lactulose content was determined using the BM Test-Combination D-glucose/D-fructose in combination with β-galactosidase, triethanolamine hydrochloride, glucose oxidase and catalase (Roche – Boehringer Mannheim, 1989). The acid soluble β-lactoglobulin content was quantified by HPLC according to the method of the International Dairy Federation (FIL-IDF, 1999). The activity of alkaline phosphatase was determined spectrophotometrically at the absorption maximum using p-nitrophenyl diso-diumphosphate as substrate following the procedure of the IDF (FIL-IDF, 1987). The lactoperoxidase activity was determined on the basis of an adopted procedure of Hernández *et al.* (1990). Before measuring the activity spectrophotometrically at 412 nm with ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) and H_2O_2 as substrate, milk proteins were precipitated by adding 1.75 M acetic acid and 1 M sodium acetate.

6. RESULTS AND DISCUSSION: SCREENING OF THE BELGIAN CONSUMPTION MILK FOR HEATING

In order to obtain an inventory of the current situation in the Belgian dairy industry, two inquiries among

eight different Belgian consumption milk producers were carried out. These inquiries resulted in 154 different consumption milk samples in which the following parameters were determined: furosine, lactulose, β-lactoglobulin, alkaline phosphatase and lactoperoxidase.

The results of both inquiries were very similar. **Figures 1**, **2** and **3** represent the average lactulose, furosine and β-lactoglobulin content of the Belgian consumption milk samples of both inquiries. As expected the difference in processing conditions was clearly reflected. Significant differences can be observed between direct and indirect UHT systems. Heat treatments by direct UHT systems result in considerably less heat damage.

The **figures 4** (for thermization and (high) pasteurization) and **5** (for UHT and sterilization) represent the relation between the average furosine and lactulose content of the samples of both inquiries. Minimum and maximum values for both parameters are indicated.

The **figures 6** (for thermization and (high) pasteurization) and **7** (for UHT and sterilization) represent the relation between the average lactulose and acid soluble β-lactoglobulin content of all investigated consumption milk samples of both inquiries. Minimum and maximum values for both parameters are indicated. In both inquiries, there was one milk sample labelled as highly pasteurized milk. The sample of the first inquiry had a soluble β-lactoglobulin content below 2000 mg/l which would be to low according to the possible future European legislation. The sample of the second inquiry had a soluble β-lactoglobulin content above 2000 mg/l. An important fraction of the UHT milk samples will not meet the expected norms : 62.5% of the indirect UHT milk samples of the first inquiry and 60% of the indirect UHT samples of the second inquiry has a lactulose content above 600 mg/l. Of the indirect UHT milk samples of the first inquiry, 37.5% has an acid soluble β-lactoglobulin content below 50 mg/l. No problems can be observed with the direct UHT systems.

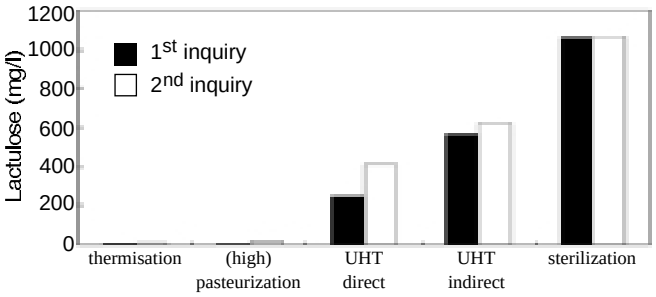

Figure 1. Comparison of the average lactulose content between the two inquiries held among the Belgian dairy industry for the different heat treatments — *Comparaison du taux moyen de lactulose pour les différents traitements thermiques et pour les deux enquêtes organisées dans l'industrie laitière belge.*

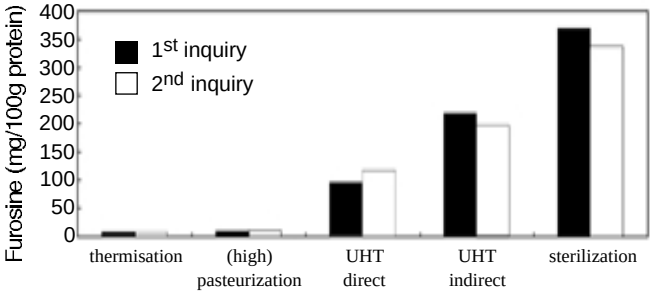

Figure 2. Comparison of the average furosine content between the two inquiries held among the Belgian dairy industry for the different heat treatments — *Comparaison du taux moyen de furosine pour les différents traitements thermiques et pour les deux enquêtes organisées dans l'industrie laitière belge.*

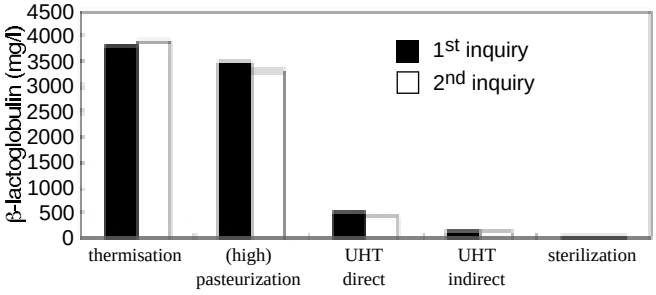

Figure 3. Comparison of the average β-lactoglobulin content between the two inquiries held among the Belgian dairy industry for the different heat treatments — *Comparaison du taux moyen de β-lactoglobuline pour les différents traitements thermiques et pour les deux enquêtes organisées dans l'industrie laitière belge*.

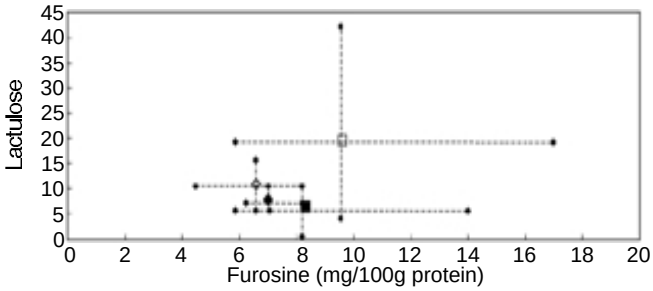

Figure 4. Relation between the average lactulose and average furosine content in the samples "thermization" of the first (♦) and second (◊) inquiry and in the samples "(high) pasteurization" of the first (■) and second (□) inquiry. Minimum and maximum values for both parameters are indicated — *Relation entre les taux moyens de lactulose et de furosine pour les échantillons "thermisés" de la première* (♦) *et de la seconde* (◊) *enquête et les échantillons "à (haute) pasteurisation" de la première* (■) *et de la seconde* (□) *enquête. Les valeurs minimales et maximales des deux paramètres sont indiquées pour les différents traitements thermiques.*

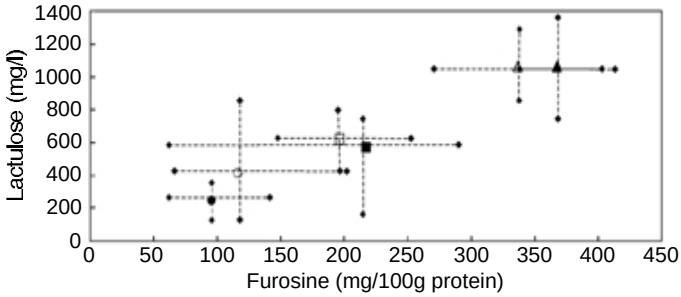

Figure 5. Relation between the average lactulose and average furosine content in the samples "UHTdirect" of the first (●) and second (○) inquiry, in the samples "UHT indirect" of the first (■) and second (□) inquiry and in the samples "sterilization" of the first (▲) and second (△) inquiry. Minimum and maximum values for both parameters are indicated — *Relation entre les taux moyens de lactulose et de furosine pour les échantillons "UHT direct" de la première* (●) *et de la seconde* (○) *enquête, les échantillons "UHT indirect" de la première* (■) *et de la seconde* (□) *enquête et les échantillons 'stérilisés' de la première* (▲) *et de la deuxième enquête* (△). *Les valeurs minimales et maximales des deux paramètres sont indiquées pour les différents traitements thermiques*.

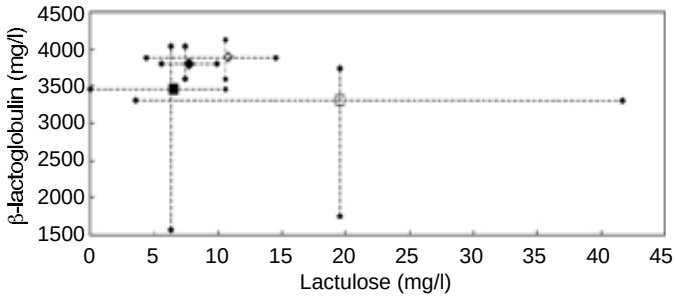

Figure 6. Relation between the average β-lactoglobulin and average lactulose content in the samples "thermization" of the first (♦) and second (◊) inquiry and in the samples "(high) pasteurisation" of the first (■) and second (□) inquiry. Minimum and maximum values for both parameters are indicated. – *Relation entre les taux moyens de β-lactoglobuline et de lactulose pour les échantillons 'thermisés'de la première* (♦) *et de la seconde* (◊) *enquête et les échantillons "à (haute) pasteurisation" de la première* (■) *et de la seconde* (□) *enquête. Les valeurs minimales et maximales des deux paramètres sont indiquées pour les différents traitements thermiques*.

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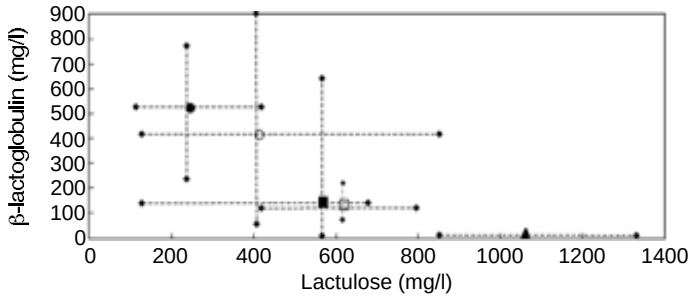

Figure 7. Relation between the average β-lactoglobulin and average lactulose content in the samples "UHT direct" of the first (●) and second (○) inquiry, in the samples "UHT indirect" of the first (■) and second (□) inquiry and in the samples "sterilization" of the first (▲) and second (Δ) inquiry. The symbols ▲ and △ can't be distinguished because the difference between both inquiries is too small. Minimum and maximum values for both parameters are indicated — *Relation entre les taux moyens de β-lactoglobuline et de lactulose pour les échantillons "UHT direct" de la première* (●) *et de la seconde* (○) *enquête, les échantillons "UHTindirect" de la première* (■) *et de la seconde* (□) *enquête et les échantillons "stérilisés" de la première* (▲) *et de la seconde enquête* (△). *Les symboles ▲ et △ du lait stérilisé ne se distinguent pas car la différence entre les deux enquêtes est trop petite*. *Les valeurs minimales et maximales des deux paramètres sont indiquées pour les différents traitements thermiques*.

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