

# Heat Load indicators and their Significance in Milk Processing

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Nowadays, milk is nearly always thermally processed before consumption. The main purpose of heating is to make milk safe for human consumption (by killing pathogenic bacteria) and to extend its shelf life. Heating causes a significant loss of organoleptic and nutritional quality (e.g. vitamin destruction, precipitation of calcium phosphate, denaturation of whey proteins, and Maillard reaction). Furthermore, an undesirable precipitation of denatured proteins and minerals can be formed on the walls of heat exchangers. The reason is heating of milk that causes different modifications in the physico-chemical state of its components, leading primarily to the denaturation of certain protein fractions (enzymes, whey proteins and the formation of Maillard reaction products). Several heat-induced changes related to these modifications have been developed in recent years to determine the quality of milk. These so-called heat-damage markers indicators can be used to control and check the heat treatments given to milk. However, only some compounds are suitable as chemical markers of the heat treatment intensity and they are called heat load indicators or time temperature integrators (TTIs) that are used to quantify the impact of thermal processes on milk (e.g. the enzymes alkaline phosphatase and lactoperoxidase; the whey protein  $\beta$ -lactoglobulin  $\beta$ -Lg; hydroxymethylfurfural, HMF; lactulose; and furosine) (Mayer *et al.*, 2010).

## Heat Load Indicators

### Type 1-indicators

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

## Enzymes

Milk contains enzymes that have the ability to catalyze specific chemical reactions. Enzyme activities can be considerably affected by thermal processing as indicated in Table 1. 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 pasteurized 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 pasteurized 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 labeled as “highly pasteurized” when a negative result is obtained. Therefore, the use of lactoperoxidase test distinguishes between the two treatments viz. Pasteurization and high temperature Pasteurization. Standard methods for alkaline phosphatase and lactoperoxidase tests include those based on spectrophotometry and fluorimetry. Other native enzymes can also be used to evaluate heat load in milk e.g.  $\alpha$ -fucosidase, phosphodiesterase,  $\alpha$ -mannosidase, but their practical use is much less extended.

## Whey Proteins

Several physical and chemical changes occur in whey protein during thermal processing of milk, and these changes (denaturation) affect the



functional and sensory properties of milk. During the heating process, whey proteins containing sulfhydryl residues undergo various changes resulting in the formation of (1) a protein complex between beta-lactoglobulin and kappa-casein, with consequent modification of rennet coagulation behavior and heat stability, (2) typical off flavors, and (3) unusual amino acids (lysinoalanine).

Whey proteins show different thermal stabilities: alpha-lactalbumin > beta-lactoglobulin > bovine serum albumin > immunoglobulins. The whole whey protein fraction, as well as its individual components, may be used as indicators of thermal treatment. While denaturation of alpha-lactalbumin can be a good parameter to describe high-temperature treatments, such as sterilization, denaturation of beta-lactoglobulin is useful to describe thermal treatments from pasteurization to UHT processing. Most important indicators are the whey protein nitrogen index (WPNI) and the heat number. The WPNI is the amount of undenatured whey protein N (soluble in saturated NaCl) expressed as milligrams per gram 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  $\beta$ -lactoglobulin. Chromatographic techniques allow these determinations with high precision and accuracy but variations in the absolute and relative concentrations of  $\beta$ -lactoglobulin in the milk may be a drawback (Mortier *et al.*, 2000).

The quantitative determination of acid-soluble  $\beta$ -Lg has been proposed to distinguish between different categories of heat-treated milk. A minimum content of 2600 mg per L for pasteurized milk, of 2000 mg per L for high-pasteurized milk, and of 50 mg per L for UHT milk is within the limits proposed by the International Dairy Federation (Mayer *et al.*, 2010, Tamime, 2009). The proposed data are indicated in Table 1 for various heat treated samples.



## Type 2-indicators

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. The most studied chemical reaction in heat-treated milk is, undoubtedly, the Maillard reaction, in which amino groups (mainly casein-bound lysine residues) and reducing sugars (mainly lactose) are the main reactants. The Maillard reaction consists of several steps, strictly dependent on temperature, pH, water activity, and type of sugar and amino group involved. Some of the end products considered as important Heat Load indicators are described below and their reported values are shown in Table 1.

## Lactulose

Lactose can isomerize to lactulose in heat treated milk. Lactulose isomerization is catalyzed by free amino groups of casein and is strictly dependent on time of heating, heating temperature, and pH. Normally, lactulose does not occur in fresh milk and highly pasteurized milk, but only in UHT and sterilized milk. Lactulose content is therefore considered a suitable indicator of heat treatment (De Block *et al.*, 1996).

Lactose-derived compounds can be used to evaluate more intensive heat treatments i.e. direct and indirect UHT treatments and sterilization. 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 and the determination methods are accurate and precise (De Block *et al.*, 1996). Lactulose is formed by isomerization of lactose during heating of milk and has been proposed as an analytical index to distinguish UHT from sterilized milk. UHT milk should have a lactulose upper limit of 600 mg/L. Lactulose is not found in pasteurized milks, although values upto 82 mg/L have been reported in commercial samples (Tamime, 2009).

A variety of methods have been reported to be used for lactulose determination e.g. GC, HPLC, CE, enzyme methods, colorimetry and continuous flow amperometry. Determination of lactulose allows distinction between pasteurized milk, UHT milk and sterilized milk.

### HMF

Components formed by the Maillard reaction can also 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 is an intermediate compound formed in Maillard reaction in heated milks. HMF can be formed by whatever reducing sugar and can also be used as heating indicator for sugar containing lactose free dairy products. Using HMF as a marker, Ferrer and coworkers, 2000, observed differences in various UHT milk samples. Free HMF was found to be present only in some stored UHT milk samples, levels ranging from 8.24 to 50.9  $\mu\text{g}/100$  ml of milk, probably due to lower milk quality. Several factors possibly affecting the use of HMF as a marker have been investigated. In particular, the total HMF level in milk appears to be affected by milk fat concentration. The HMF level in commercial UHT milk (stored below 50°C) is also related to temperature and time of storage, and increases with higher temperature (Keeney and Bassette, 1959).

Traditionally, HMF is determined by a colorimetric method, but this method has low specificity. At present, capillary electrophoresis and RPHPLC appear to be the most powerful techniques for HMF determination.

### Furosine

The early stage of the Maillard reaction can be monitored through the amount of furosine in milk. Maillard reaction products, such as lactuloselysine, results from heating and it is transformed into furosine by acid hydrolysis. 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 liquid milk. Furosine is present in concentrations of 3-5 mg/100g protein in raw milk and 5.2-7.5 mg/100g protein in pasteurized milk. In sterile milk samples furosine level varies within a wide range, but it is usually higher in milk processed by indirect than by direct UHT treatment. A furosine content of 8 mg per 100 g protein has been suggested as upper limit for pasteurization, of 20 mg per 100 g protein for high pasteurization, and of 250 mg per 100 g protein for UHT processing (Mayer *et al.*, 2010). However, overlapping of values in milk submitted to different heat treatments is the main difficulty encountered when using furosine as chemical indicator to distinguish among commercial sterilized milks. Moreover, the furosine level may increase during storage of UHT milk.

High concentrations of furosine are also 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 market milk. Determination of this ratio allows demonstrating improper additions of reconstituted milk powder in milk (pasteurized milk and UHT milk). Addition of reconstituted milk powder during the production of market milks 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 (Mortier *et al.*, 2000).

Furosine can be determined by a number of RP-HPLC methods using different columns, anion-exchange chromatography with pulsed amperometric detection, CE and GC. Since furosine is partially degraded during gas chromatography analysis, this method cannot be recommended for routine analytical application. The international standard is based on an ion-pair RP-HPLC system.



**Table 1 - Assessment of heat load indicators**

	Raw	Therm.*	Past.*	High Past.*	UHT Direct	UHT Indirect	Steri.* milks	References
Temperature (°C)	< 40	< 65 for few mins	72 for 15 s	127 for 3-4 s	150 for 3-4 s	138 for 3-4 s	121 for 15-20 mins	-
Phosphatase	+	+	-	-	-	-	-	Zehetner <i>et. al.</i> (1996)
Peroxidase	+	+	+	-	-	-	-	
Native $\beta$ -Lactoglobulin (mg/l)	3600	3400	3000	1800	800	200	Nil	Pellegrino <i>et. al.</i> (1995); Kondal-Reddy <i>et. al.</i> (1999)
Lactulose (mg/l)	< 10	< 10	< 10	< 100	< 200	200 to 600	> 600	De Block <i>et. al.</i> (1996)
Furosine (mg/100g protein)	< 10	< 10	< 20	< 100	< 200	100 to 250	200 to 500	Ferrer <i>et. al.</i> (2000)

\* Past. = Pasteurized, Therm. = Thermized, Steri. = Sterilized

### Conclusion

Milk heat treatments can produce very complex effects among milk constituents. Simultaneous study of several heat-induced parameters improves the classification of industrial processed milks and provides deeper knowledge of what actually happened in heat-treated milk. As a result, the quantitative determination of heat load indicators has been proposed to distinguish between different categories of heat-treated milk. Thus, heat Load Indicators or TTIs has an important role to play for controlling the nutritional and organoleptic quality of liquid milk in the future. Since dairy companies are obviously not aware of the negative effects caused by overheating of liquid milk, there is an urgent need for establishing obligatory threshold levels (limits) for market milks regarding TTIs (e.g. acid-soluble  $\beta$ -Lg, furosine, and lactulose). However, amongst the known heat load indicators furosine and lactulose are good thermal indicators of heat damage since high correlations were observed in their levels in most studies.

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