MAILLARD REACTION EVALUATION BY ε-FRUCTOSYL-LYSINE DURING MILK THERMAL PROCESSING: FIRST STEP

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In order to increase the shelf life of milk and to guarantee microbiological safety, a heat treatment is applied. The evaluation of the heat treatment is a very important point for assessing milk quality. In fact, these heat treatments are necessary both as regards the use of milk in the productive process and for the obvious commercialization requirements. Nevertheless, the high temperatures used in some processes (UHT milk, powdered milk) denature the proteins and produce lower quality milk. For certain cheese productions it is mandatory by law to use only fresh milk and the presence of significant quantities of compounds derived from particular heat treatments indicates the presence of adulteration.

The Maillard reaction causes milk to turn slightly brown. In the initial phase, this reaction takes place between an amino group of the lysine and the aldehydic function of the lactose in the milk. It leads to the formation of the Shiff base and, subsequently, aided by the temperature, to the formation of ε-FRUCTOSYL-LYSINE. ε-FRUCTOSYL-LYSINE is the product of the reaction, which we quantify to verify the type of heat treatment that the milk was subjected to. At the present time, one of the tests most often used to determine the presence of derivatives of the Maillard reaction is furosine, which is very expensive and requires the use of an HPLC instrument with dedicated column.

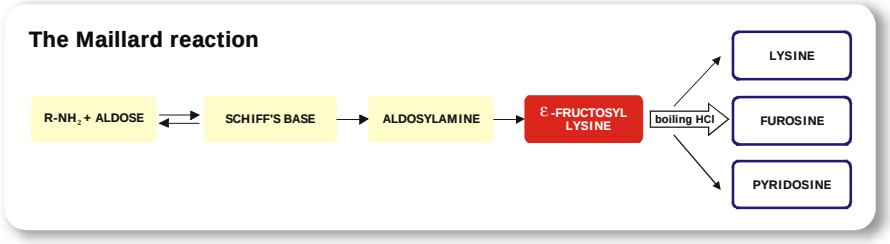

fig.1

Material and Methods

The ε-Fructosyl-lysine content was tested in 5 samples of milk subjected to different heat treatments: raw milk, pasteurized whole milk, UHT milk, powdered milk, powdered milk for infants. The samples were selected in order to have the same protein content. Furthermore, repeatability tests were carried out on samples of pasteurized, UHT and powdered milk (Table 1). Increasing quantities of UHT and powdered milk were subsequently added to a pasteurized whole milk base (Table 2).

Instrument: the samples were tested using the innovative Food*Lab* system by CDR, formerly used for testing other parameters on milk such as urea, ammonia, lactic acid and chlorides. The FoodLab instrument, thanks to a special photometric technology, allows testing samples of whole or skim, raw or pasteurized milk without any preliminary treatment of the sample.
Reagents*:* the Food*Lab* method uses a redox reaction in which tetrazolium salt, in an alkaline medium, reacts with ε-Fructosyl-lysine and forms a violet complex, whose intensity, read at 545nm, is proportional to the ε-Fructosyl-lysine concentration in the sample. The kinetics data are shown in Fig.3.
Operating procedures**:** The FoodLab system is very easy to use. The kit includes previously dispensed single use cuvettes and a dropper containing R2. 150 µL of milk and 2 drops of R2 are added to a preheated cuvette. The cuvette is placed in the reading cell at 37°C and the reaction is followed for 1 minute (Fig. 2). At the present time the result is expressed by (ΔE/min)*1000, awaiting the completion of the studies and its conversion into units of concentration per 100 gr. of proteins.

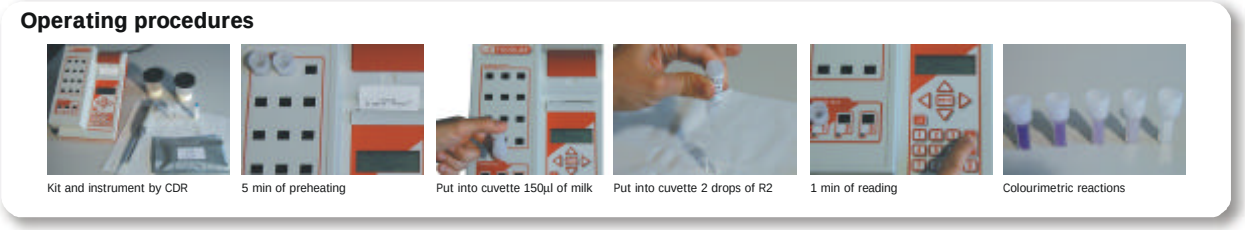

Fig.2

Results and Discussion

The system used is reliable and precise. The reaction is followed for the first minute when the kinetics is still of order 0. The slope of the regression line obtained is calculated and this allows eliminating any interferences and having high sensitivity even when the signal is very low, as, for example, in pasteurized milk.

Fig. 3 shows the kinetics data of pasteurized milk and UHT milk: the slope of the UHT milk is clearly greater than that of the pasteurized milk and the R^2 = 0.99 in both samples indicates the high sensitivity of the system.

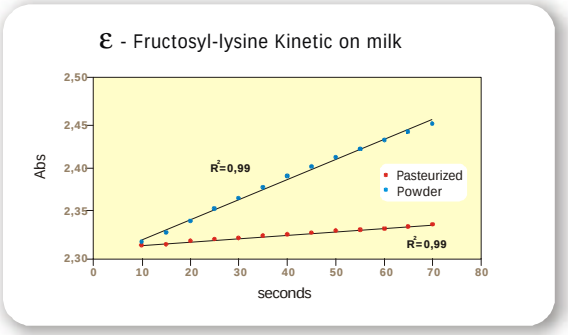

Fig.3

Table 1 shows the system's repeatability on 3 milk samples subjected to different heat treatments.

Repeatability trials

	Pasteurized milk	UHT	Powder milk
	30	69	141
	32	70	144
	28	66	138
	26	72	143
	28	69	142
Mean	29	69	142
DS	2,3	2,2	2,3

Tab.1

Figure 4 shows the concentration of ε-Fructosyl-lysine obtained from different milk samples. The heat treatment, ever higher, affects the content of ε-Fructosyl-lysine present.

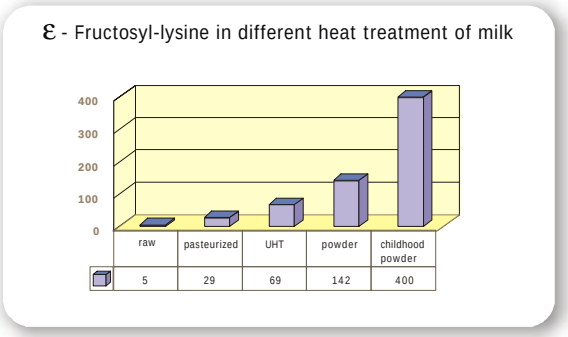

Fig.4

Figure 5 shows the concentrations of ε-Fructosyl-lysine in samples of pasteurized milk to which increasing percentages of UHT and powdered milk have been added (Table 2). The system distinguishes the various sample types well, obtaining excellent linearity.

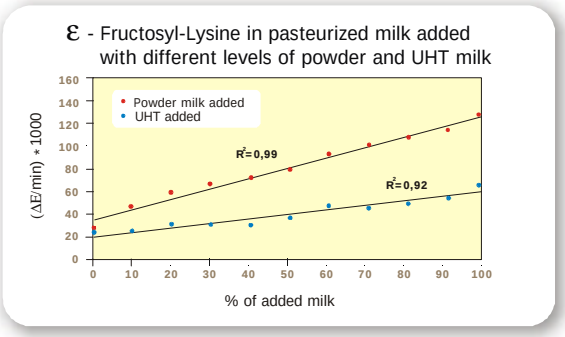

Fig.5

Sampling procedures

% of added milk	UHT	Powder milk
0	only pasteurized milk	only pasteurized milk
10	10 mL UHT + 90 mL pasteurized	10 mL powder + 90 mL pasteurized
20	20 mL UHT + 80 mL pasteurized	20 mL powder + 80 mL pasteurized
30	30 mL UHT + 70 mL pasteurized	30 mL powder + 70 mL pasteurized
40	40 mL UHT + 60 mL pasteurized	40 mL powder + 60 mL pasteurized
50	50 mL UHT + 50 mL pasteurized	50 mL powder + 50 mL pasteurized
60	60 mL UHT + 40 mL pasteurized	60 mL powder + 40 mL pasteurized
70	70 mL UHT + 30 mL pasteurized	70 mL powder + 30 mL pasteurized
80	80 mL UHT + 20 mL pasteurized	80 mL powder + 20 mL pasteurized
90	90 mL UHT + 10 mL pasteurized	90 mL powder + 10 mL pasteurized
100	only UHT	only powder milk

Tab.2

Conclusion

The study is still in progress but, from the data obtained up to now, we deduce that there is an excellent correspondence between ε-Fructosyl-lysine content and heat treatment of milk. The correlation between ε-Fructosyl-lysine and Furosine will be studied by dr. Toppino P.M. This allows using the test to guarantee the milk quality, its shelf-life or to discover any adulterations (ex. the addition of powdered milk to fresh milk). Furthermore, the simplicity and inexpensiveness of the FoodLab system make this a user-friendly test.

References

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