



PEROXIDE IN MILK

RAPID METHOD FOR THE DETERMINATION OF PEROXIDE IN MILK

INTRODUCTION

Lactoperoxidase is an enzyme present in milk in very big quantity. It is associated to the serum proteins. It is inactivated by thermal treatments with considerably high temperatures (70° C for 15 minutes or 80°C for 30 seconds) that are conditions more drastic than those required for a normal pasteurization process. For this reason, the persistence of lactoperoxidase activity in pasteurized milk can be used as a good indicator for the quality of the product. A "light" temperature treatment that preserves this enzyme can be done only to a raw milk of good microbiologic quality. In this case chemical-physical and nutritional characteristics of the milk are altered at the minimum level.

The different sensitivity to heat of some enzymes (in particular alkaline-phosphatase and lactoperoxidase) has permitted the development of different methods to be used to verify the efficiency of thermal treatment of milk through pasteurisation. These methods anyway are qualitative methods and only indicate the presence or absence of the enzyme giving only an indication about the fact that a thermal treatment has occurred. A quantitative result of lactoperoxidase though makes possible to determine the nutritional quality of the milk: the higher is the lactoperoxidase value, the lower are the alterations of the original milk characteristics.

MATERIALS AND METHODS

In order to have lactoperoxidase evidence in milk we need to add hydrogen peroxide and a compound that receives the oxygen, to act as an indicator.

FOODLAB innovative method permits to quantify the lactoperoxidase in milk in a very easy and quick way. It is used a pre-filled single use cuvette and microquantities of the sample.

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Sistema Qualità certificato ISO 9001 ed. 2000



Materials:

- FOODLAB Instrument
- Pre-filled single use cuvettes
- Chromogen bottle
- Starter reagent bottle
- Micropipette for taking 5 μ L
- Micropipette for taking 50 μ L

Prepare the reagent contained into the cuvette adding 50 μ L of chromogen reagent.
 Using the specific pipette add 5 μ L of sample into the cuvette and then, after 5 minutes of incubation into the specific cells of the instrument, put 50 μ L of starter reagent.
 After 5 minutes the instrument prints out the result expressed as U/L of lactoperoxidase.

Reagent	Sample Volume	Wavelength	Analysis type	Unit of measure	Calibration availability
3 compounds	5 μ L	505 nm	End point	U/L	YES

Linearity	Accuracy	Repeatability	Corr. coefficient	Sensitivity	Test time	Test/hour
8000 U/L	+ - 5%	CV < 5%	R2>0,99	100 U/L	10 minutes	60

RESULTS AND DISCUSSION

The quantitative analysis of peroxide is an innovative test and therefore it is very difficult to find commercial samples to be used as standards or official reference methods.

CDR has compared the analysis of different milk samples done with Foodlab method with the values reported in the technical sheet of the Reflectoquant® reflectometric method.

** Reflectoquant® Peroxidase test in Milk - Reflectometric determination after conversion of a specific substrate*



The values have been compared and the results are good.

Values reported for tests done with Reflectoquant® :

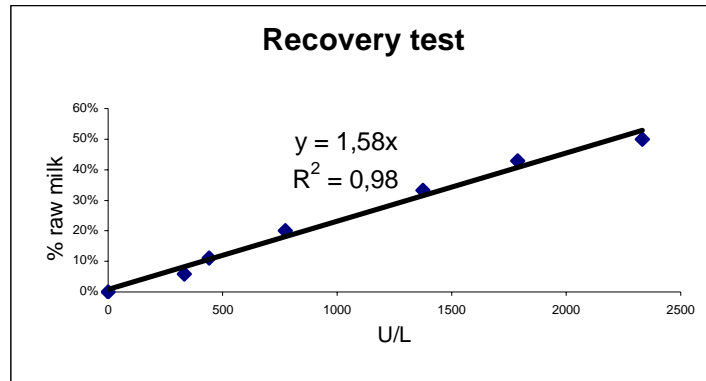
Sample	Reflectoquant® [U/L]
Raw milk	5900
Pasteurized milk A	155
Pasteurized milk B	555
Pasteurized milk C	1785
Pasteurized milk D	1948
Pasteurized milk E	2791
Pasteurized milk F	3118
Pasteurized milk G	3121

Values obtained with Foodlab method in CDR laboratory:

Sample	Foodlab Method [U/L]
Raw milk a	5686
Raw milk b	6107
Raw milk c	5913
Pasteurized milk 1	138
Pasteurized milk 2	656
Pasteurized milk 3	1442
Pasteurized milk 4	2387
Pasteurized milk 5	2691
Pasteurized milk 6	3948



In order to verify the specificity of the test it has been done some recovery tests, adding to milk without peroxidase, incremental quantities of raw milk. The results are very good.



Repeatability tests with Foodlab method have been done in CDR laboratories. The results are very good.

Milk sample	Foodlab method [U/L]
Test 1	2387
Test 2	2339
Test 3	2408
Test 4	2546
Test 5	2519
Mean	2440
SD	88,58
CV	3,6%



CONCLUSIONS

FOODLAB method for peroxide test on milk is very simple, quick and reliable. The quantitative method gives precise indications about the thermal treatment of milk and in addition is an indicator of milk quality: the higher is the peroxidase value, the lower is the importance of the alterations of the original characteristics that it has encountered.

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Peroxidase test in Milk - Reflectometric determination after conversion of a specific substrate. Reflectoquant®