



## ALKALINE PHOSPHATASE (ALP) IN MILK

### RAPID METHOD FOR THE DETERMINATION OF ALKALINE PHOSPHATASE IN MILK

#### INTRODUCTION

Alkaline Phosphatase (ALP) is an enzyme normally present in raw milk and it is inactivated when thermal treatment conditions are slightly higher than those required for the destruction of the pathogens bacteria.

Therefore detecting ALP value in pasteurized milk is useful to determine if the process have been done correctly. Pasteurized milk can maintain a maximum "contamination" value of raw milk of 0,1% that corresponds to 350-500 mU/L.

#### MATERIALS AND METHODS

The best substrate for the dosage of phosphatase alkaline is experimentally demonstrated, 4-Nitrophenyl phosphate. This compound is hydrolyzed by phosphatase giving as result a product of hydrolysis 4-Nitrophenol, that in alkaline environment develops a yellow colour.

FOODLAB innovative method simplifies the official method. It uses microquantities of sample as it is and a reagent pre-filled in single use cuvettes.

#### Materials:

- FOODLAB instrument
- Pre-filled single use cuvettes
- Bottle with starter reagent
- Micropipette to take 150  $\mu$ L

Using the specific pipette add 150  $\mu$ L of sample into the cuvette and, after 10 minutes of incubation in the specific cells, add 50  $\mu$ L of starter reagent. After 15 minutes of reaction, the instrument prints the result expressed as U/L of ALP.

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Sistema Qualità certificato ISO 9001 ed. 2000



Reagents	Sample Volume	Wavelength	Analysis type	Unit of measure	Calibration available
2 compounds	150 uL	405nm	End point	U/L	YES

Linearity	Accuracy	Repeatability	Correl. Coefficient	Sensitivã	Test time	Test/hour
5 U/L	+ - 10%	CV < 10%	R2 > 0,98	0,1 U/L	30 minutes	25

## RESULTS AND DISCUSSION

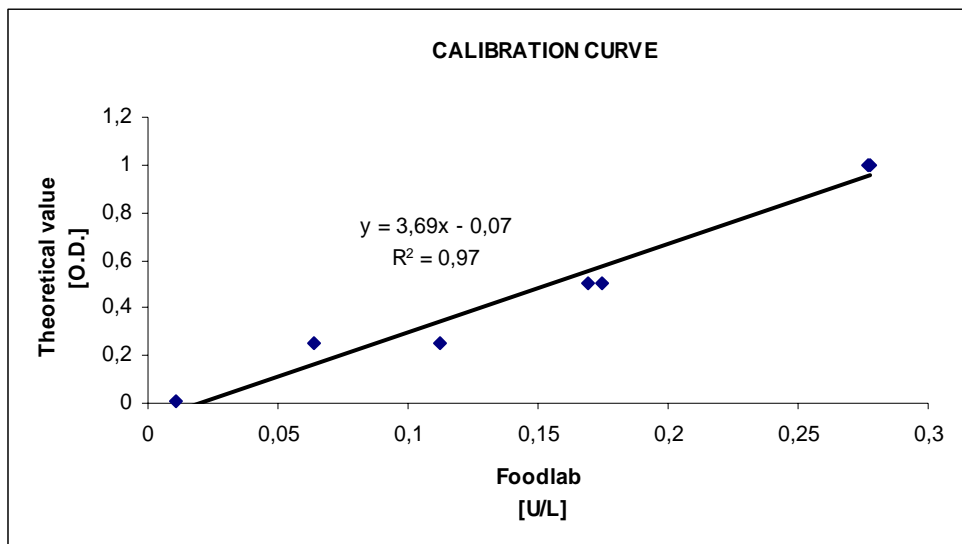
Calibration curve has been determined using additional quantities of raw milk to pasteurized milk. These values are in agreement with what is reported in literature ("*ALP testing for milk pasteurization*" Cornell University - Dairy science facts – 1998).

% of raw milk	THEORETICAL VALUE of the sample [U/L]
100% pasteurized milk	0,01
0,05% of raw milk	0,25
0,1% of raw milk	0,5
0,2% of raw milk	1



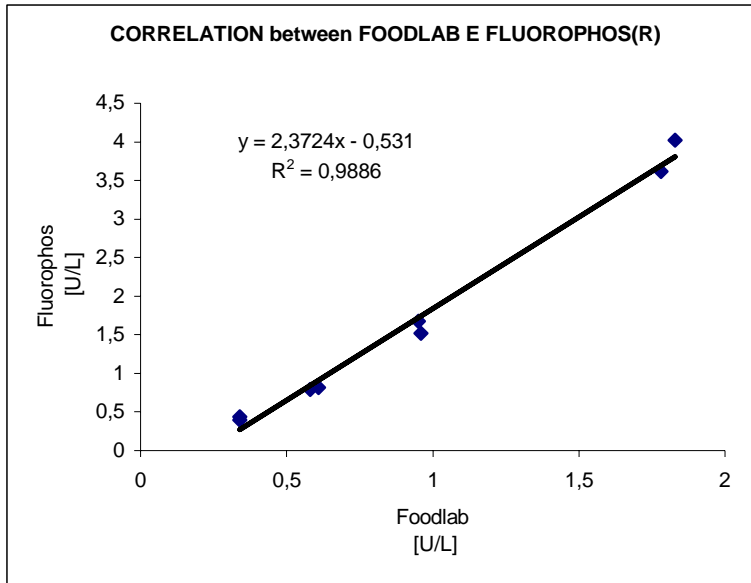
The calibration curve has been done using a double reading of the samples, and shows a very good coefficient of correlation.

Foodlab [O.D.]	Theoretical value [U/L]
0,011	0,01
0,064	0,25
0,112	0,25
0,169	0,5
0,175	0,5
0,278	1
0,277	1



After instrument calibration, some comparative tests, between FOODLAB e FLUOROPHOS® (Advanced Instruments), have been done.

The results show a good correlation between the 2 methods.



Some repeatability tests have been done in CDR laboratories.

Milk Sample	ALP [U/L]
Test 1	0,52
Test 2	0,58
Test 3	0,67
Test 4	0,55
Test 5	0,57
Test 6	0,55
Test 7	0,57
<b>Mean</b>	<b>0,57</b>
<b>SD</b>	<b>0,05</b>
<b>CV</b>	<b>8,6%</b>

## CONCLUSIONS

FOODLAB method for ALP analysis is very simple, quick and reliable: it is an ideal answer to the need of controlling "in-line" pasteurization process.



## **BIBLIOGRAPHY**

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