THERMAL INACTIVATION KINETICS OF ALKALINE PHOSPHATASE IN EQUINE RAW MILK

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INTRODUCTION

REVIVAL OF MARE'S MILK

- Mare's milk is not a new fad, but it is coming back into fashion.
- In the early 20th century it was so popular in Germany that it was delivered door to door.
- More recently it has enjoyed a revival in continental Europe, with equine dairy farms springing up in Belgium, France, the Netherlands and Norway.

INTRODUCTION

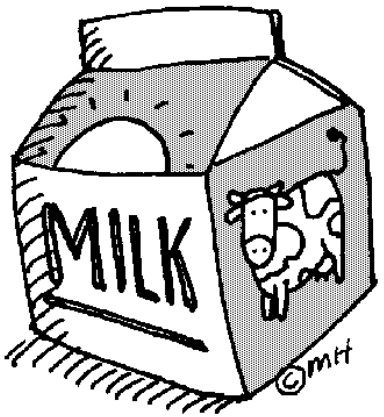
ALKALINE PHOSPHATASE (ALP):

- Enzyme present in the milk of all mammals
- Universal usage of ALP test as index of the efficiency of HTST pasteurisation

  (assessment of the thoroughness of heat treatments and/or the addition of raw milk to heated products)
- The most important milk enzyme from a dairy technology viewpoint

INTRODUCTION

HTST PASTEURISATION OF MILK:



- Mild and continous heating process
  (73-76°C during 15s)
- **Reduction of the number of bacterial pathogens in milk** to levels that do not consitute a risk to human health
- To obtain **safe** pasteurised products for human consumption, a **6D reduction of pathogens** is required

INTRODUCTION

EUROPEAN LEGISLATION:

- In order to have a safe pasteurised product, the residual level of ALP after pasteurisation should be **less** than **350 mU/L** in **BOVINE** milk

No information available on the usefulness of equine ALP as an indicator!!

OBJECTIVE

- Since equine milk is also a vector for pathogenic bacteria, a test for correct pasteurization or contamination with raw milk is of great significance to public health

- For milk of many species ALP is an adequate marker

- **Is this also the case in equine milk?**

SPECIES VARIABILITY in equine and bovine milk

Milk identification number	Breed	Number of animals	ALP activity[a]
EM1	Belgian 'Brabant' Draft horses		8077 ± 40
EM2	Haflinger horses		3371 ± 207
EM3	Haflinger horses		3517 ± 94
EM4	New Forest Pony		6604 ± 119
EM5	New Forest Pony		20 814 ± 119
EM6	Mix[b]		3121 ± 57
EM7	Mix[c]		6079 ± 46
EM8	Mix[d]		11 423 ± 172
BM1	Holstein cows		805 833 ± 8422
BM2	Holstein cows		1 050 267 ± 10 683
BM3	Holstein cows		740 433 ± 7366
BM4	Holstein cows		855 033 ± 3301
BM5	Mix[e]		764 633 ± 19 092

Raw equine milk contains ± 35-350 times less ALP activity than bovine milk

Distribution of alkaline phosphatase in raw equine and bovine milk

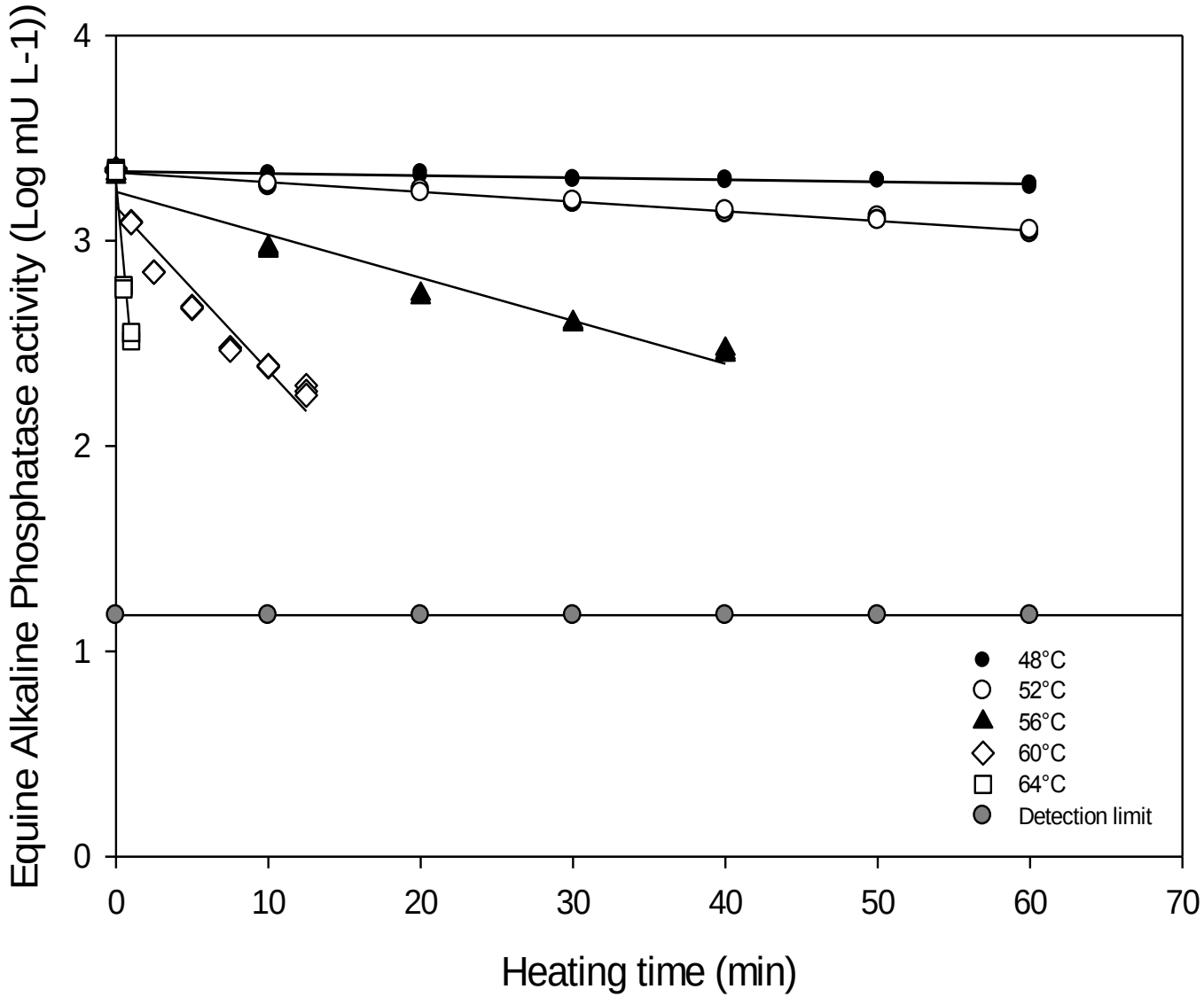
Milk/milk fraction	Total volume (mL)	Protein concentration (g 100 mL^{-1})	Lactose concentration (g 100 mL^{-1})	Fat concentration (g 100 mL^{-1})	ALP concentration (mU 100 mL^{-1})	% ALP	ALP/lactose ratio
Equine milk							
Whole milk	100	1.74	7.08	0.96	351.7	100	49.68
Fat-enriched fraction	19.90	1.16	7.04	3.72	320.7	18.15	45.59
Skim milk fraction	78.90	1.93	7.14	0.19	381.9	85.67	53.52
Bovine milk							
Whole milk	100	3.50	4.86	3.82	76 460	100	15 730
Fat-enriched fraction	6.30	1.61	3.02	50.00	449 500	37.04	149 000
Skim milk fraction	91.83	3.64	4.78	0.73	49 600	59.57	10 380

In **bovine milk,** ALP is partly associated with the **milk fat fraction** or with the **MFGM**, while in **equine** milk this association is negligible.

Heat inactivation of alkaline phosphatase in equine milk as a function of time and temperature

- Raw equine milk samples were heated in 100 µl glass capillaries in a water bath.
- Alkaline phosphatase activity was determined by the fluorimetric reference method (IDF, 2006).
- D-values (time required for 90% inactivation) were calculated by linear regression analysis.
- The Z-value (number of degrees Celsius needed for a 10 fold decrease in D-value) was calculated

Heat inactivation of equine alkaline phosphatase in raw equine milk at 5 different temperatures



Kinetic Parameters

EQUINE ALP	
D_{48}-value (min)	998
D_{52}-value (min)	211
D_{56}-value (min)	47.8
D_{60}-value (min)	7.8
D_{64}-value (min)	1.2
E_a-value (kJ mol^{-1})	155
z-value (°C)	5.31

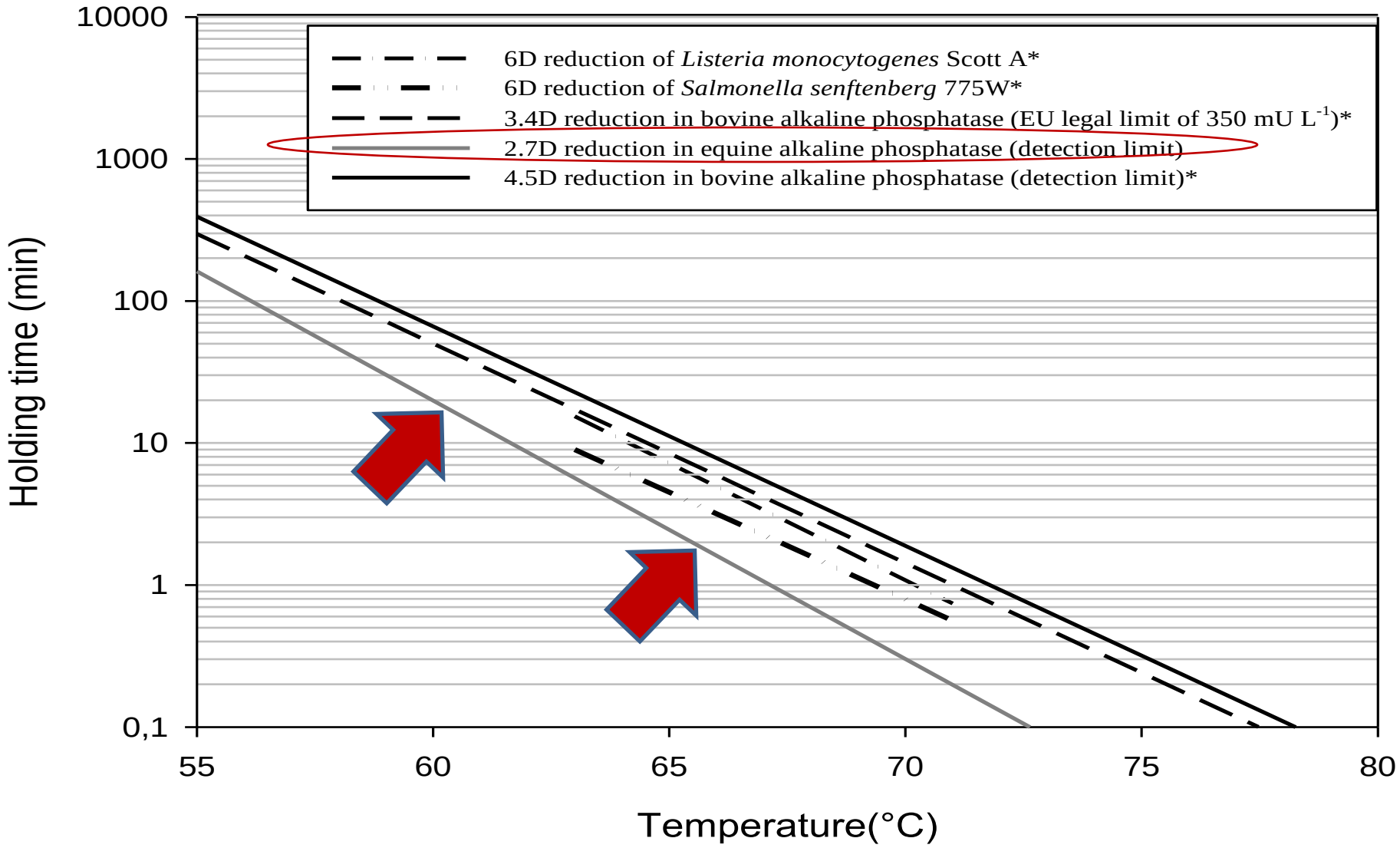
This Z-value is in the range of those reported in the literature for bovine ALP

Detection Limit

Raw milk contamination in heat-treated (phosphatase free) equine milk (%)	Average equine ALP activity (mU L^{-1})
100	2275 (± 149)
50	1082 (± 24)
20	431 (±16)
10	237 (± 14)
5	109,2 (± 4,3)
2	47,8 (± 4,1)
1	29,8 (± 1,3)
0,5	18,07 (± 3,07)
0,2	**15,9 (± 3,2)**
0.1	<10
0	<10

- **Equine** milk: ± 2200 mU/L → detection limit 0,2%: **2.7** decimal reduction in ALP
- Bovine milk: ± 1000000 mU/L → detection limit 0.0035%: **4.5** decimal reduction in ALP

Evaluation of the suitability of equine ALP as indicator for proper pasteurisation in equine milk



Conclusions

- Equine alkaline phosphatase is **not suitable** as indicator for proper pasteurization of equine milk
- **Small (< 0.2%) contaminations of raw milk** will **not be detected** in the pasteurized product
- This is due to the **low intrinsic equine ALP levels** and due to the subsequent detection **limitations of the method**

Thank you for your attention!

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