The Lactoperoxidase System – a natural antimicrobial system

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Hansen (1924) First report of peroxidase activity in milk

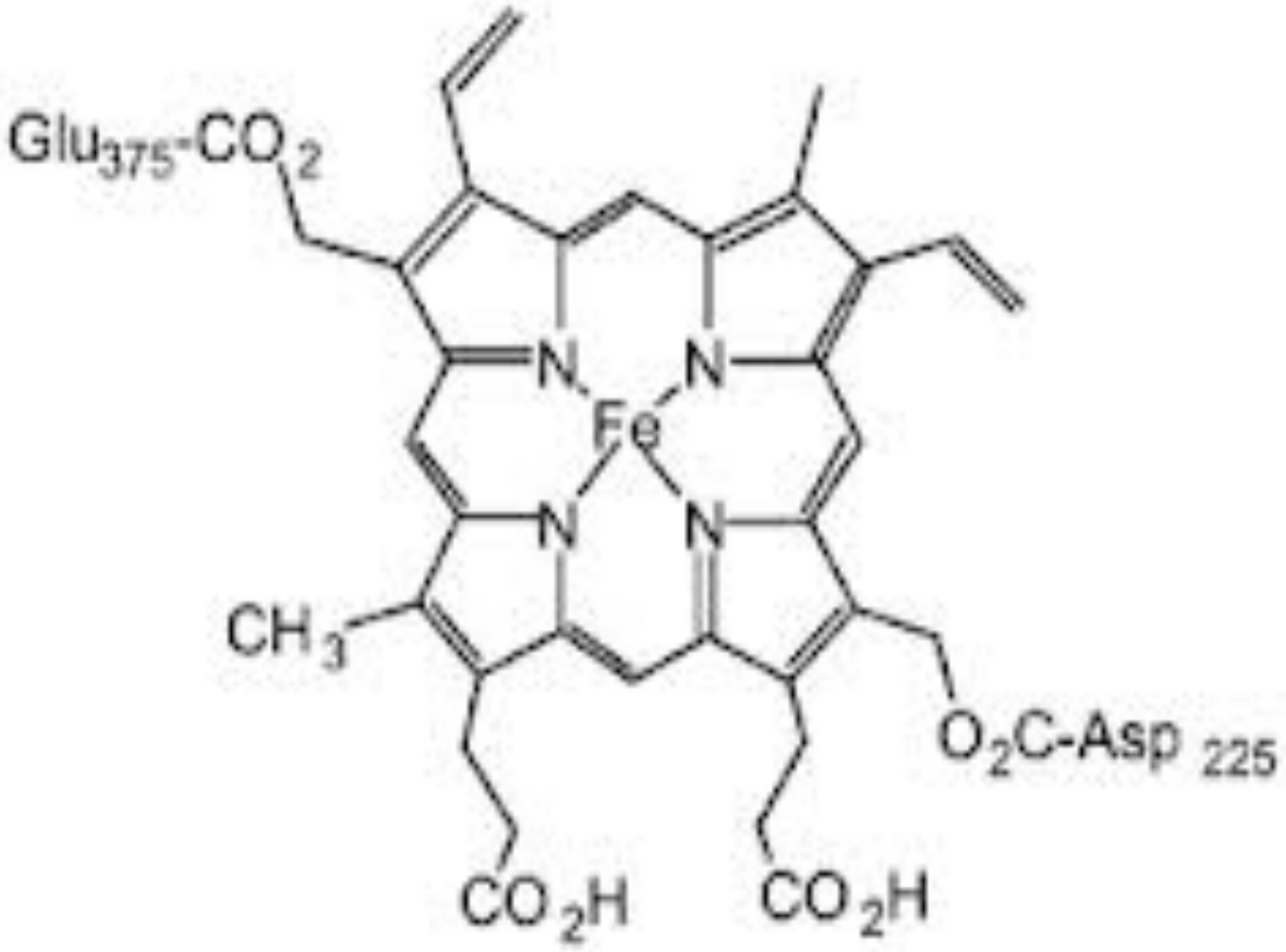
Realisation of potential benefits of system in bovine milk - studies in 1970s – e.g. Reiter, Marshall, Björck

The Lactoperoxidase system – 3 components:

- Lactoperoxidase enzyme
- An electron donor (thiocyanate SCN^- ; Halides I^-, Br^- but not Cl^-)
- A source of H_2O_2 (added H_2O_2 ; Catalase negative organisms – e.g. starter bacteria; Addition of sodium percarbonate; enzymic methods – e.g. glucose oxidase; presence of leucocytes)

Properties of lactoperoxidase

- MWt – approx 78 k
- 10 % carbohydrate
- Contains haem group with 1 atom of iron per molecule
- IEP 9.2-9.9 (NB most whey proteins on acid side of neutral)


Glu375-CO2
N
N
Fe
N
N
CH3
O2C-Asp 225
CO2H
CO2H

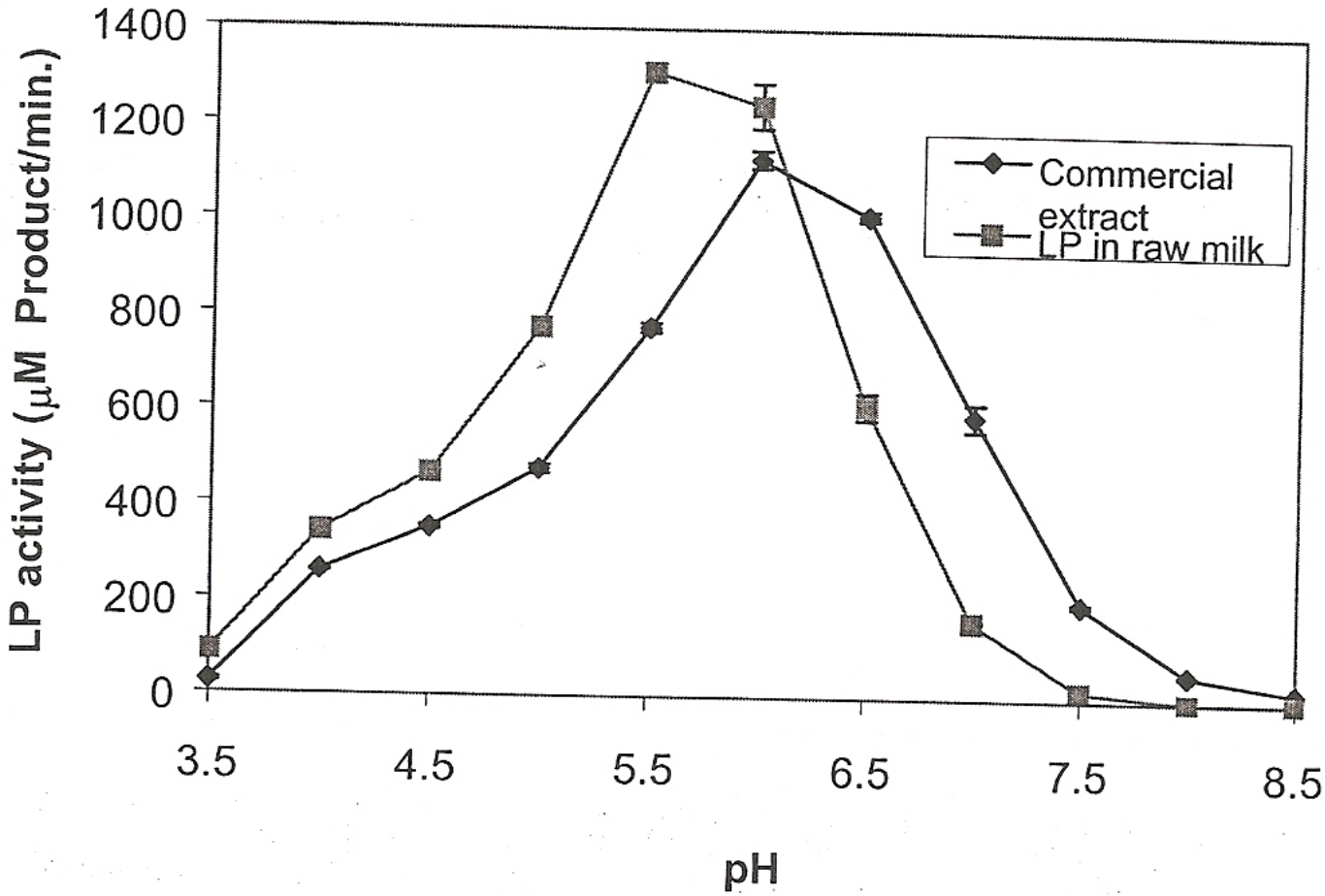
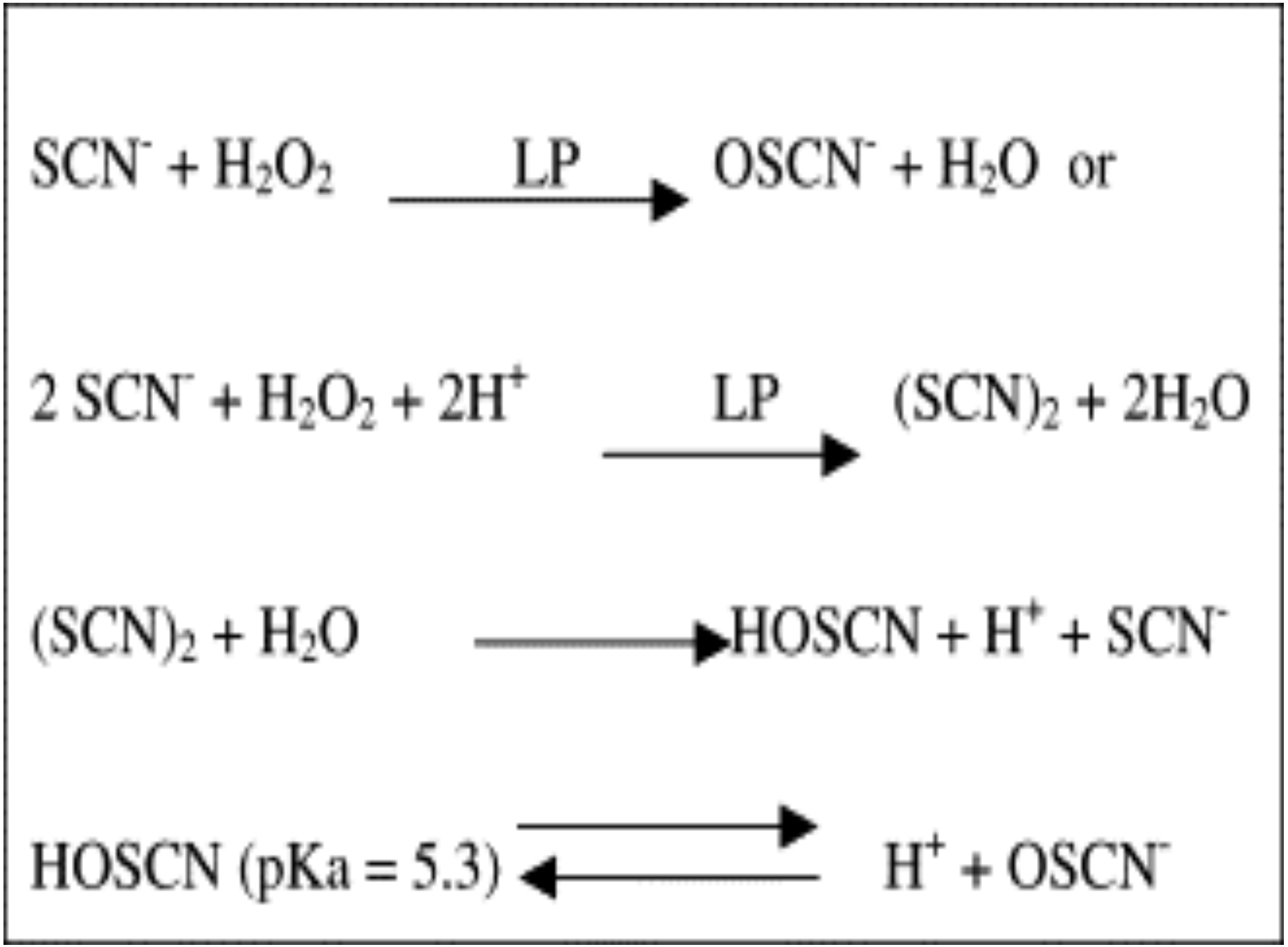


Figure 5.3. pH profile of LP activity in raw milk and in the commercial extract. Error bars represent standard deviations (n = 6).

Occurrence of lactoperoxidase

- Normal component of bovine and human milk and has been detected in milks from all species tested (1% of whey protein i.e. 10-30 µg/ ml milk in bovine milk).

- Lactoperoxidase (or similar peroxide activity) also occurs in saliva, cervical mucus, mucus from lungs, sweat, tears and nasal glands
- Peroxidases are synthesized in exocrine glands and secreted onto mucosal surfaces

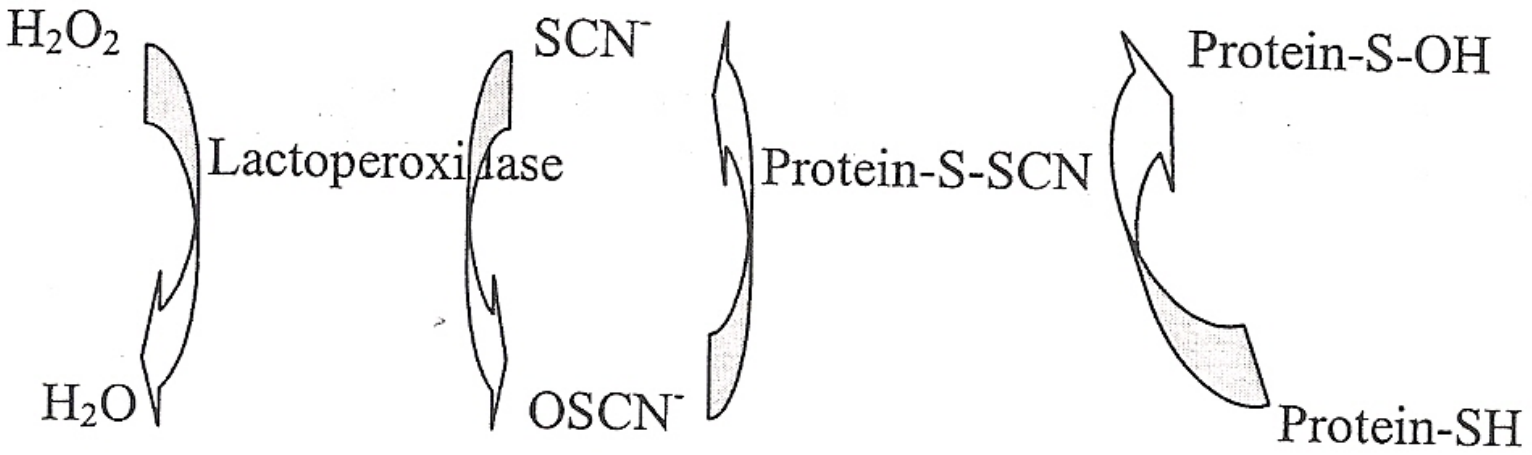

SCN- + H2O2 LP OSCN- + H2O or
2 SCN- + H2O2 + 2H+ LP (SCN)2 + 2H2O
(SCN)2 + H2O HOSCN + H+ + SCN-
HOSCN (pKa = 5.3) H+ + OSCN-

Anti-bacterial agents

- Hypothiocyanite $OSCN^-$
- Hypoiodite OI^-
- Hypobromite OBr^-

Effects of hypothiocyanite ($OSCN^-$) or hypoiodite (OI^-) on bacteria

- Oxidation of SH groups in cytoplasmic membrane proteins
- Loss of ability to transport glucose
- Leaking of K^+, amino acids, peptides etc


H_2O_2
H_2O
Lactoperoxidase
SCN^-
$OSCN^-$
Protein-S-SCN
Protein-S-OH
Protein-SH

Effects on micro-organisms

- Bacteriostatic but not lethal against Gram-positive organisms
- Bacteriocidal against many Gram-negative organisms
- Some reports of activity against moulds, yeasts, mycoplasma, protozoa and viruses

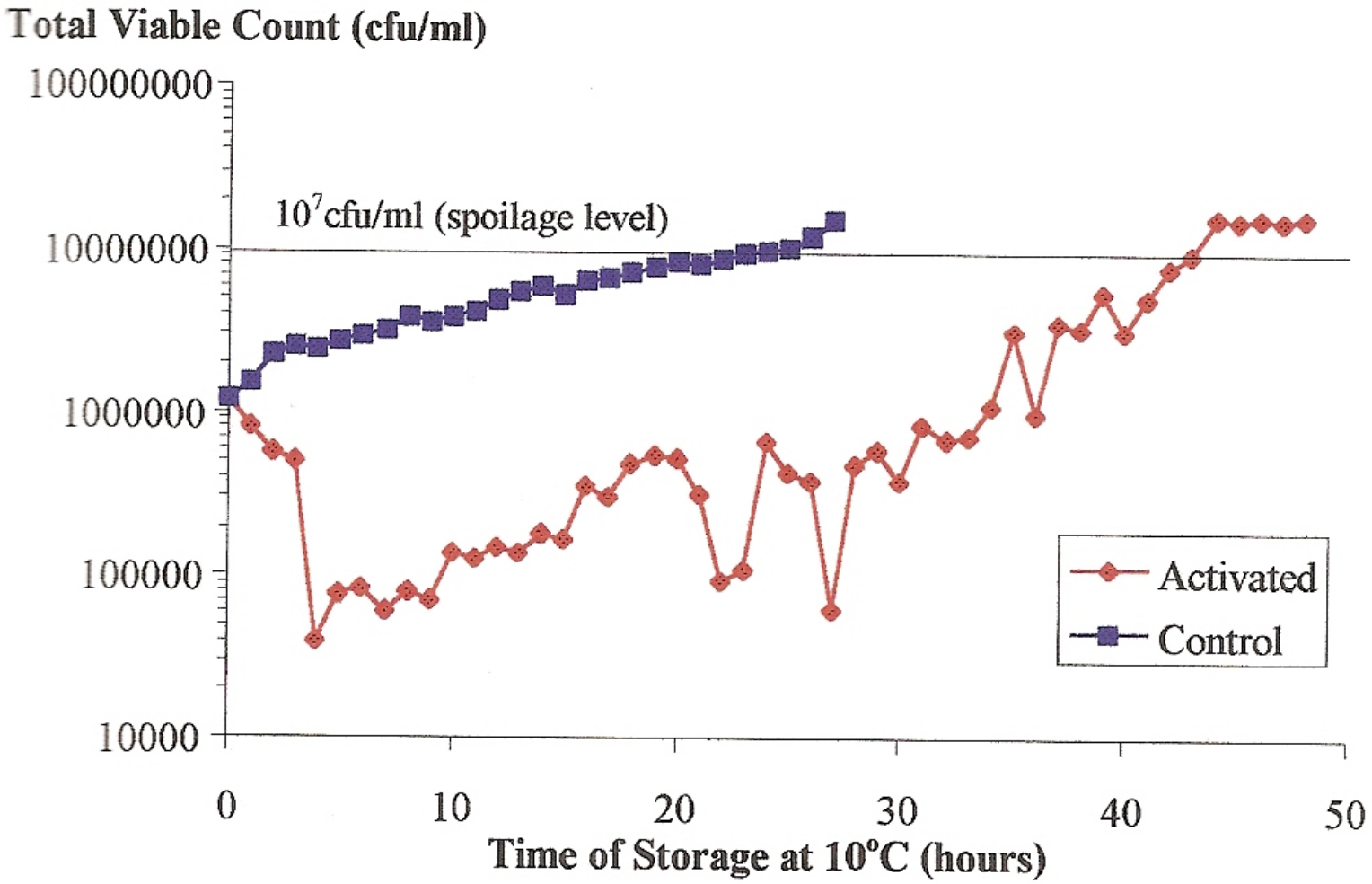
Biological significance of lactoperoxidase

- Protection of mammary gland against infection
- Protection of nursing calf against infection
- Limit the accumulation of toxic levels of peroxide in the udder

Activation of LPS

- Fresh milk contains small amounts of SCN^- and virtually zero H_2O_2 - LPS is inactive
- Addition of small quantities of SCN^- (or halide) followed by source of H_2O_2 kicks the system into action

Figure 3.1 Variation in Total Viable Count over 48 Hours between Control and LPS-Activated Raw Milks



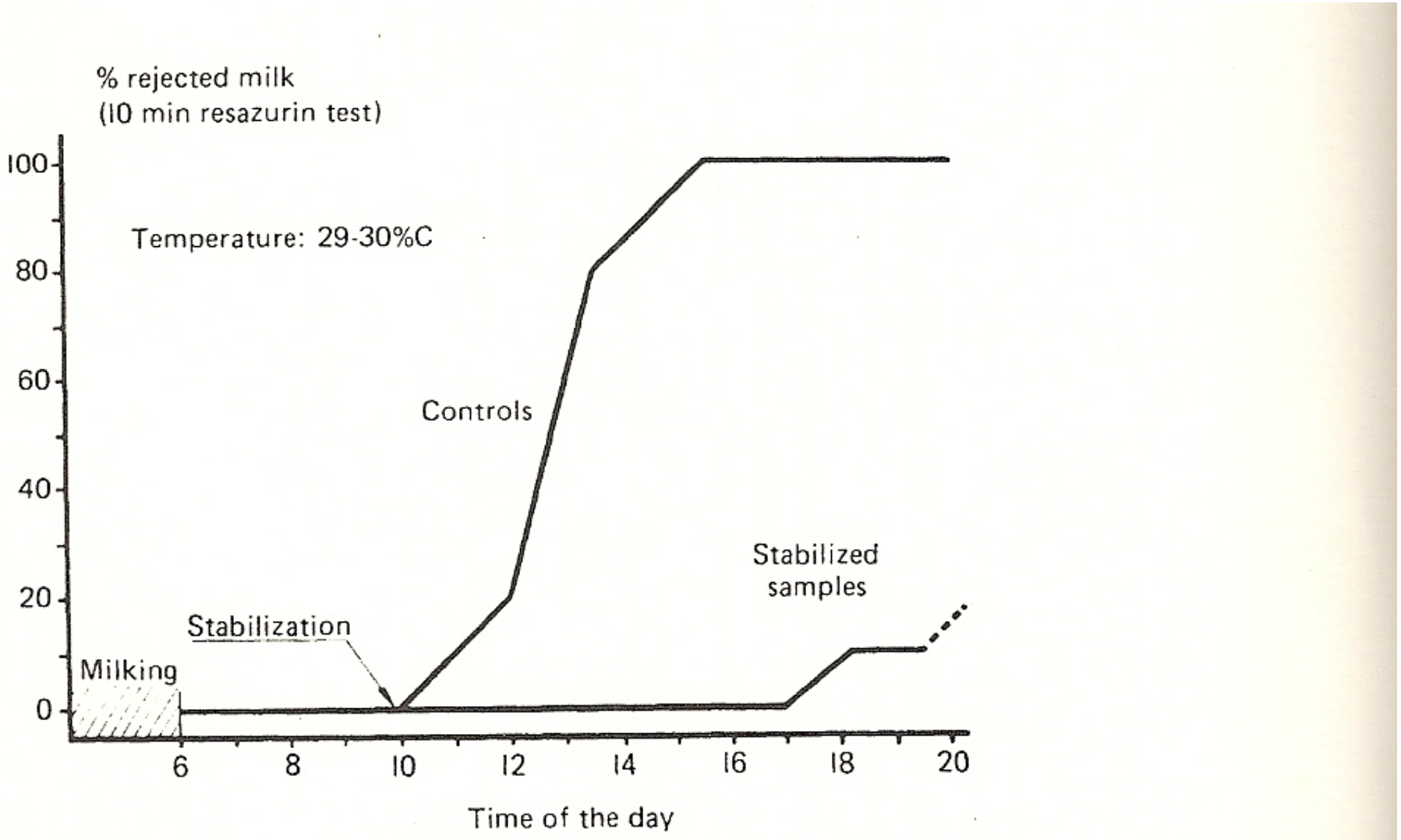
Effects of temperature on shelf-life of raw milk (based on titratable acidity 0.18%)

a– raw milk

b– 10 ppm SCN^- ; 80 ppm H_2O_2 : c - 10 ppm SCN^- ; 80 ppm percarbonate

d– 10 ppm I^- ; 80 ppm H_2O_2 : e – 10 ppm I^- ; 80 ppm percarbonate

	Shelf life (hours)										
	Experiment 1						Experiment 2				
	a	b	c	d	e		a	b	c	d	e
4°C	192	384	300	360	444		264	432	480	576	768
19°C	25	40	35	55	76		28	48	48	75	100
30°C	18	25	21	43	50		7	24	22	31	50


% rejected milk
(10 min resazurin test)
100
80
60
40
20
0
Temperature: 29-30%C
Controls
Stabilized
samples
Stabilization
Milking
6
8
10
12
14
16
18
20
Time of the day

Lactoperoxidase activation kits

Sachets containing:

Na or K Thiocyanate – 14 mg/Litre

Followed by

Sodium percarbonate – 10 mg/Litre

Cost US$ 0.0025-0.01 per litre of milk


STABILAK
Activador del sistema lactoperoxidasa, forma natural de defensa de la leche cruda en refrigeración
D-50 L
STABILAK
2 STABILAK POLVO
1 STABILAK POLVO

MANUAL ON THE USE OF THE LP-SYSTEM IN MILK HANDLING AND PRESERVATION

Potential problems

- No negative organoleptic problems
- No nutritional contraindications
- Clear potential for inhibition of starter bacteria but problems very limited in practice

Legal position

- Codex adopted “Guidelines for the preservation of milk using the lactoperoxidase system” in 1991 to facilitate application in situations where technical, economic or practical reasons do not allow cooling facilities
- Emphasised that system should not be used for products intended for international trade
- FAO/WHO recommendation to reverse the latter rejected in 2006/7

Isolation of lactoperoxidase

- From acid or sweet whey
- Cation exchange – IEP 9.2-9.7

1

eBay Buyer Protection

ation

outh Fluoride Toothpaste,Fresh Mint Original,4.5oz

New

25 days 7 hours (28 Oct, 2012 18:07:19 GMT)

More than 10 available / 12

£10.35

Buy It Now
Buy It Now

Postage: £1.50 Read item description or contact seller for details.

Returns:
Returns Accepted

Shop with confidence. Learn More


C.E.T.
Enzymatic
Toothpaste
for Dogs & Cats
virbac


12 pack
biotène
Dry Mouth Gum


BreathRx
introductory kit

Biovert

Description: Natural Protection System

Benefits: Safe, very mild and effective alternative to traditional preservative systems

INCI Name: Glucose (&) Glucose Oxidase (&) Lactoperoxidase

Manufacturer: Lonza

BioVert-

Natural preservative base which consists of the following listed ingredients- **used in all except PHA**.

Glucose- a monosaccharide or simple sugar. In our products it is a component of the natural preservative BioVert. It is the substrate for the BioVert Enzyme whicis is glucose oxidase and lactoperoxidase.

Lactoperoxidase- Enzyme extracted from premium quality bovine milk. When combined with glucose oxidase it forms the preservative enzyme in BioVert which works with oxygen to prevent microbial activity.

Glucose Oxidase- is found in honey and acts as a natural preservative when combined with lactoperoxidase. a non-irritant active ingredient with anti-macrobial activities. It promotes normalization of skin sebum production. Excellent for acneic skin conditions; significantly reduces blackheads.


BURT'S BEES
BURT'S BEES®
Earth Friendly Natural Personal Care for The Greater Good™
BURT'S BEES
Peppermint
FOOT
LOTION
BURT'S BEES
Naturally
Nourishing
BURT'S BEES
Radiance
BURT'S BEES
BURT'S BEES
Baby Bee
Tear Free


SIMPLY
ORGANIC
EVERYDAY
BODY LOTION


LipoCeutical
nanosome technology
VITISES
GEL
REGULATING GEL FOR CUTANEOUS DEPIGMENTATION
GEL REGULADOR DE LA DEPIGMENTACIÓN CUTÁNEA
3.4 Fl. oz
LipoCeutical
nanosome technology
VITISES
GEL
REGULATING GEL FOR CUTANEOUS DEPIGMENTATION
GEL REGULADOR DE LA DEPIGMENTACIÓN CUTÁNEA
Pseudocatalase / Lactoferrin - Lactoperoxidase SOD / EDTA
3.4 Fl. oz


episilk
instant facelift
serum
with hyaluronic acid
episilk
instant facelift
serum

Pampered Prince


ZYMOX
OTIC
Enzymatic Solution
For Veterinary Use Only
ZYMOX
OTIC
Enzymatic Solution
For Veterinary Use Only


Nature's Cure
Two-Part
Body Acne Treatment


Clearasil
ULTRA
ACNE SOLUTION SYSTEM
4

Addition to calf or piglet milk replacers

Potential medical applications:

- **Wound healing**
- **Tumour therapy**
- **Anti-viral agent**

Raw milk stored for different periods before pasteurisation

Keeping quality assessed

Milk stored for 3-4 days before pasteurisation has longer KQ (post pasteurisation) than milk pasteurised immediately

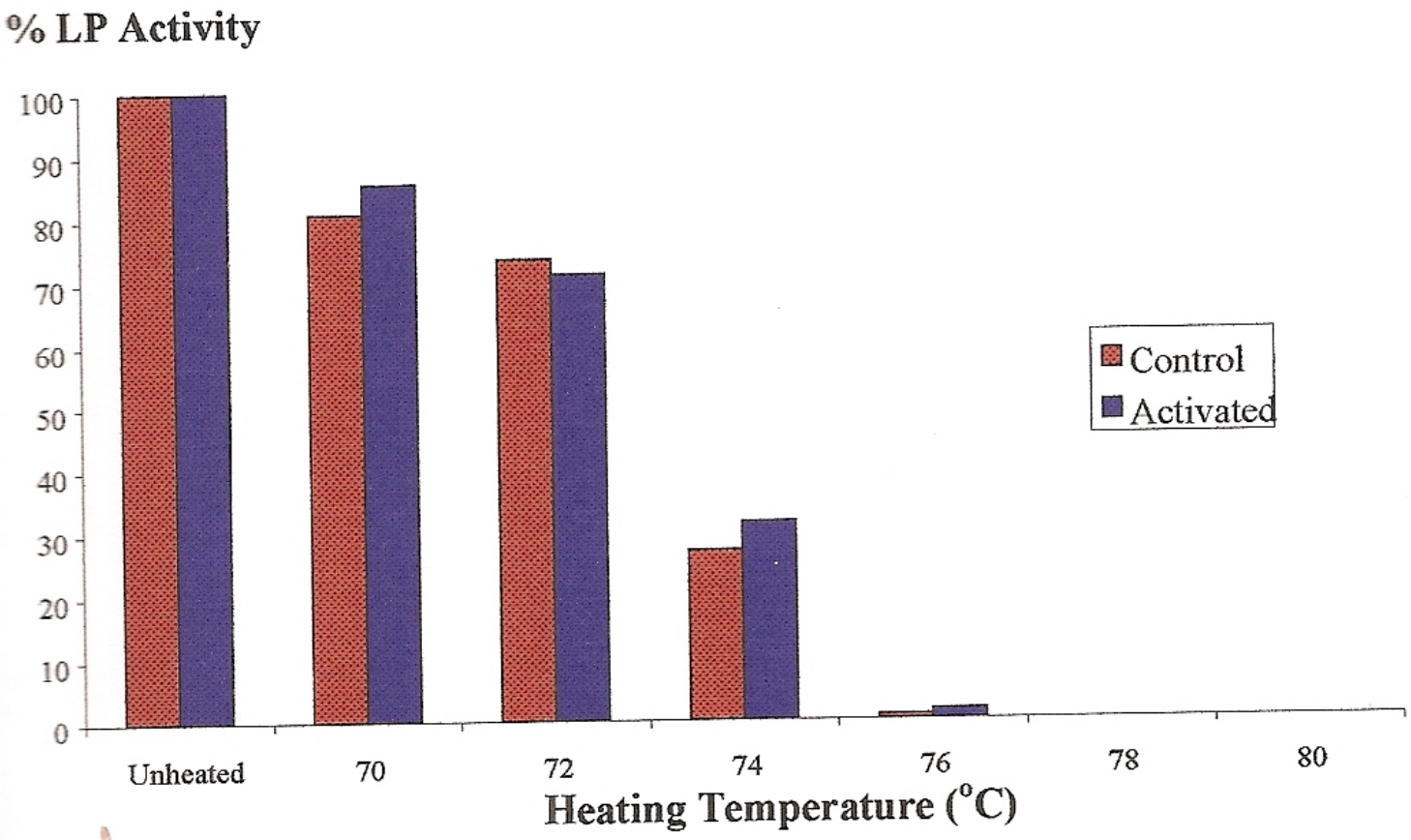
Counterintuitive?
LPS sytem??

Work involving Mike Lewis plus others including Florence Fonteh, Leonard Fweja, Nicky Barrett

Thermal inactivation

- No measurable loss in activity below 65^0 C
- 70% of LP activity remains after standard HTST pasteurisation (72^0 C for 15 s)
- Virtually zero activity remains after heating to 80^0 C

Figure 4.9 Average % Heat Inactivation of LP in a Continuous Pasteuriser



Does LP system contribute to keeping quality of pasteurised milk?

- Challenge test the system in pasteurised milk against

  - *Pseudomonas aeruginosa* & *Staphylococcus aureus* (post pasteurisation contamination)

  - *Streptococcus thermophilus*

Tests used to measure the keeping quality of milk processed at 72°C and 80°C for 15 s.

Processing conditions	**72°C/15 s**	**80°C/15 s**
Tests used	**Keeping quality (days at 8°C)**	
Unfresh aroma	17 (20)	14 (17)
Clot on Boiling	19 (22)	19 (21)
Stability in 68% alcohol	17 (20)	15 (18)
TVC>10^7	13 (15)	13 (16)
Lactic acid increase >0.04%	16 (20)	15 (18)
pH to fall below 6.5	15 (18)	13 (16)
Dip in dissolved oxygen level	14 (17)	13 (15)

( ) repeat experiment

UHT Milk (sterile/no LP)

↓

Control or added LP enzyme

↓

Pasteurise at either 72 or 80^0 C/15 s

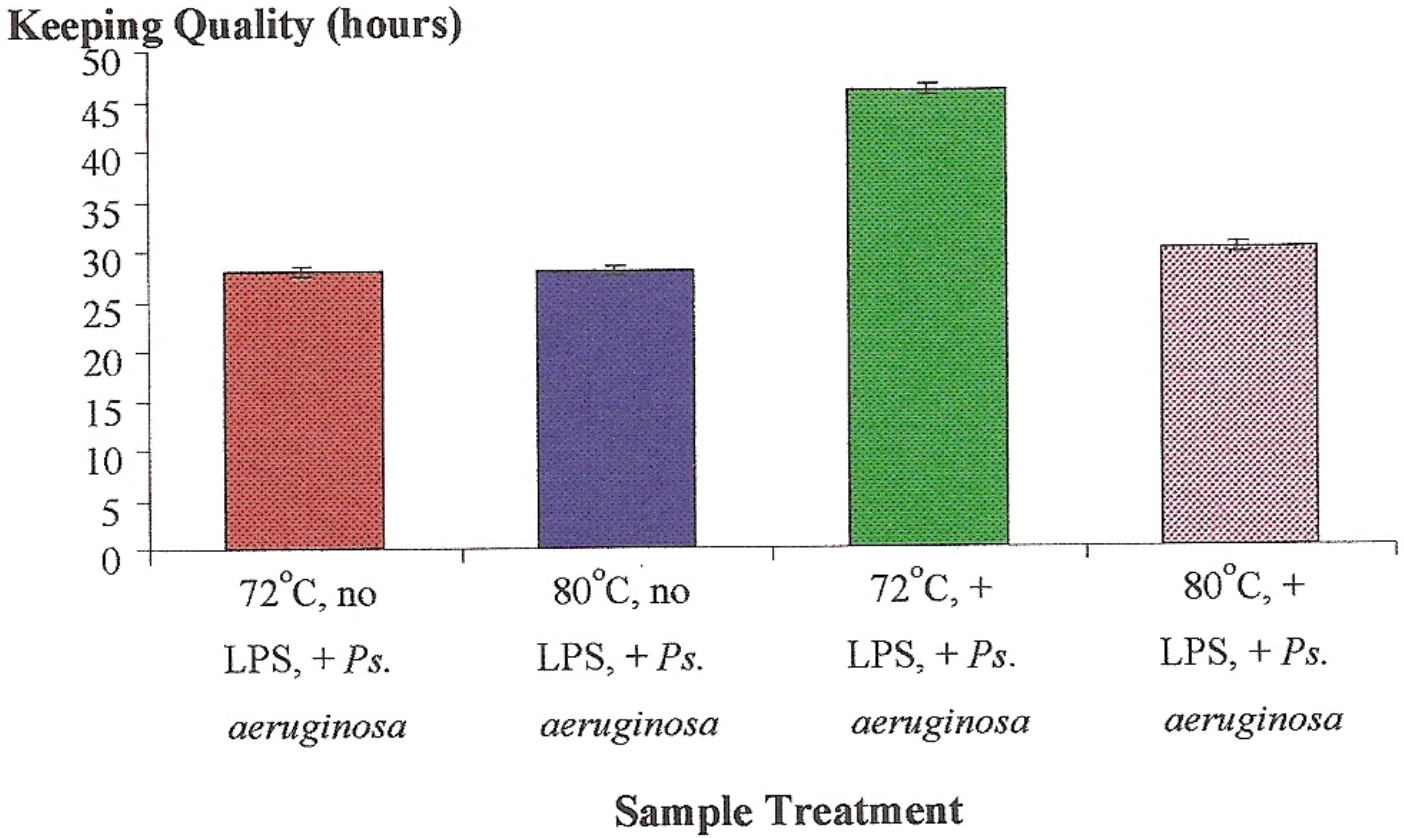
↓

Add SCN^-/H_2O_2

↓

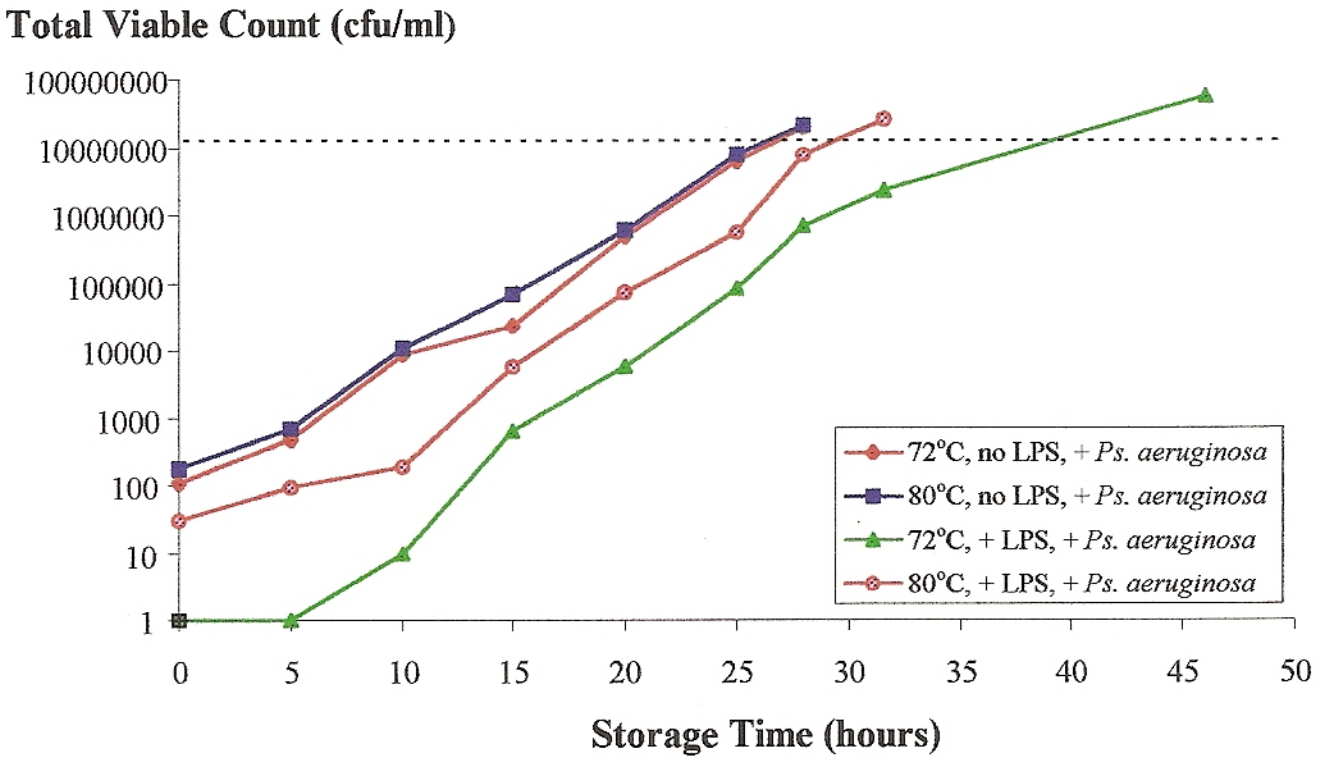
Add PPC spoilage organisms

Figure 5.2 Keeping Quality of Milks stored at 25°C, Challenged with *Pseudomonas aeruginosa*



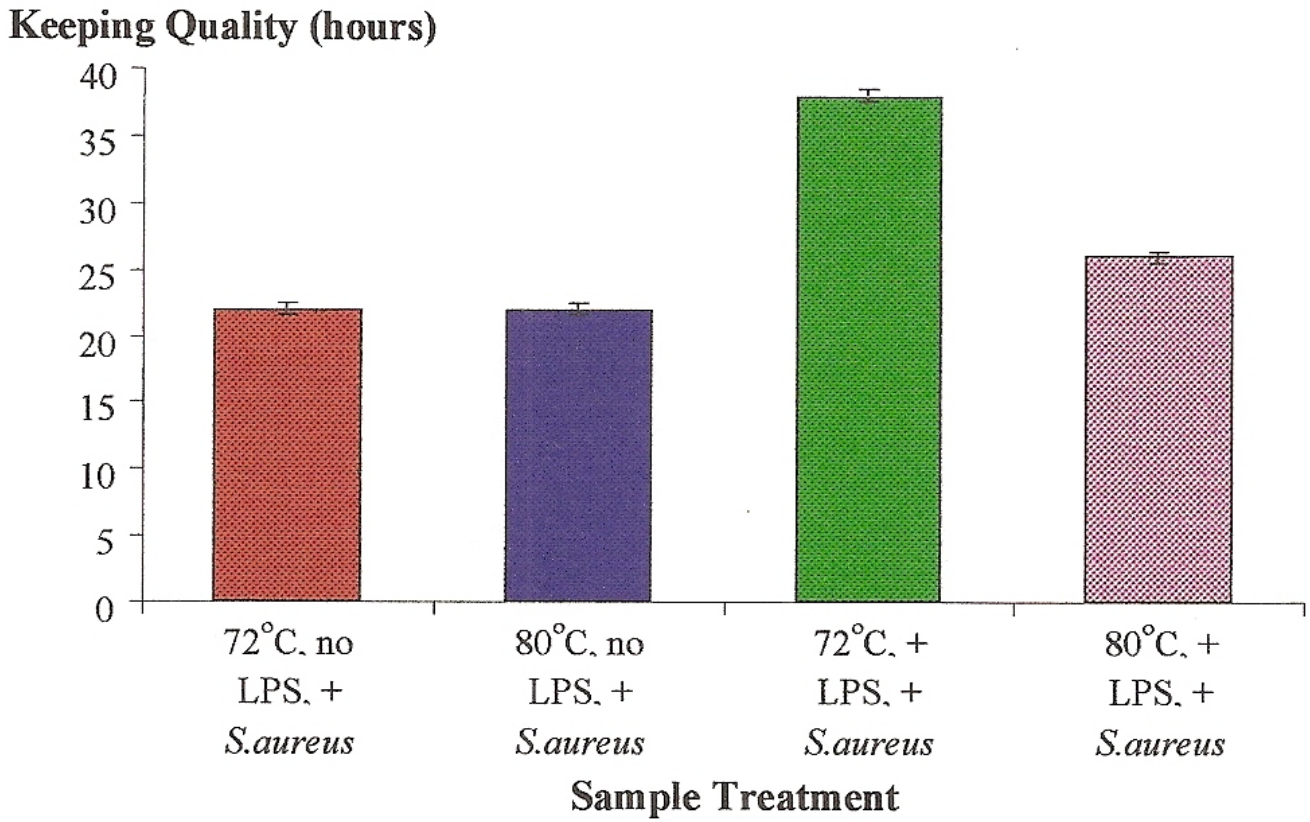
(Keeping Quality measured by titratable acidity and alcohol stability as described in section 5.2.1.3)

Figure 5.3 Total Viable Count of Milks stored at 25°C, Challenged with *Pseudomonas aeruginosa*



(Total Viable Count carried out on Plate Count Agar as described in section 5.2.1.4)

Figure 5.4 Keeping Quality of Milks stored at 37°C, Challenged with *Staphylococcus aureus*



(Keeping Quality was determined by titratable acidity and alcohol stability as outlined in section 5.2.1.3)

UHT Milk (sterile/no LP)

↓

Control or added LP enzyme

↓

Add thermophilic bacteria

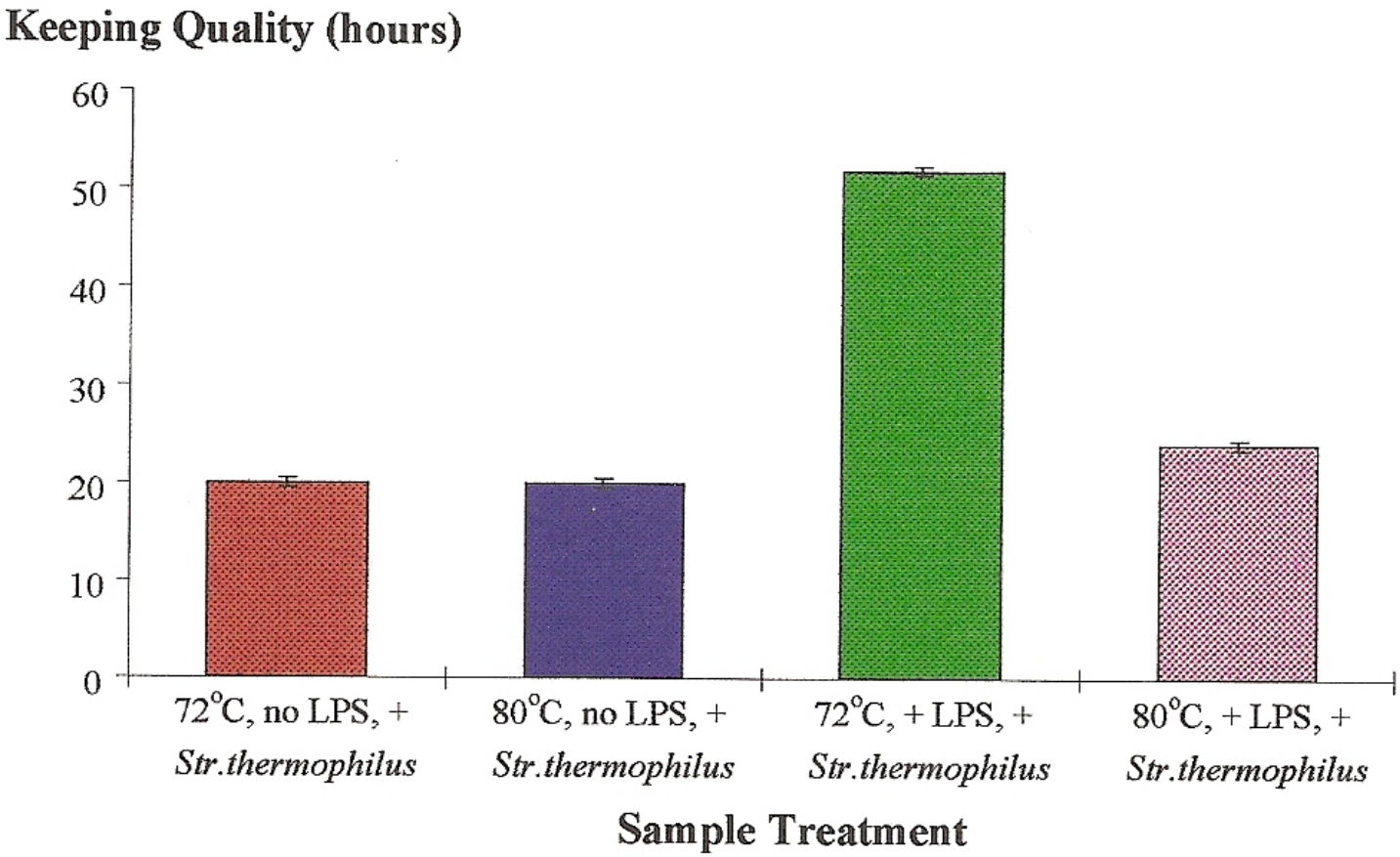
↓

Pasteurise at either 72 or 80^0 C/15 s

↓

Add SCN^-/H_2O_2

Figure 5.7 Keeping Quality of Milks stored at 37°C, Challenged with *Streptococcus thermophilus*



(Keeping Quality determined by titratable acidity and alcohol stability as outlined in section 5.2.1.3)

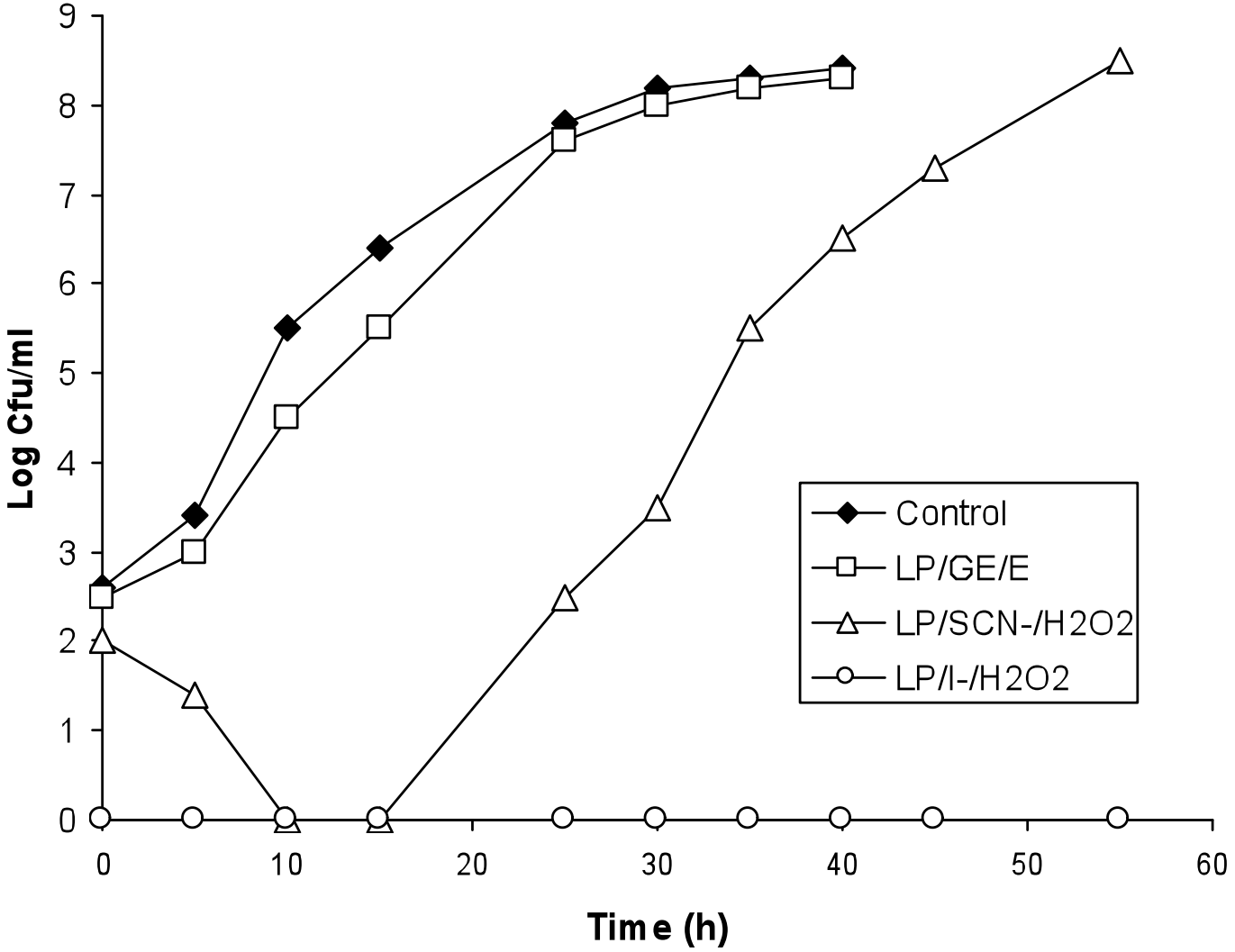
Conclusion

- LPS may well contribute to keeping quality of pasteurised milk
- LP activity may be the real reason why milk pasteurised at 72^0 C has better keeping quality than 80^0 C
- Recommendations to increase pasteurisation conditions to improve safety may not be appropriate!

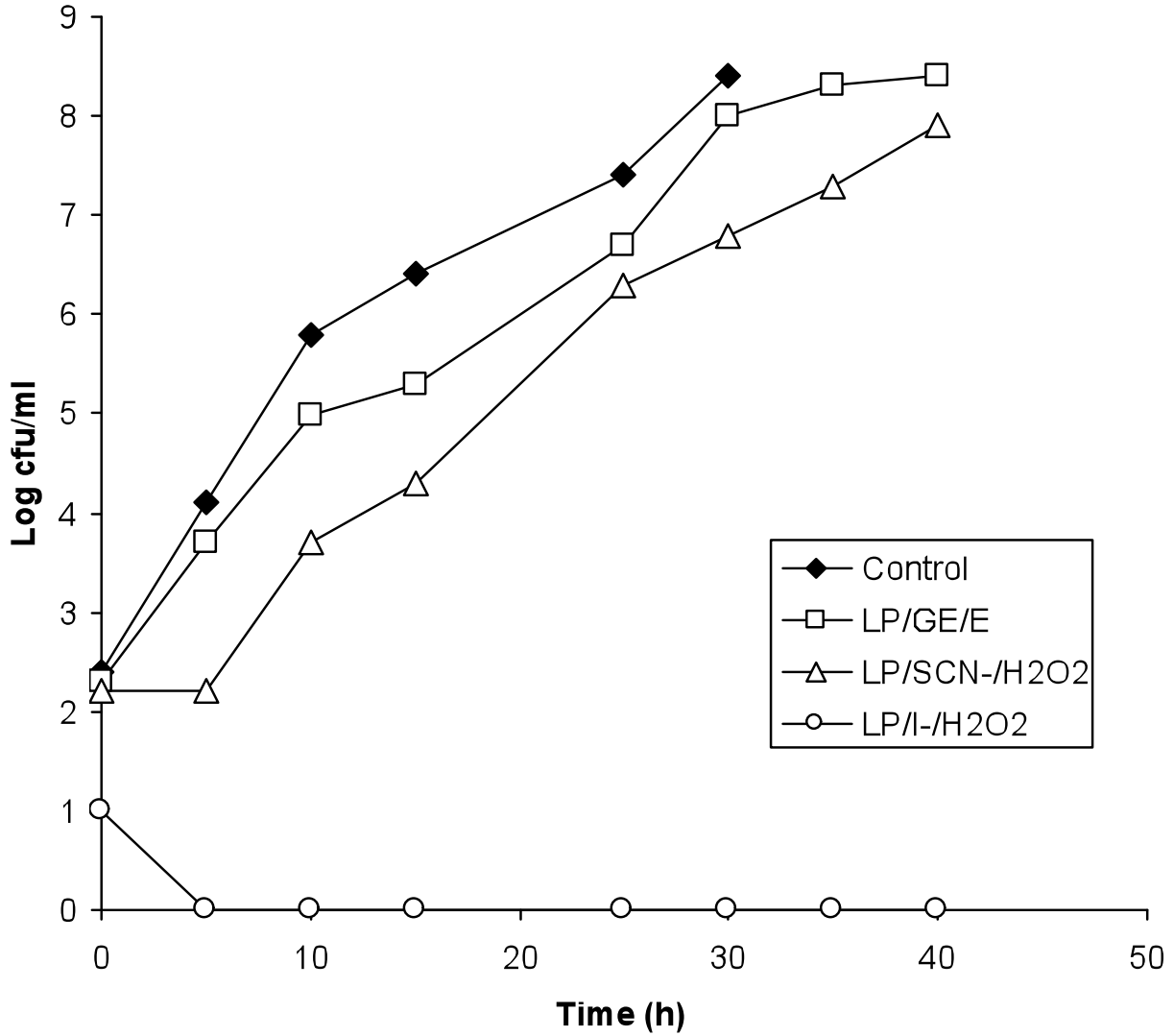
What is the best method of activating the system?

- UHT milk inoculated with pure cultures of test bacteria
- 30 mg/L purified lactoperoxidase and different LPS activators (thiocyanate, Iodine, garlic extract!) added and incubated at optimum growth temperature
- Keeping quality and growth pattern monitored over time

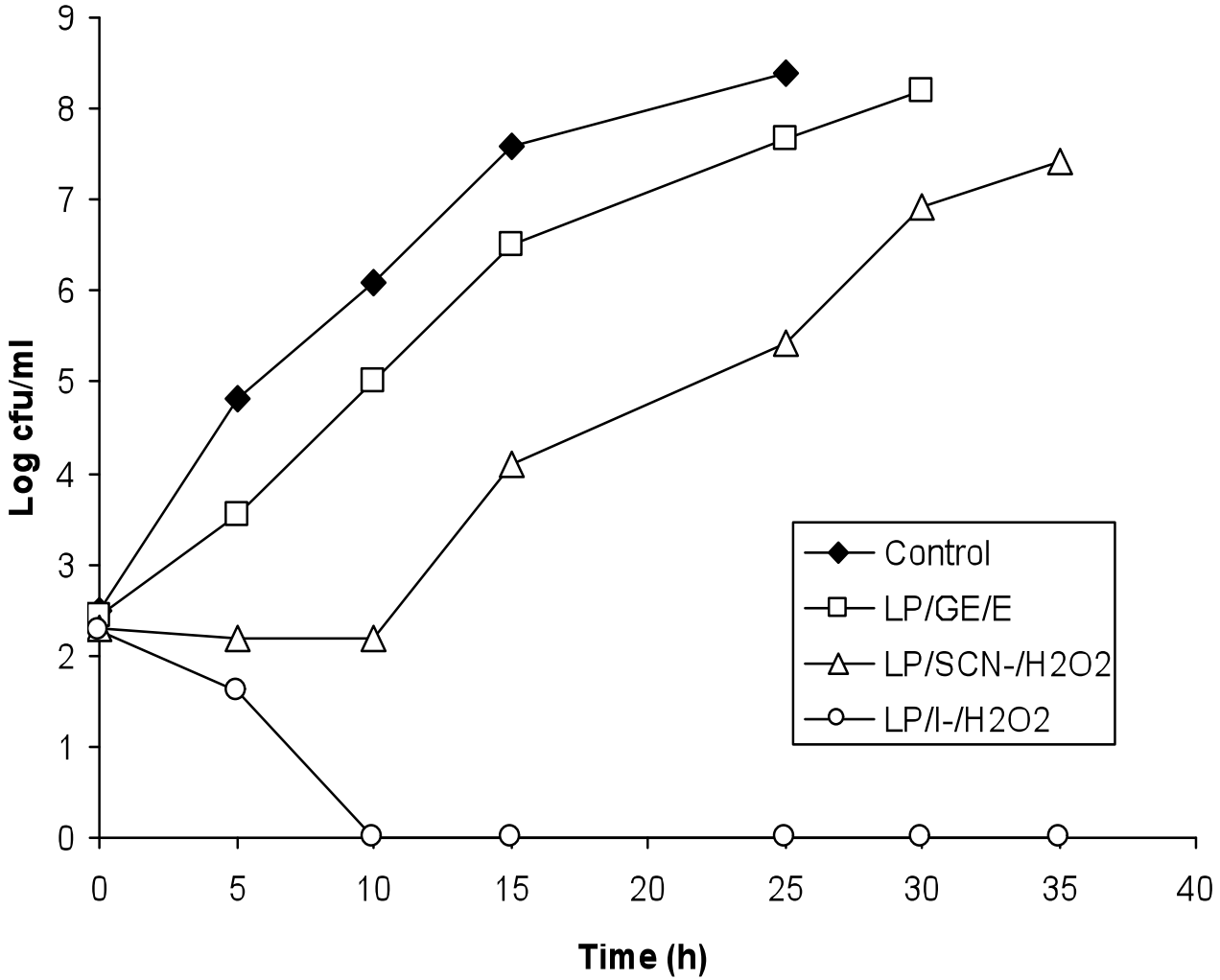
The antimicrobial effects of the different lactoperoxidase systems (LP+I^-+H_2O_2 , LP +SCN^-+H_2O_2 and LP+GE+E) against *Pseudomonas aeruginosa* in UHT milk



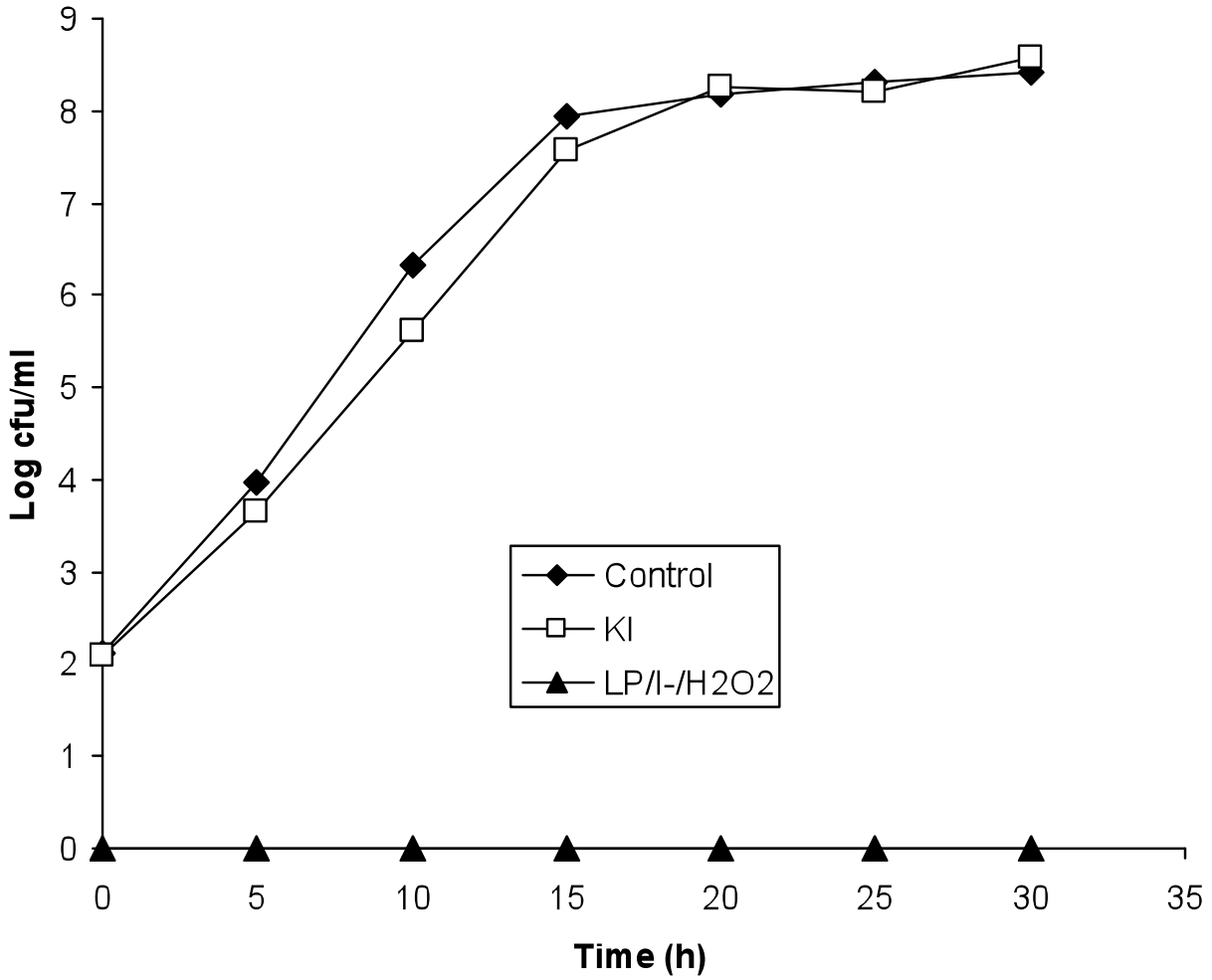
The antimicrobial effects of the different lactoperoxidase systems (LP+I-+H_2O_2, LP +SCN-+H_2O_2 and LP+GE+E) against *Staphylococcus aureus* in whole UHT milk



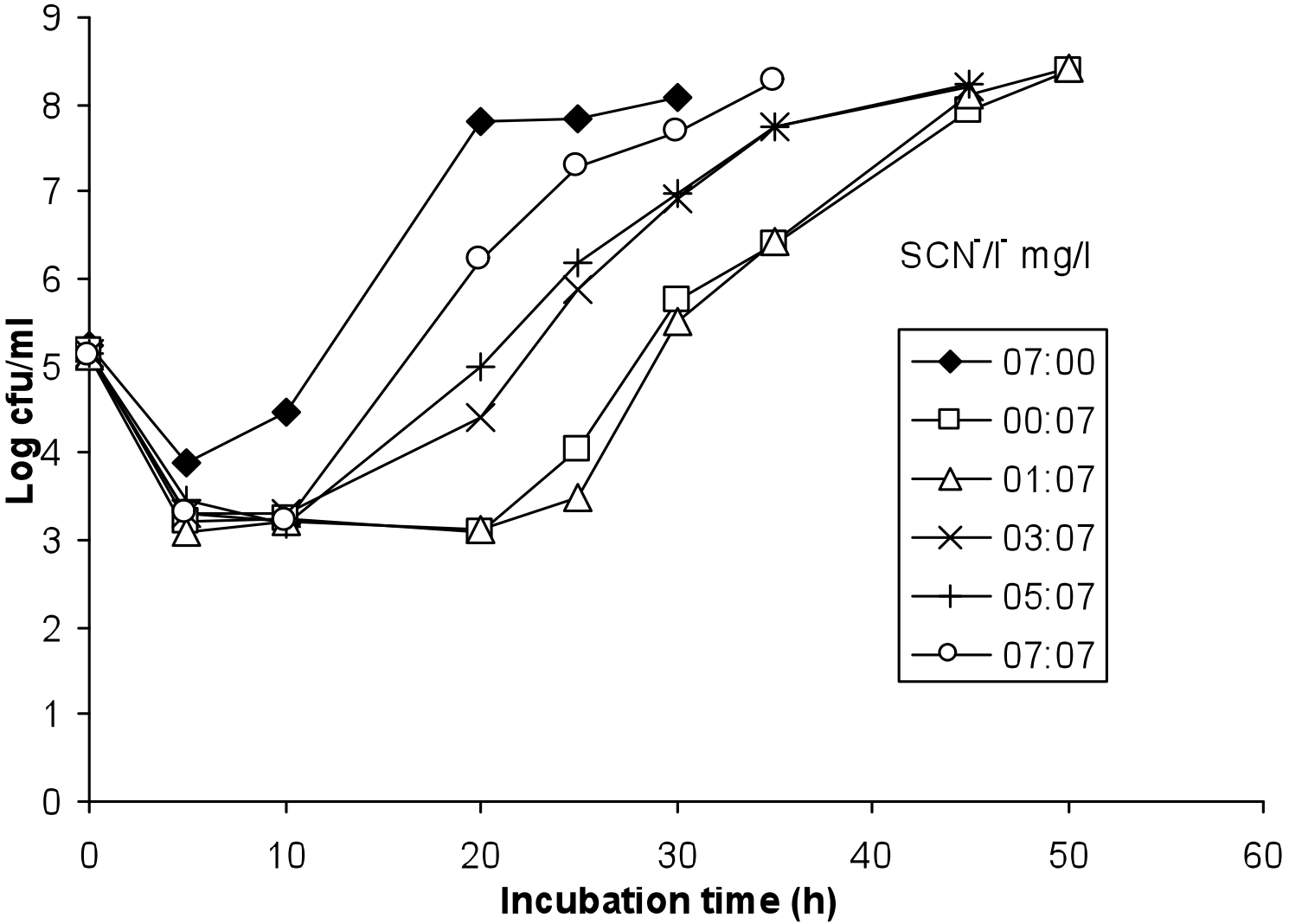
The antimicrobial effects of the different LP systems (LP+I^-+H_2O_2, LP+SCN^-+H_2O_2 and LGE) against *Bacillus cereus* in whole UHT milk.



The antimicrobial effects of iodide (7mg/kg) alone, and in the presence of H_2O_2 (80 mg/kg) and lactoperoxidase against *Staphylococcus aureus*



The antimicrobial effects of LP-s activated using different combination levels of SCN-: I in whole UHT milk inoculat*ed* with a high initial level (10^5) of mixed cultures *(Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa*)



Conclusions

- Iodide is more effective than thiocyanate as an activator of LPS against different bacteria
- Garlic extract is ineffective (and makes the milk taste very odd!!)
- Iodide and thiocyanate do not act synergistically

Variation in LP levels

- Between species
- Between bulk samples
- Between individuals

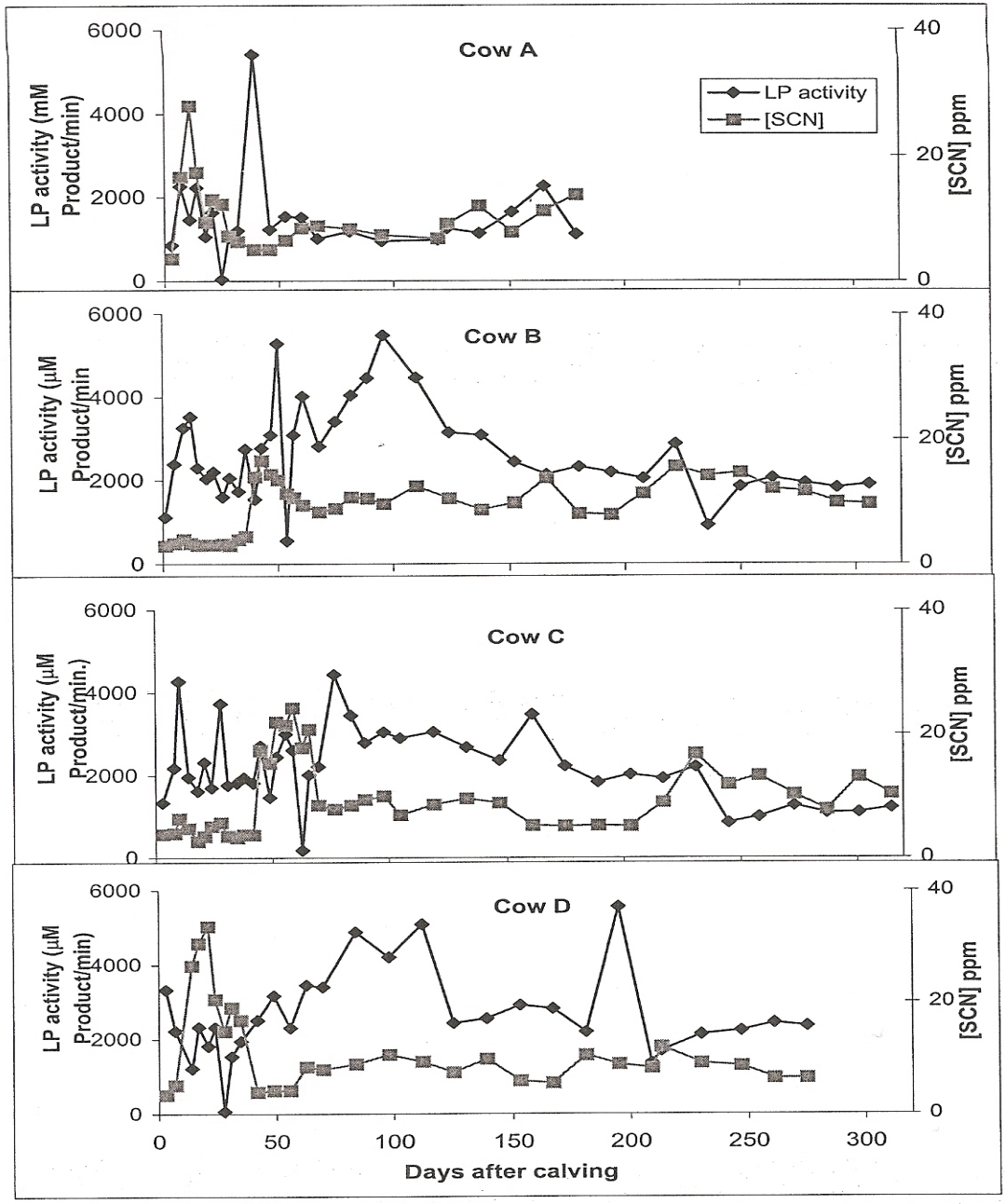


Figure 4.7. Variations in LP activity (◆) and thiocyanate content (■) in cows' milk throughout lactation.

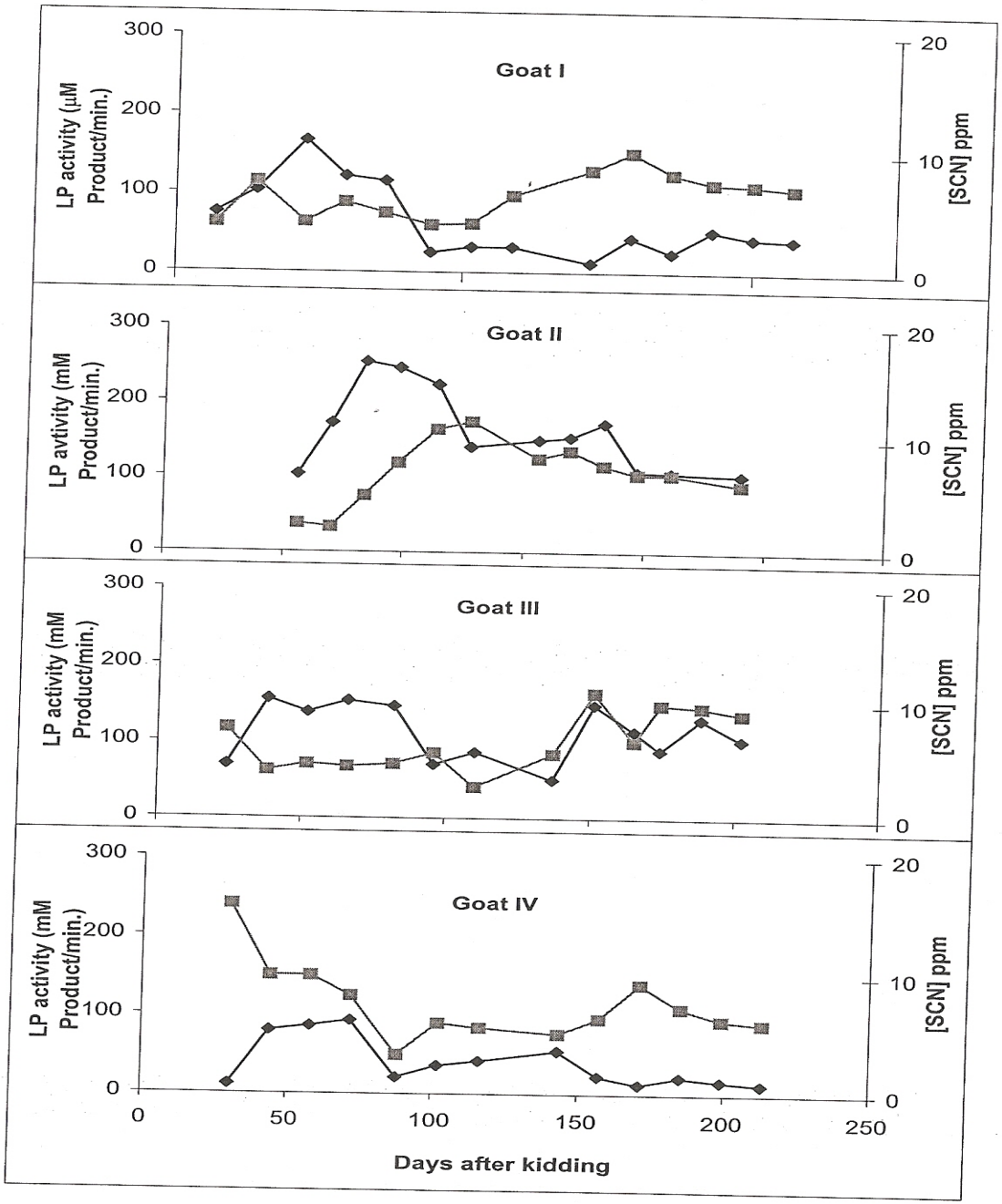


Figure 4.11. Variations in LP activity (◆) and thiocyanate content (■) in goats' milk throughout lactation.

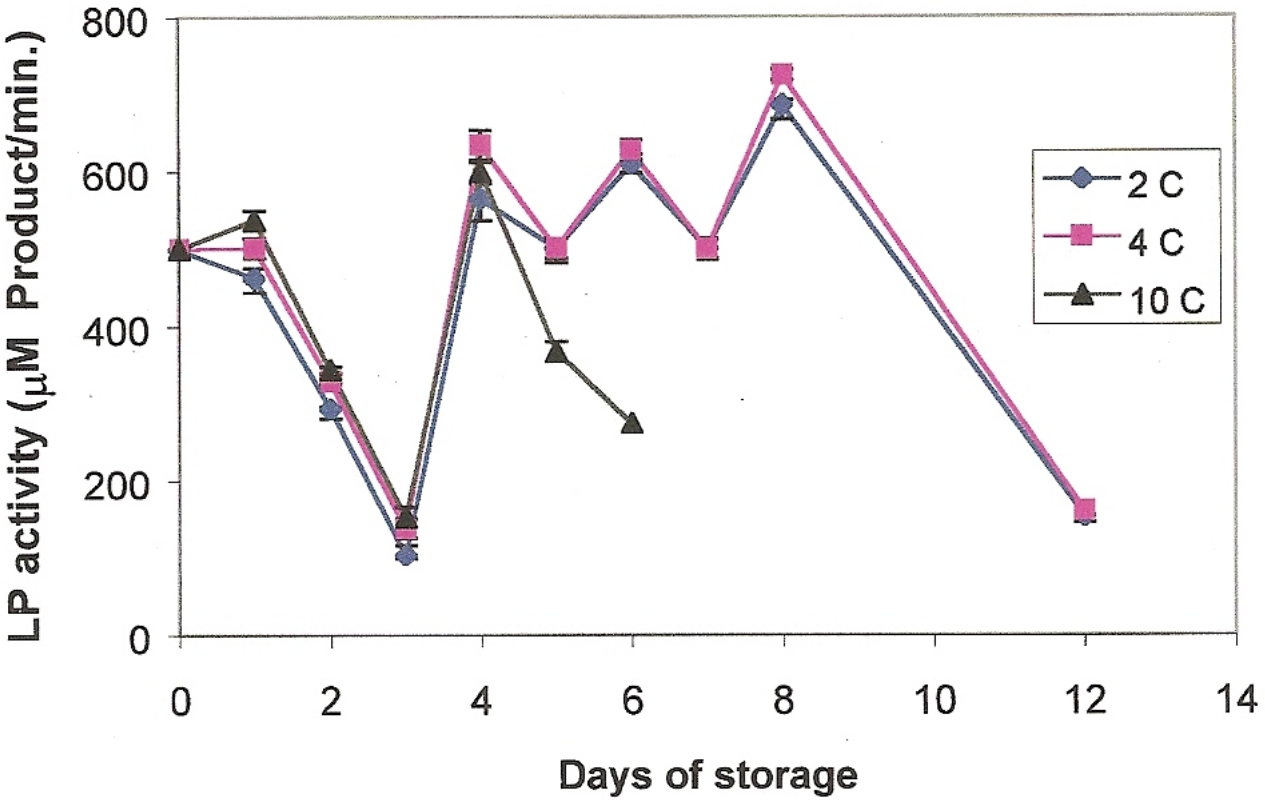


Figure 4.1. Variations in LP activity during storage of cow's bull milk at different temperatures. Error bars represent standard deviations (n = 6).