

# Selection of “thermophilic” Lactobacilli for cheese production

## Content:

- Experience
- Market demands
- Selection criteria for different cheese types
- Examples of results and final selection ...
- Example of blends and their performance ...
- Example of final products for tasting

# Experience

- Grana P. ,Parmeggiano R., Piave and other Italian types
- Emmenthal a.o. Swiss types
- Comte and other French types
- Gouda a.o. with extra umami flavour etc.
- Gorgonzola a.o. blue cheeses
- Taleggio a.o. soft (smear) cheeses

# Market demands changing

- Direct inoculation, not only bulk set/whey cultures
- Phage alternatives needed
- Good cheese also from pasteurized and bactufuged or micro filtered milk (= better Propionibact. also needed for Swiss cheese)
- Better stability/longer shelf life...
- And more demands for each specific cheese type

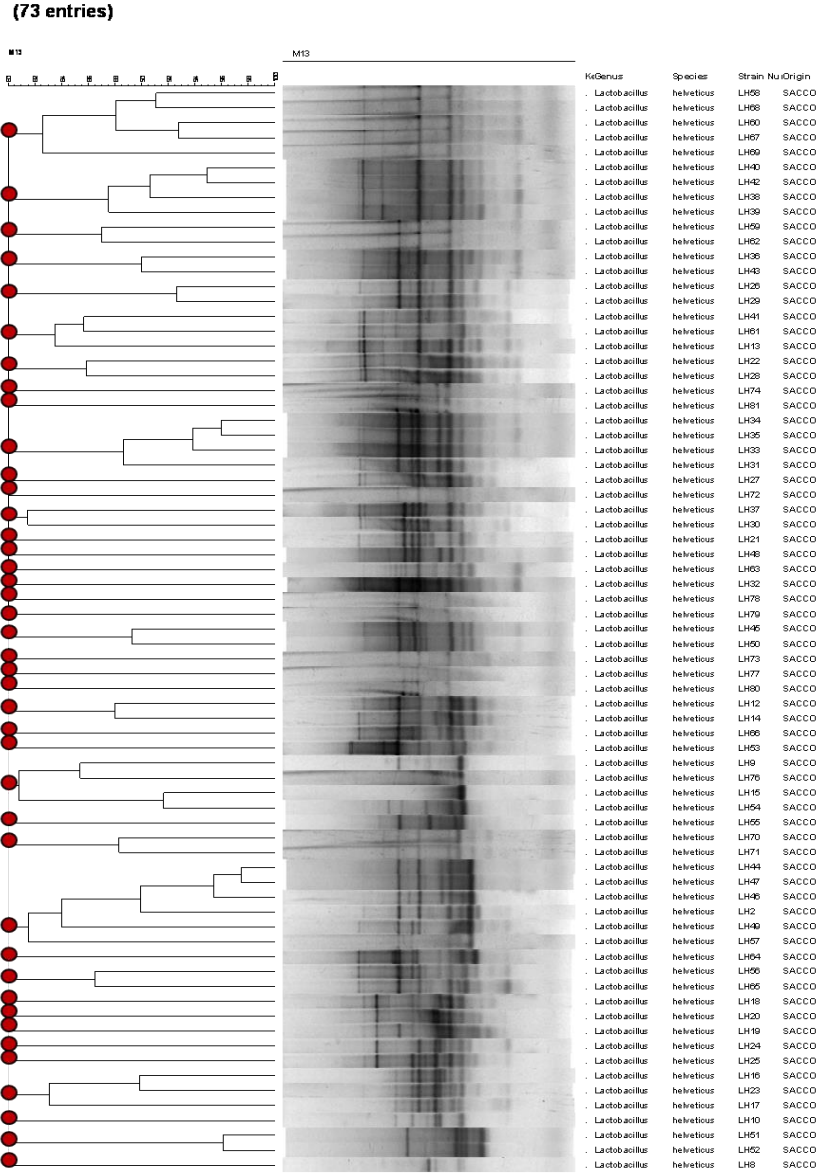
# Demands and selection criteria for hard cheese like Grana, Emmenthal etc.

- Fast acidification after cooking to 53-55° - some areas only 48-49°
- Lysozyme resistance - Lysozyme still used against Clostridia by some
- Depletion of Galactose - else growth of heterof. giving BA...
- Fast autolysis for fast peptidase activity - flavour and PAB growth
- High peptidase activity (and high transaminase activity) - flavour
- But no de-carboxylation activity - no biogenic amines
- Low proteinase activity for Emmental a.o. - no cracks wanted
- Phage alternatives needed – so min. 2-4 strains strains of LH and 2 LL

# Demands and selection criteria for semi-hard types with umami flavour and crystals




- Extra fast growth at 34+/-4°
- Fast lysis for fast release of peptidase activity
- High peptidase activity - umami flavour and crystals
- High transaminase activity – (fruity) flavour
- No de-carboxylation activity - no BA
- Not too high proteinase activity - sliced cheese - short texture unwanted
- Phage alternatives less critical – still min. 1-2 LH and 1-2 LL wanted

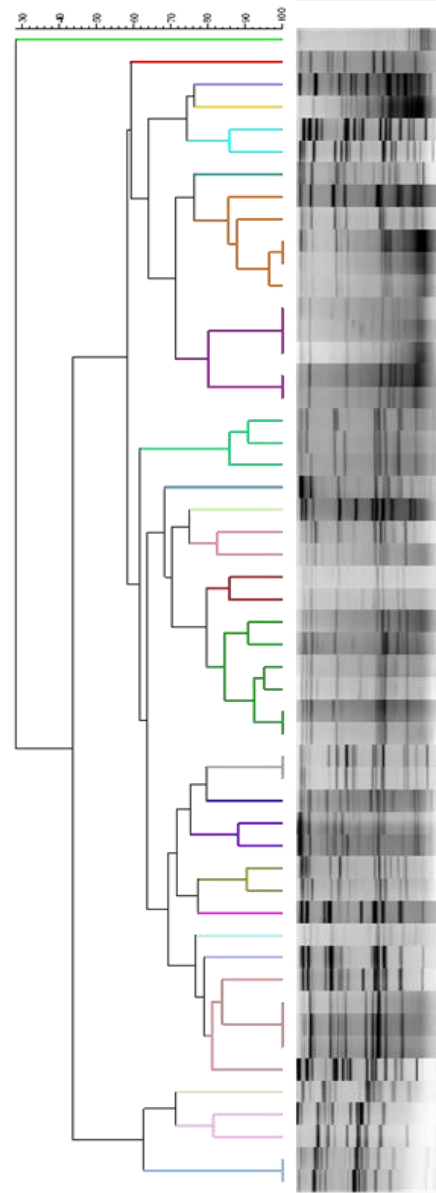
# Genotypic characterization



- DNA extraction (lysozyme and Proteinase K)
- RAPD – PCR method using M13 primer (GAGGGTGGCGGTTCT)
- Fingerprinting profiles( *Bionumerics*)
- On left example with first 73 of the strains
- The many strains grouped in 41 clusters
- Wide infraspecific genotypic heterogeneity

PFGE (RsrII - Opt. 1% - Tol. 1.5% - UPGMA)

-  LH fast group I
-  LH fast group II
-  LH slow



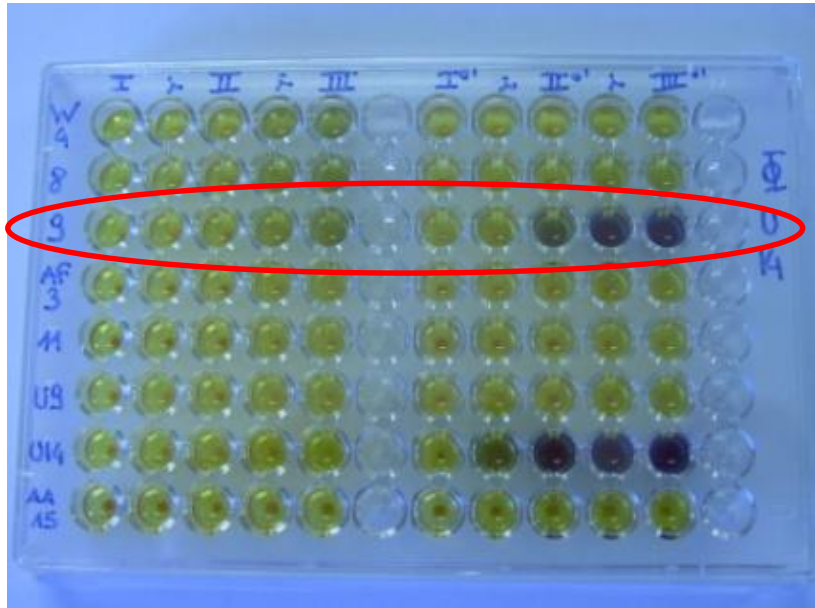
SIGLA CEPPO	PULSOTYPE	CLUSTER
LH53	21	01
LH87	43	02
LH46	20	03
LH45	19	04
LH15	8	05
LH02	1	05
LH65	22	06
LH88	35	07
LH66	23	07
LH38	7	07
LH28	7	07
LH13	7	07
LH62	18	08
LH51	18	08
LH43	18	08
LH89	34	08
LH91	34	08
LH96	40	09
LH95	39	09
LH94	38	09
LH26	13	22
LH86	33	10
LH25	12	11
LH24	11	11
LH23	10	12
LH17	9	12
LH85	32	13
LH93	37	13
LH84	15	13
LH83	31	13
LH90	29	13
LH82	29	13
LH78	27	14
LH77	27	14
LH73	26	15
LH72	25	16
LH69	24	16
LH10	3	17
LH08	2	17
LH92	36	18
LH27	14	19
LH37	17	20
LH79	28	21
LH67	16	21
LH34	16	21
LH32	16	21
LH101	5	21
LH104	6	23
LH100	4	24
LH99	42	24
LH88	41	25
LH97	41	25

# Phage characterization

- With 20 different phages



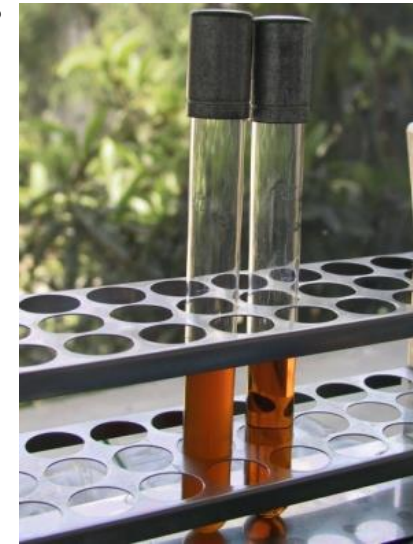
Agar-based method



Lysis in 96 wells microplates

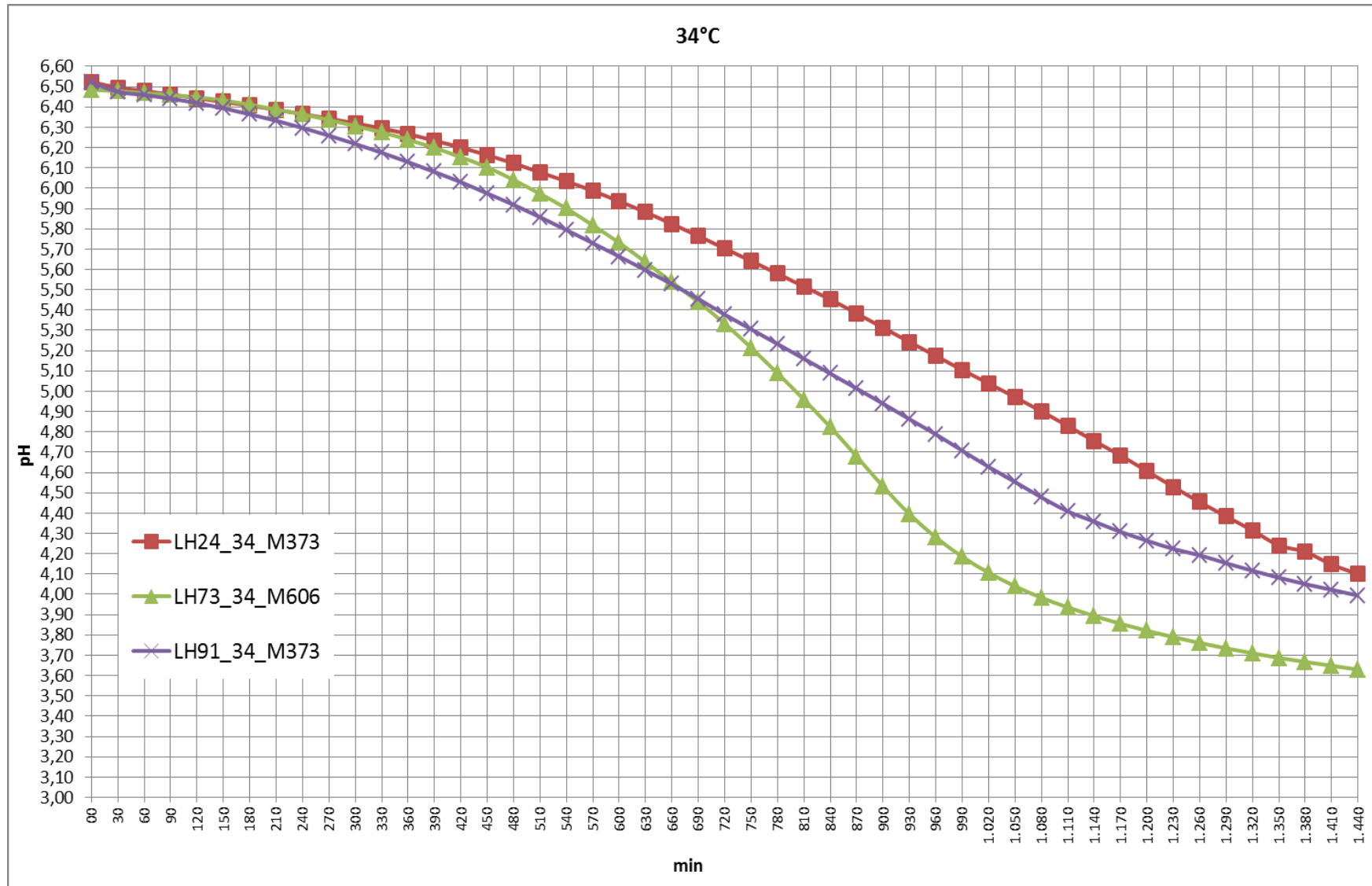
Positive strains

Lysis in test tubes

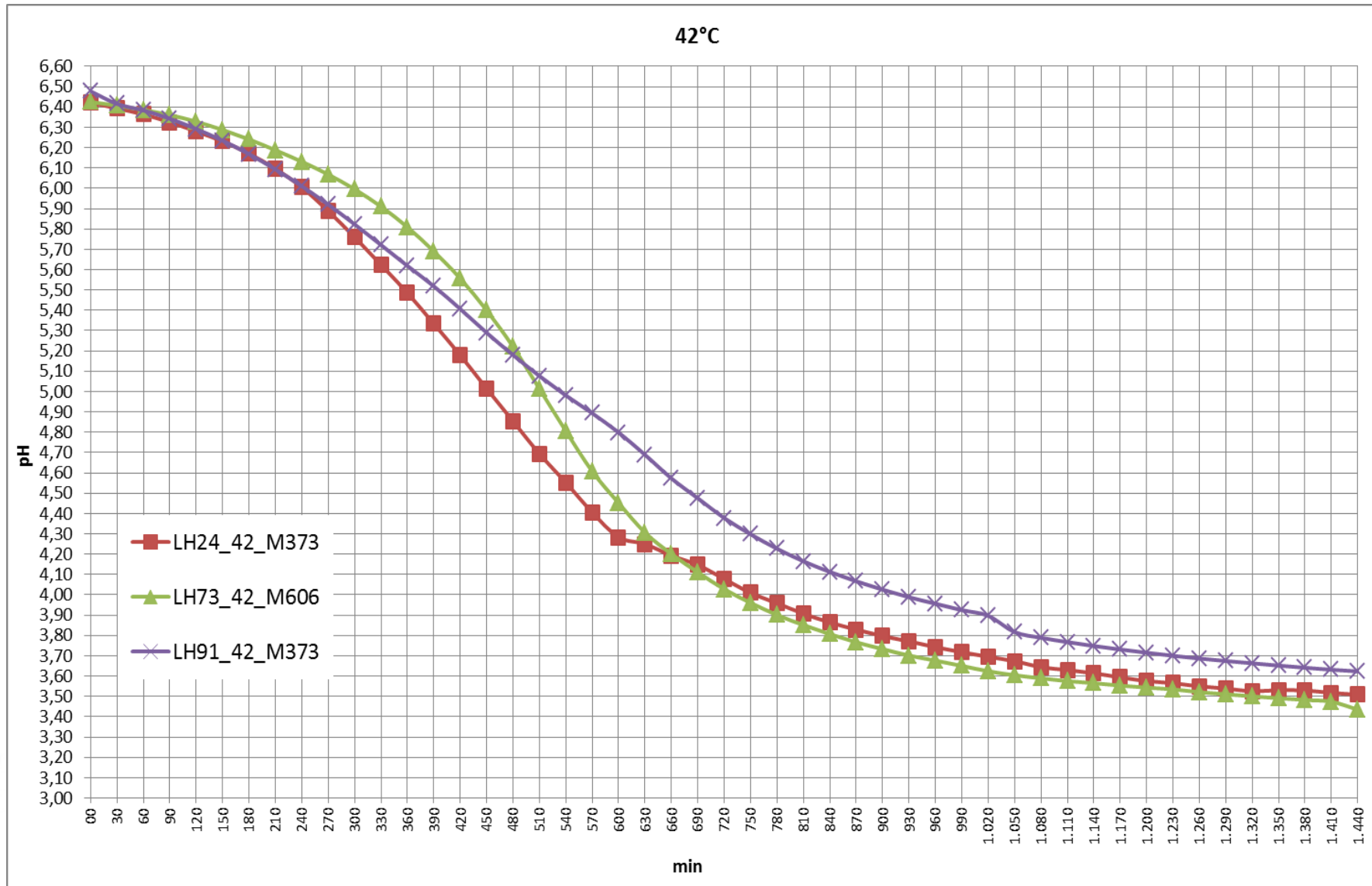




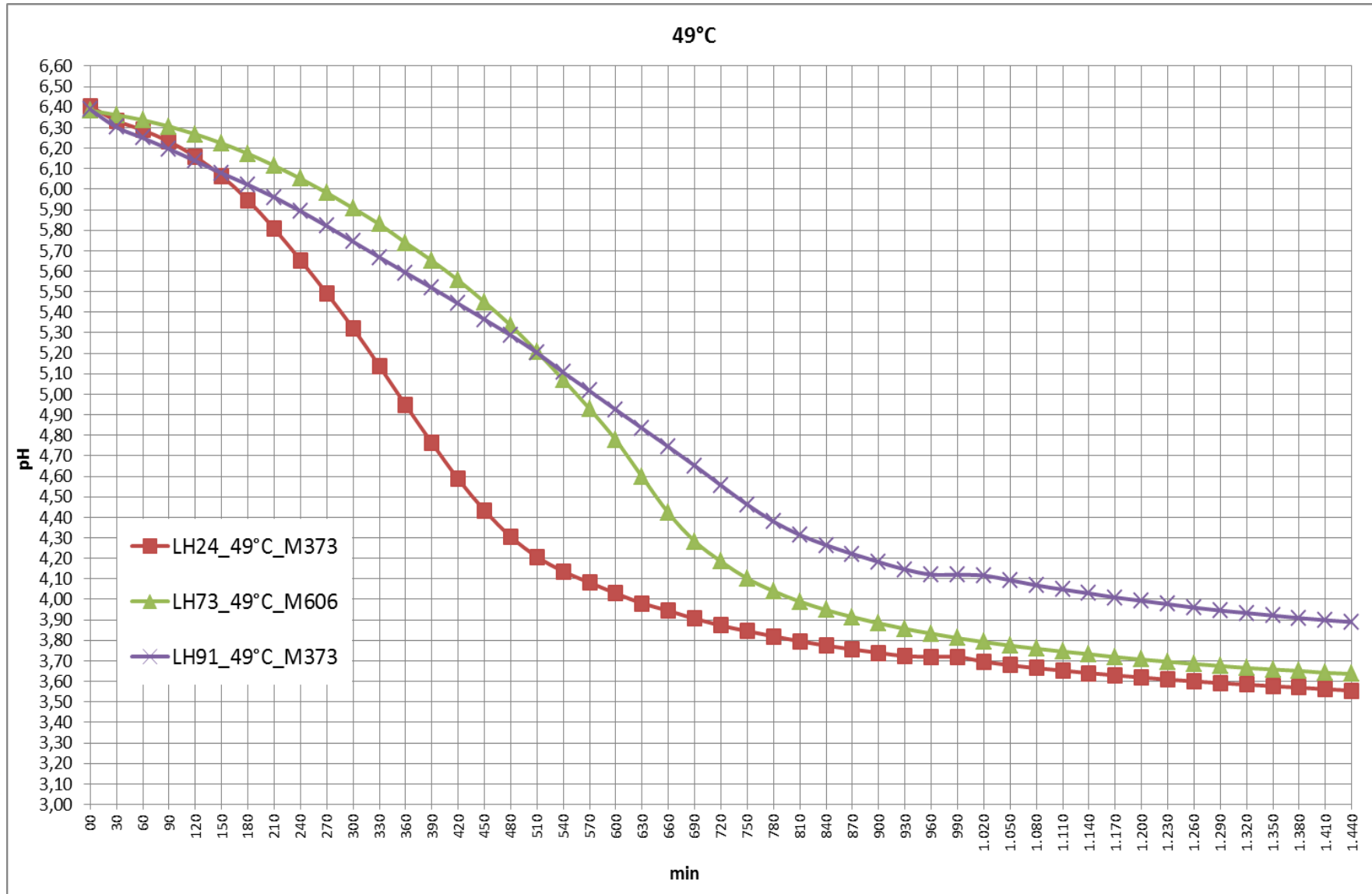
# Examples of LH acidification at 34°



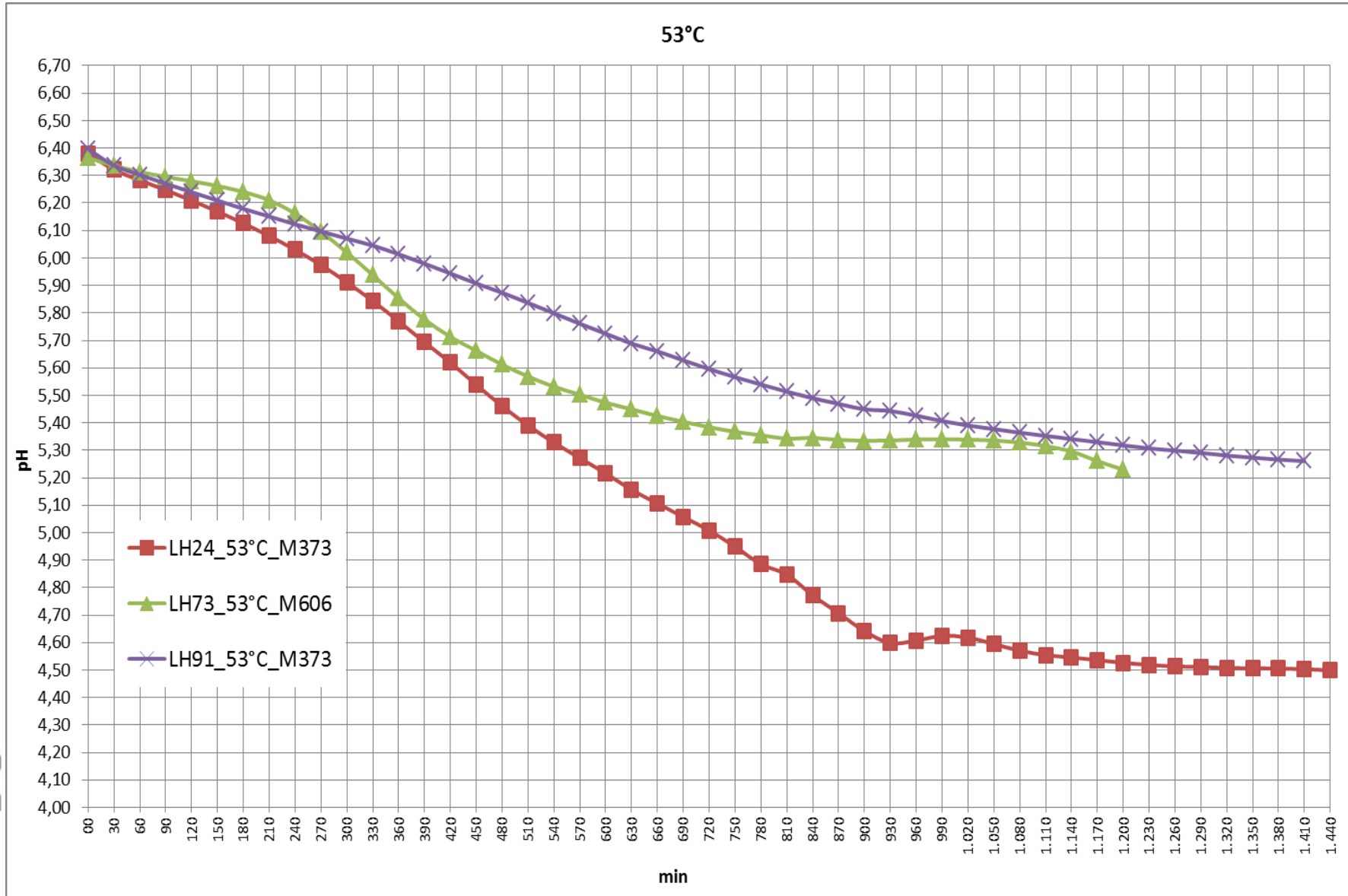
# Examples of LH acidification at 42°



# Examples of LH acidification at 49°

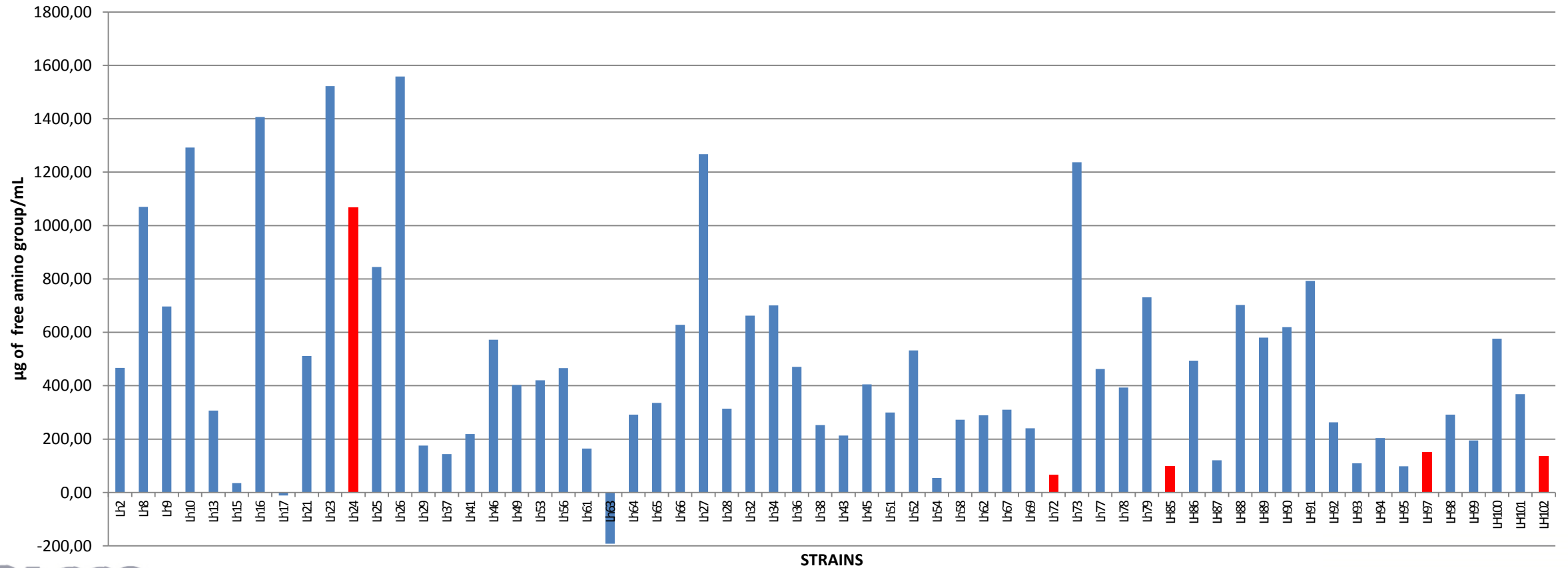


# Examples of LH acidification at 53°

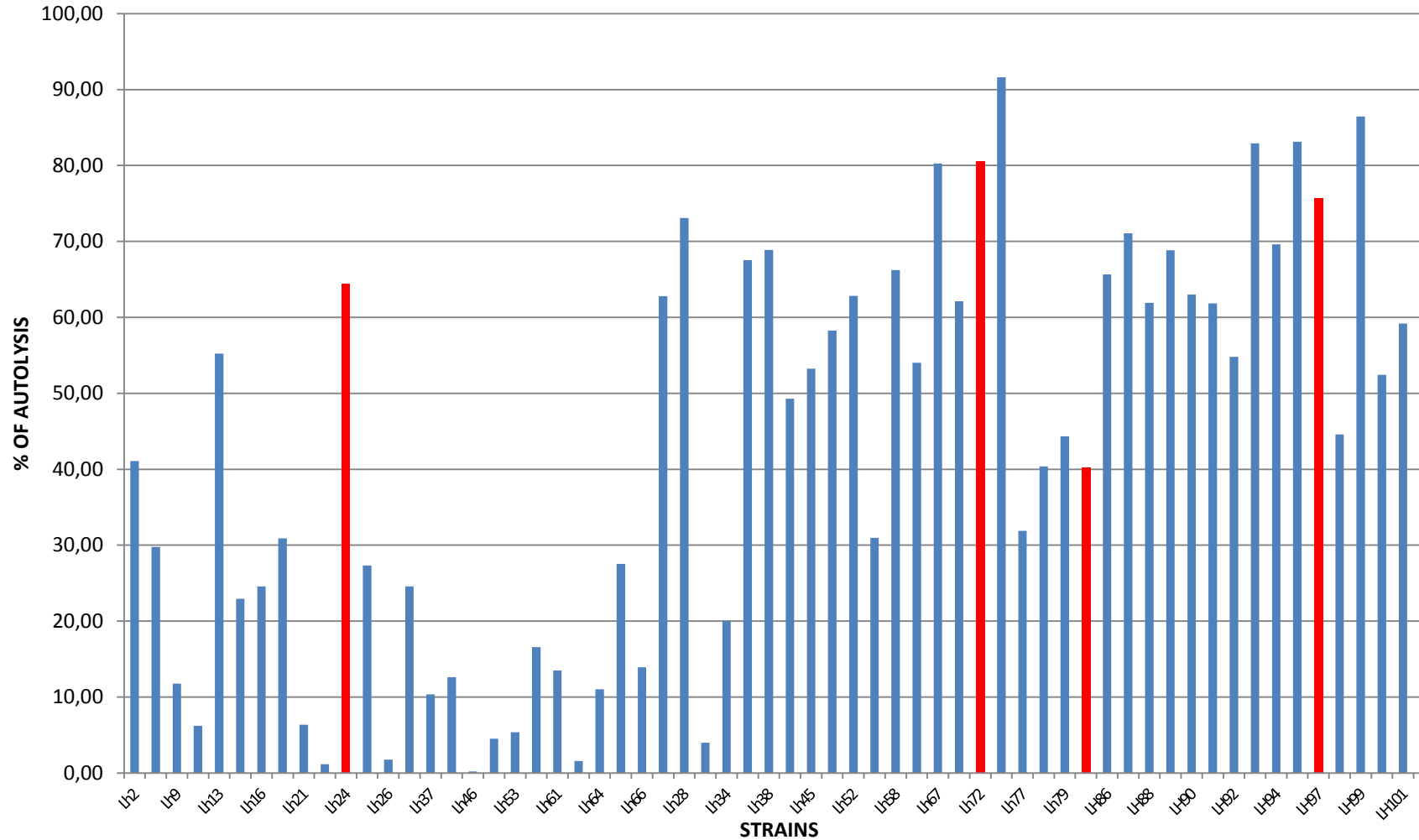


# Proteinase activity of some LH strains

## Proteolysis

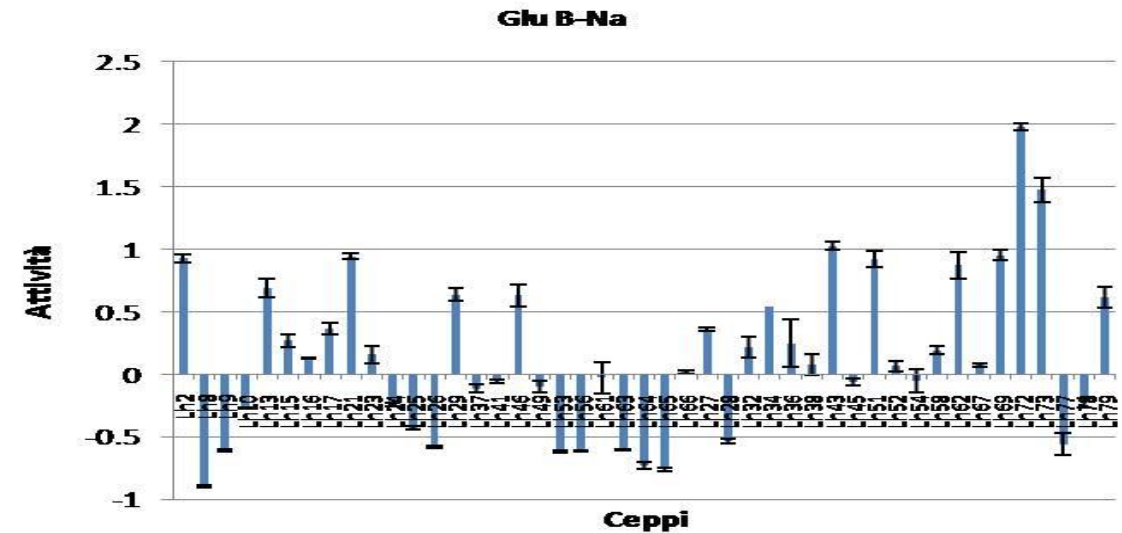
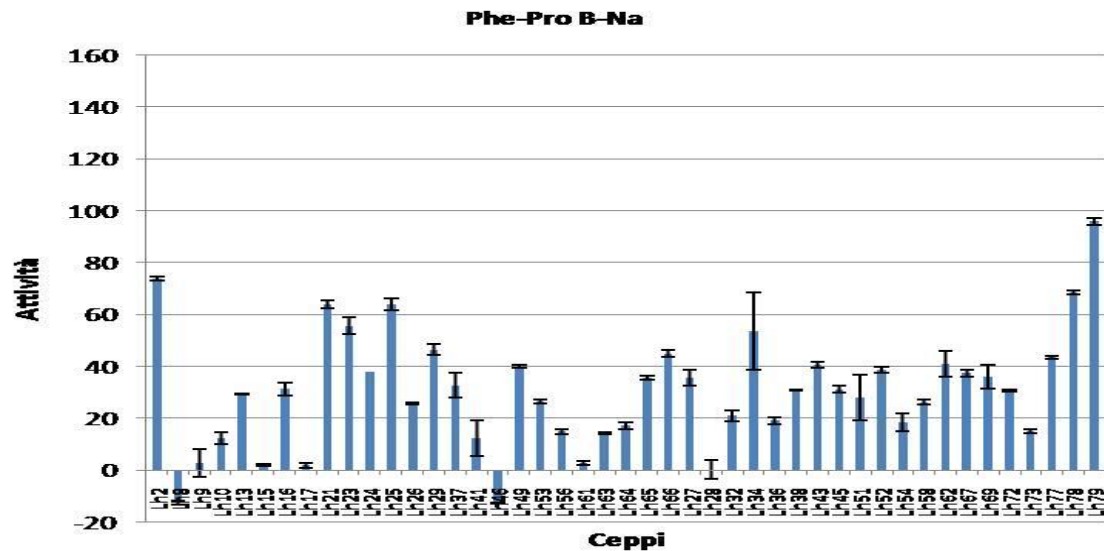
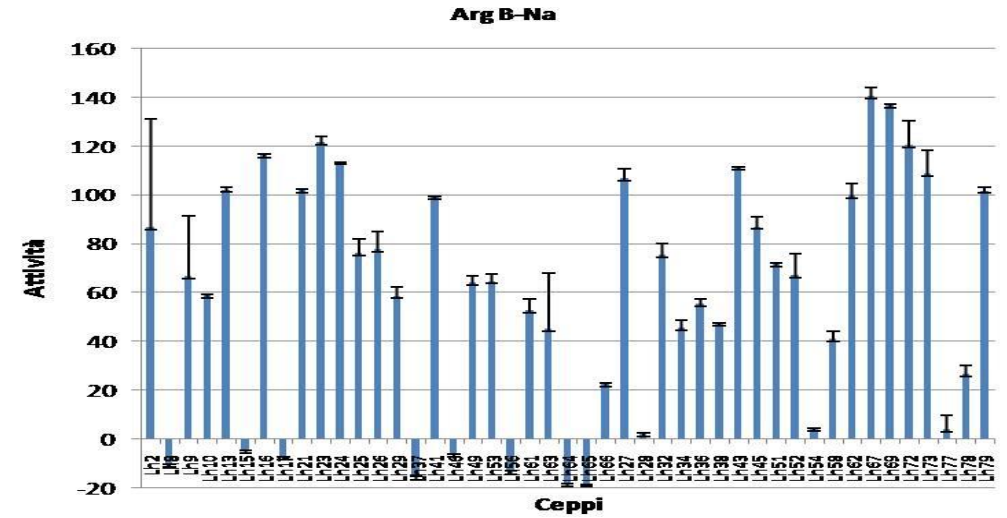
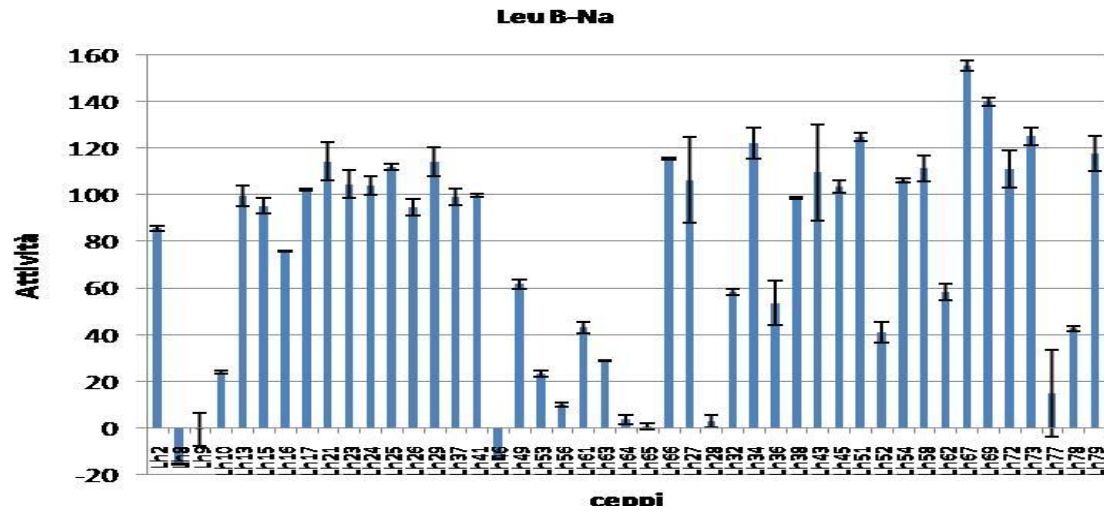


# Autolysis of some LH



 Selected strains

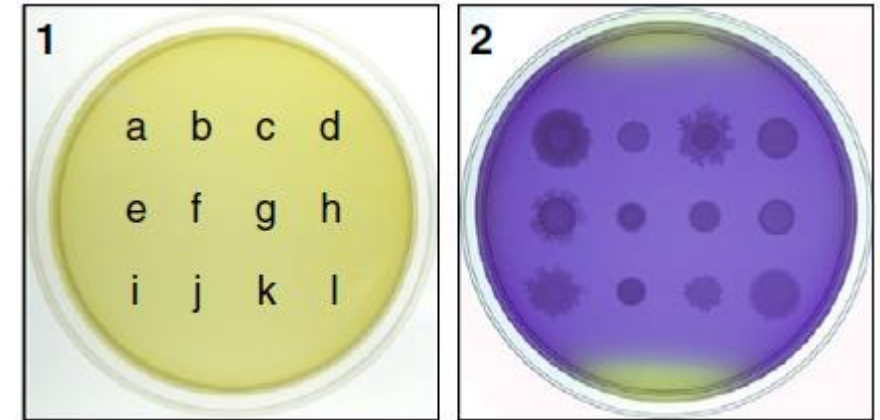
# Peptidase activity of some LH strains



# Test for Biogenic Amine production

Only strains selected with no production, but most had none!

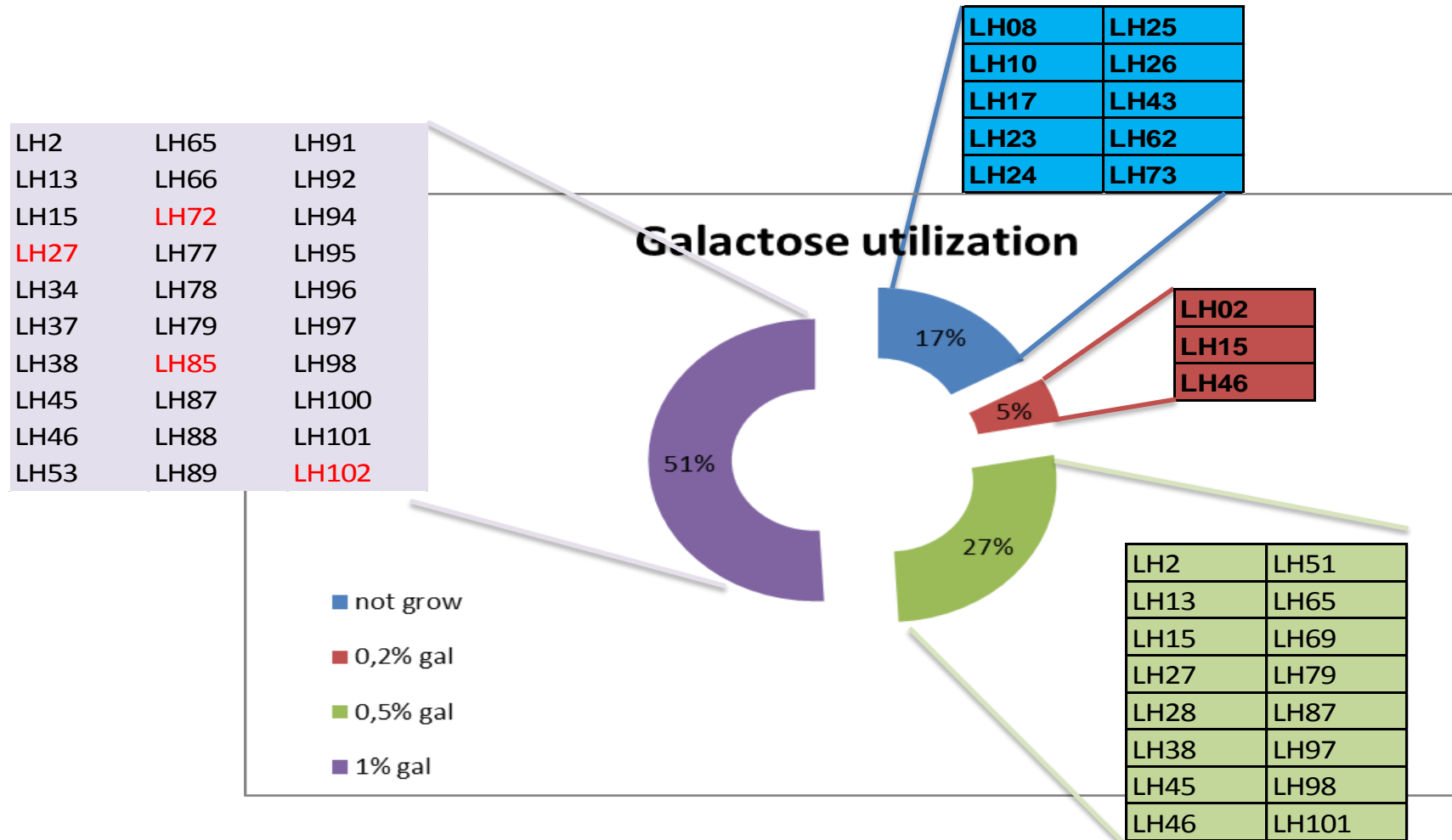
Aminoacid	Biogenic amines
Histidine	Histamine
Tyrosine	Tyramine
Lysine	Cadaverine
Hornithine	Putrescein



Release of the carboxylic group resulting in a color change for pH increase



# Galactose utilization - LH



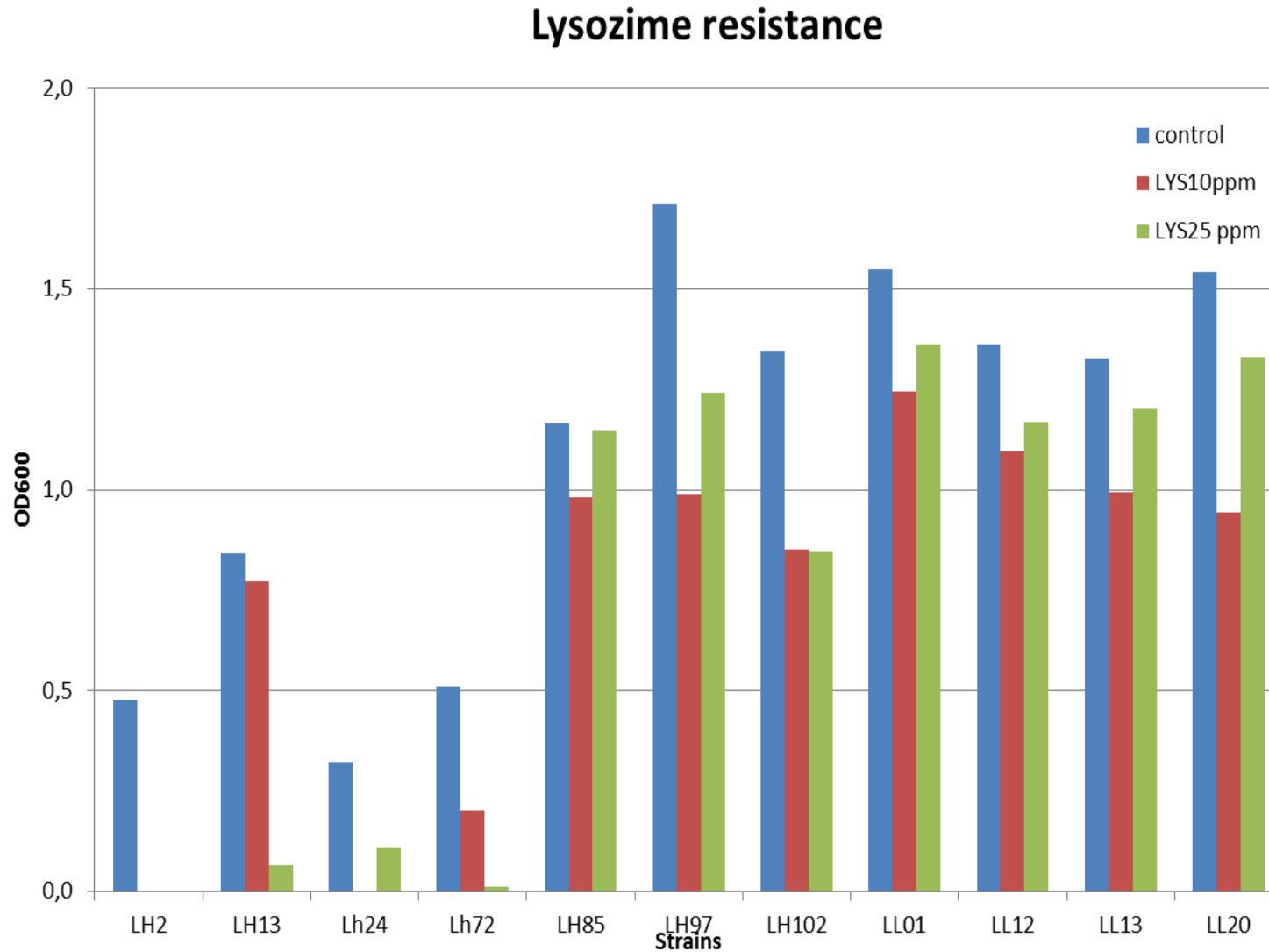
# Examples of salt sensitivity

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<b>Sensitive (growth only with 2% NaCl in moisture)</b>	<b>Normal (growth with 2% and 4% NaCl in moisture)</b>	<b>Insensitive (growth also with 6% NaCl in moisture)</b>
LH88	LH13	LH24
LL01	LH72	LH45
LL12	LH97	LH89
LL13	LH102	LH91

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# Examples of Lysozyme resistance

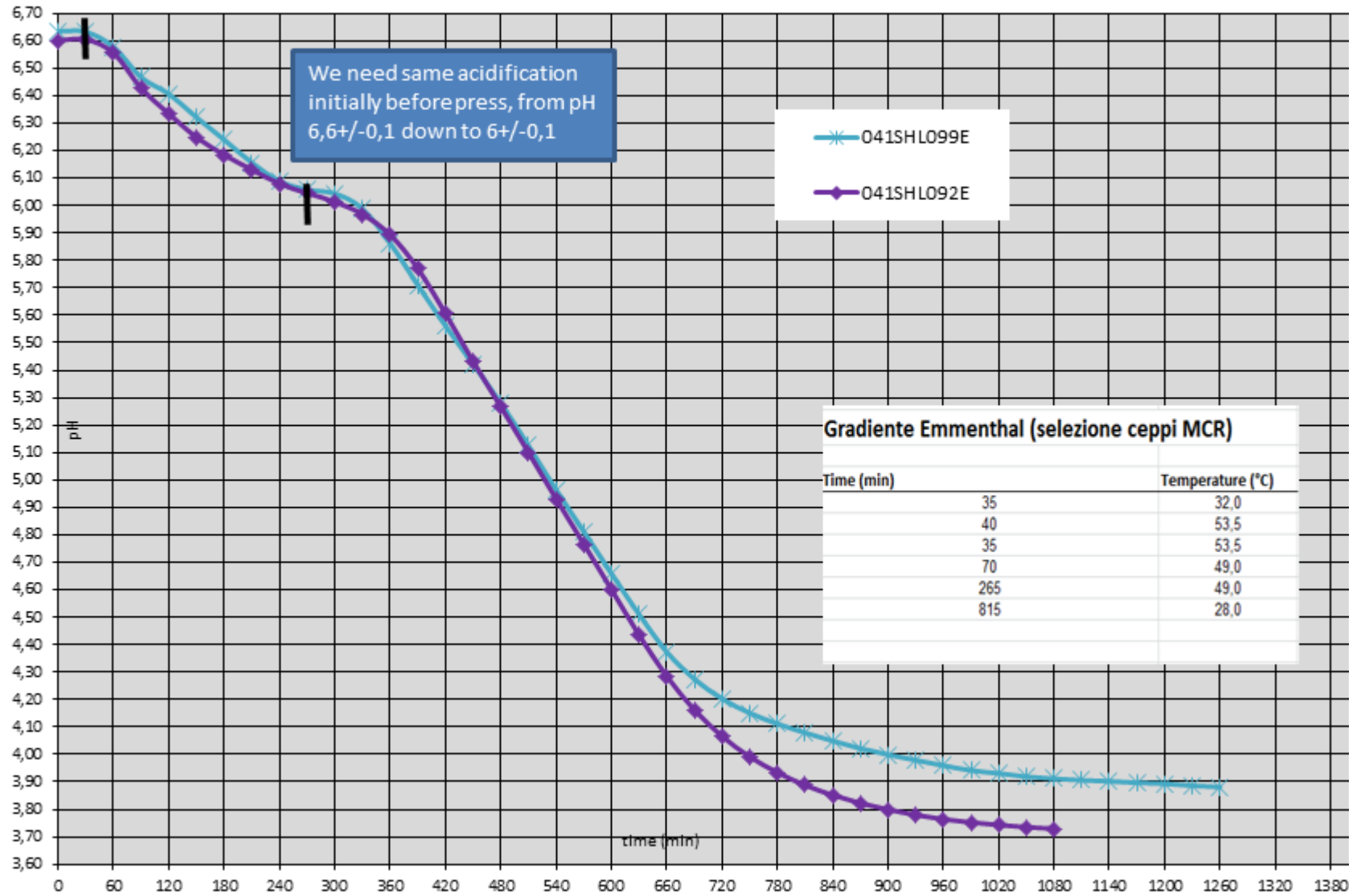


# Overview of selected strains for semi- and hard cheese

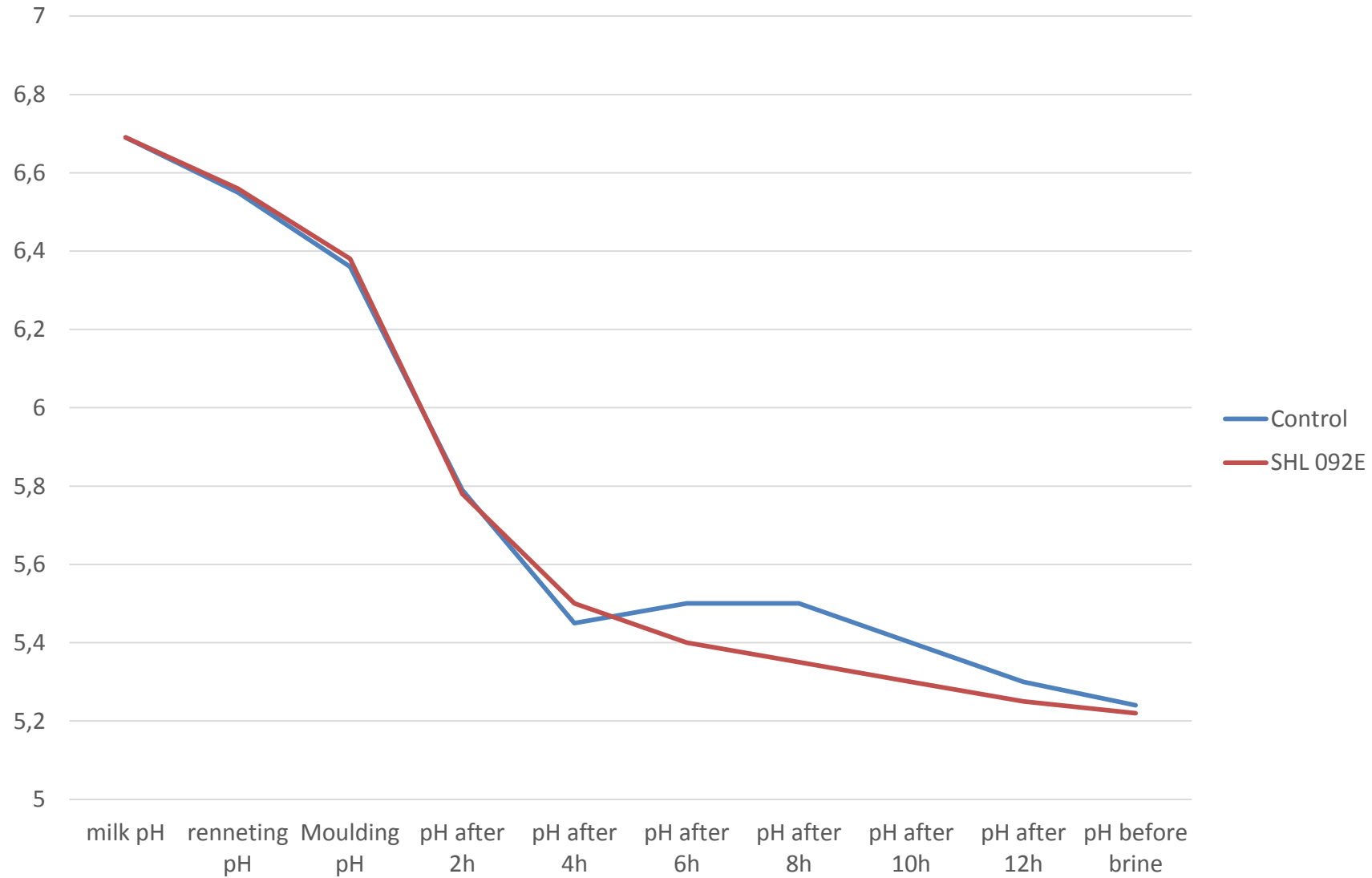
Strain	34°C	42°C	49°C	53°C	Gal+	Prot+	A.lysis	Arg+	Leu+	Glut+ All L	Phe+	NaCl s.	Lysoz. r	Appl.
LH13	M	F	M	L	M	L	M	H	H	L	L	M	M	S(+H)
LH88	F	F	F	F	L	M	H	M	H	L	M	H	M	S+H-E
LH97	M	M	M	M	M	L	H	L	L	L	M	M	M	H-E
LH102	M	M	F	F	H	L	H	H	M	L	H	M	M	H-E
LL01	M	F	F	F	-	L	H	L	L	L	M	H	H	S-E
LL12	M	F	F	F	M	L	M	M	M	L	H	H	H	S+H-E
LL13	M	F	F	F	M	L	M	H	H	L	H	H	H	S+H-E
LL20	M	F	F	F	L	M	M	L	L	L	M	H	H	S+H-E

# Development of SHL 092/9E Cryofast for Emmenthal

Acidification curve in LPM614 with emmenthal temperature profile



# pH Emmenthal production



# Emmenthal chemical composition milk and cheese

Sample	Milk Fat%	Milk Prot.%	cheese Fat%	cheese DM%	cheese FDM%	cheese MFFC%	Cheese Y. L Milk/Kg	
Control f.	3,00	3,50	30,0	62,7	47,8	54	na	
SHL 092E f.	3,00	3,50	30,0	62,7	47,8	54	na	
Control 100 days	-	-	30,2	63,3	47,7	52,6	16,5	
SHL 092E 100 days	-	-	30,1	63,1	47,7	52,8	16,6	

# Emmenthal sensory and volatiles (104 days)

sample	Int. app. 100 days	Texture 100 days	Flavour 100 days	Int. app. 300 days	Texture 300 days	Flavour 300 days	Comments
Control	5	5	5	5	4,5	4	Good
SHL 092E	5	5	5	5	5	5	Very good

Sample	Acetic a. mg/100g	Propion. a. mg/100g	Iso.But.a. mg/100g	Butyric a. mg/100g	Iso.Val.a. mg/100g	Valeric a. mg/100g	Caproic a. mg/100g	Caprylic a. mg/100g	Proteol. TVA mmol/Kg
Control	310	520	<1	10	1	<1	1	1	173
SHL 092E	293	593	<1	20	2	<1	2	2	204



# Cheeses for sensory testing

- Emmenthal from Arla Taulov.

Made with MW 039N, SHL 092E and PF7 Cryofast from bactufuged and pasteurized milk, cooked to 49°

- Primeo Samsøe type crystal cheese from Noerup M..

Matured min. ½ year made with standard DL-culture + LH13/88 and LL12+13 Cryofast, scalded to 39°