EFFECT OF STORAGE ON PROTEOLYSIS AND LIPOLYSIS OF GOAT MILK CHEESES

Young W. Park

Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA 31030,

and

Department of Food Science and Technology, University of Georgia, Athens, GA 30602, U.S.A.

Three primary maturation processes occur during cheese ripening

- Glycolysis:
  1. **Lactose** ⟶ **Lactic acid (by the starter bacteria)**
  2. About 0.8-1.5% lactose or lactic acid remains in cheese curd.
  3. This residual lactose is quickly metabolized under normal conditions, predominantly to L-lactate.
- Proteolysis:
  1. Proteins ⟶ Peptides and amino acids
  2. Proteolysis before, during and after cheese manufacture.
- Lipolysis:
  1. Fat, triglycerides ⟶ Short, medium chain and free fatty acids.

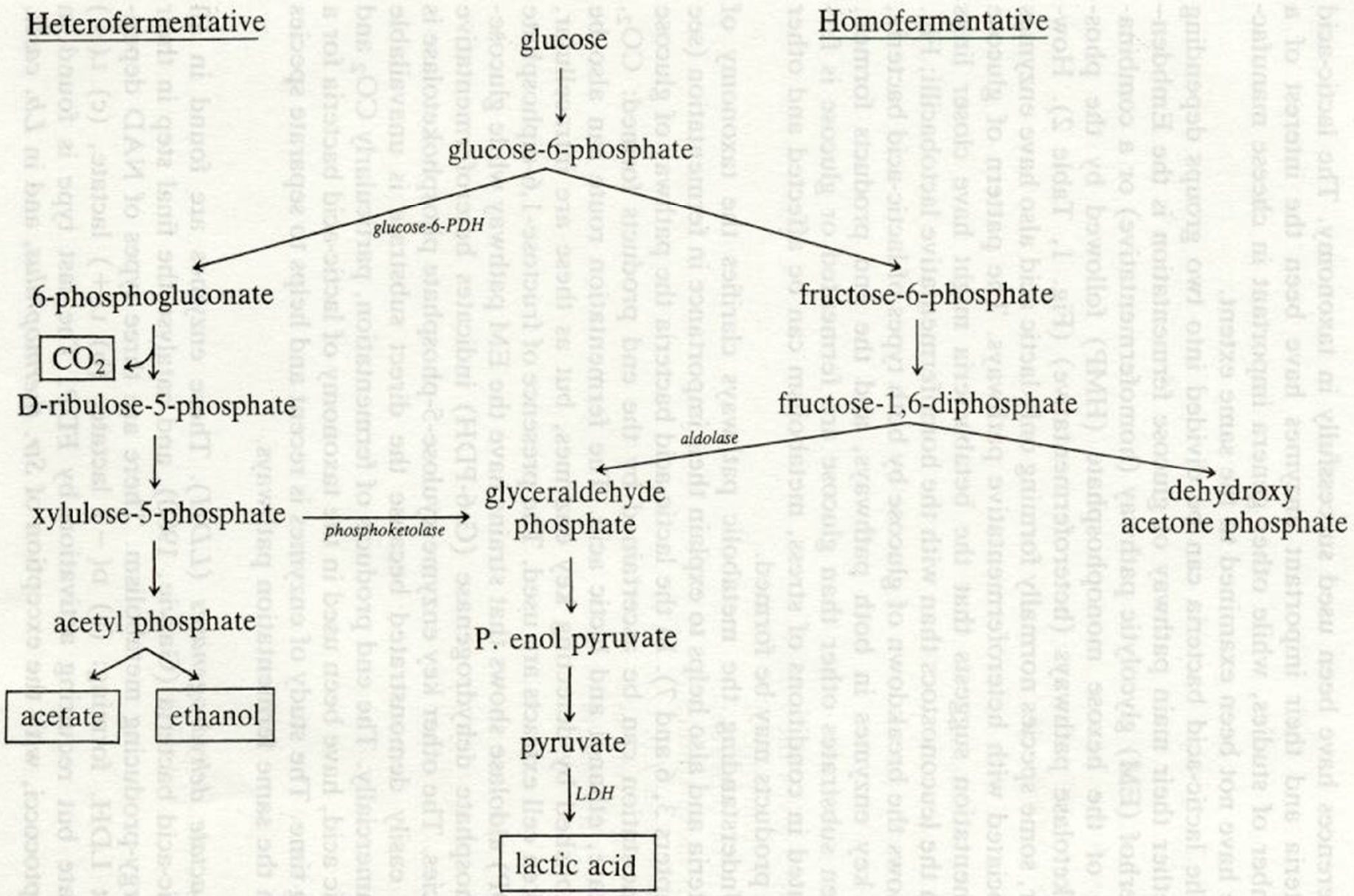


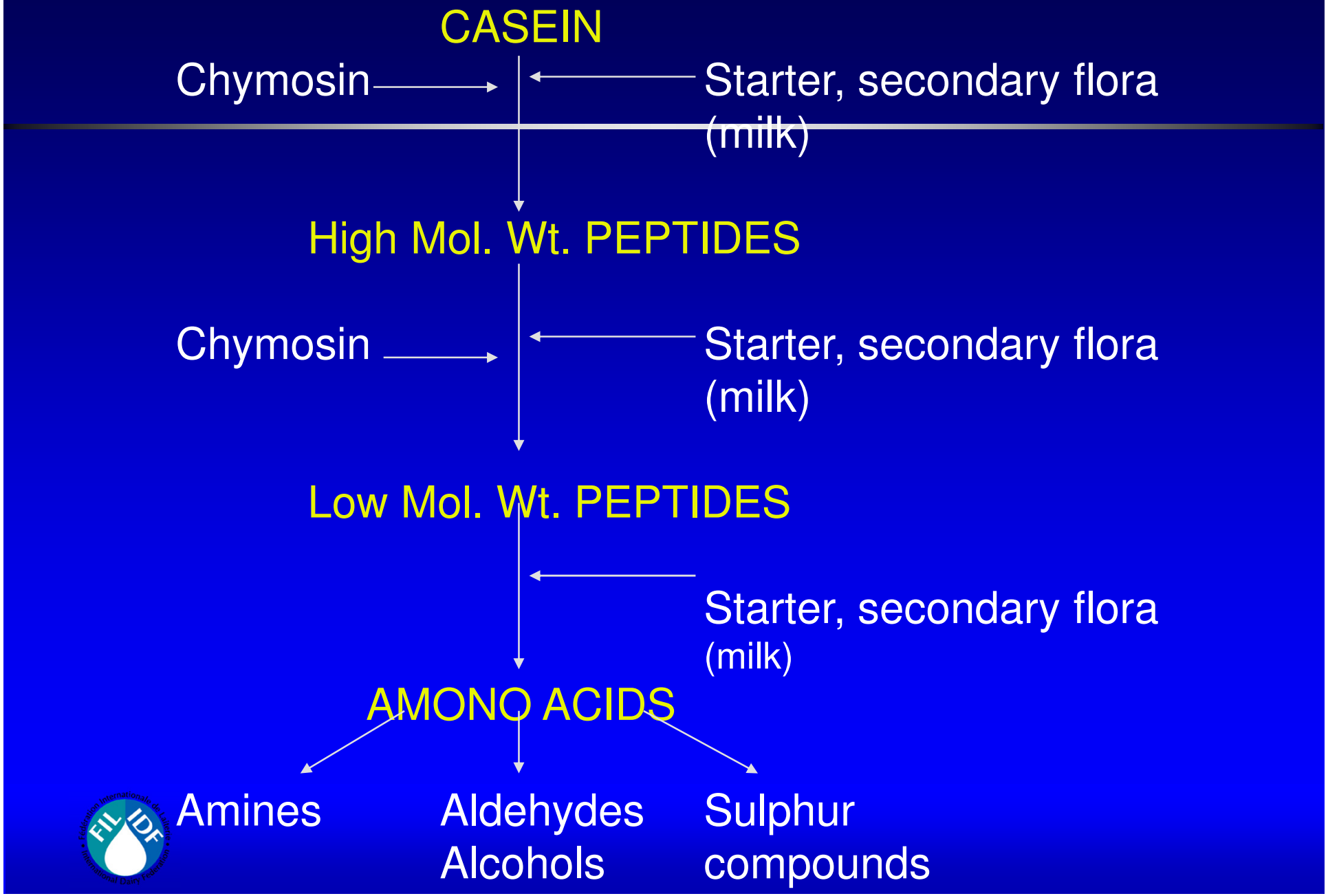
Fig. 1. Glycolytic pathways in lactic-acid bacteria.

Comparison of Nitrogen and Casein Contents between Goat and Cow Milk[a]

N and Casein Compounds	Goat Milk	Cow Milk[b]	Range (Goat Milk)
Total N (g/l)	27.2	32.0	19.1 – 33.6
Casein (g/l)	21.1	27.0	15.8 – 26.0
Non-protein N (%)	6.3	4.5	3.1 – 13.2
Casein (% of total)			
αs_1	5.6	38.0	0 – 20
αs_2	19.2	12.0	10 – 30
β	54.8	36.0	43 – 68
κ	20.4	14.0	15 – 29
α-La/β-Lg	0.63	0.4	0.33 -1.1

aReneuf and Lenoir, IDF Bull, No. 202:69, 1986
bFriesian-Holstein cows

Proteolysis Of Casein During Cheese Ripening



EFFECTS OF PROTEOLYSIS ON CHEESE RIPENING

1. Changes in Texture through:
    a. Degradation of protein network
    b. Increase in pH
    c. Higher water binding by the newly formed amino and carboxyl group

2. Development of flavor components, such as amino acids and peptides.

3. Changes in pH by the formation of NH_3.

4. Greater release of sapid compounds during mastication.

FIVE PROTEOLYTIC AGENTS

1. Indigenous enzymes in raw and/or pasteurized milks.
2. Coagulating enzymes, rennet or its substitute such as chymosin, pepsin or microbial proteinases.
3. Starter culture bacteria and their enzymes after the cells are lysed.
4. Enzymes from secondary starters, such as propionic acid bacteria, and yeasts and molds.
5. Nonstarter bacteria opportunistically entered during cheesemaking.

Factors Affecting Proteolysis of Cheese

- Chymosin
- Plasmin
- Protease from starter and non-starter bacteria
- pH of curds
- Moisture content of curds
- Salt content of cheese (curds)
- Salt-to-moisture ratio
- Storage temperature
- Storage time
- Humidity of aging room

LIPOLYSIS IN MILK

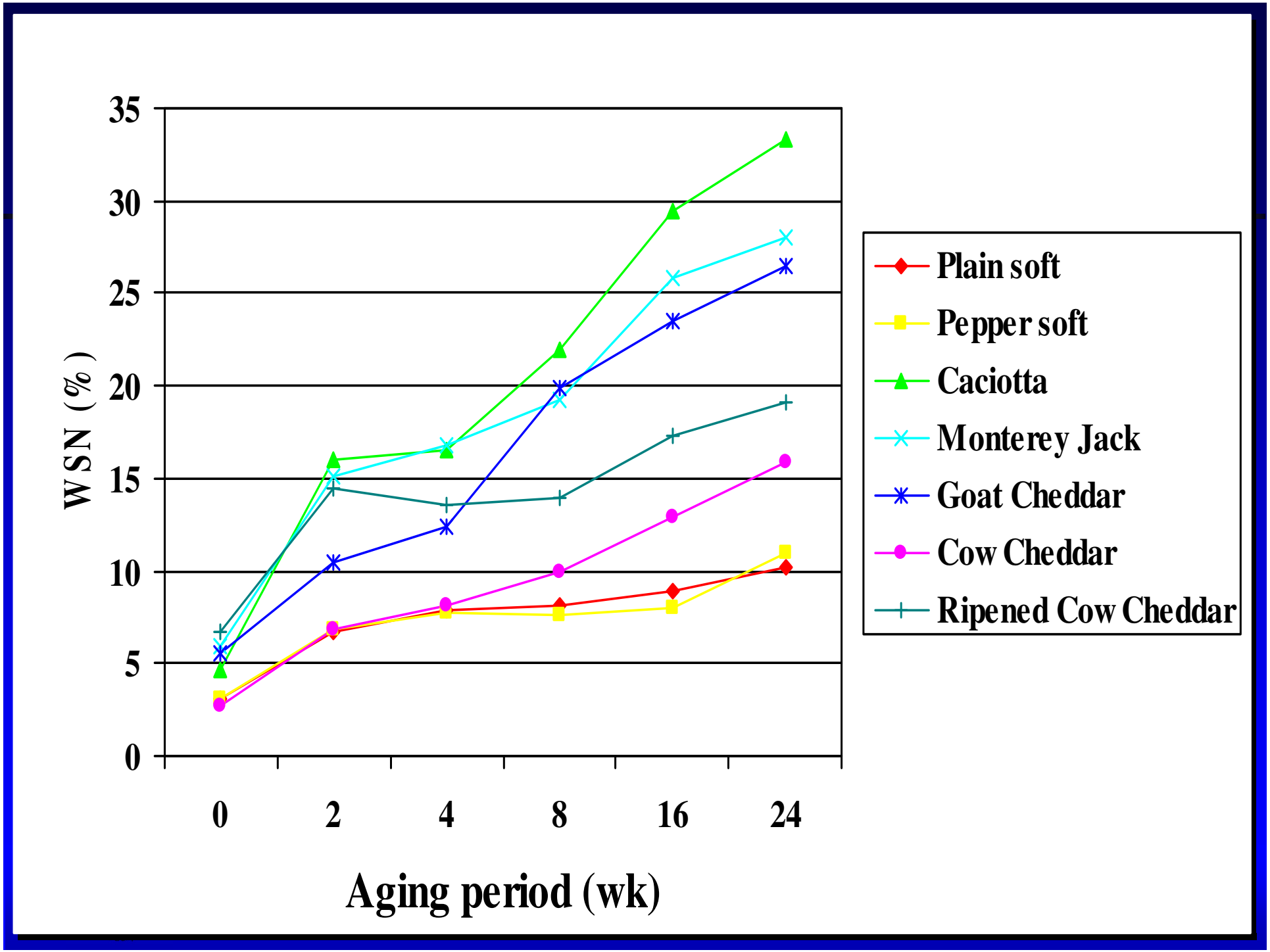
1. Induced Lipolysis:
   a. Processing factors; Agitation, foaming, homogenization, and freezing and thawing (Activation by temp changes).
   b. Temperature factors; During transportation, storage and processing
   c. Farm factors; Milking machines, pipelines, pumping, bulk tank

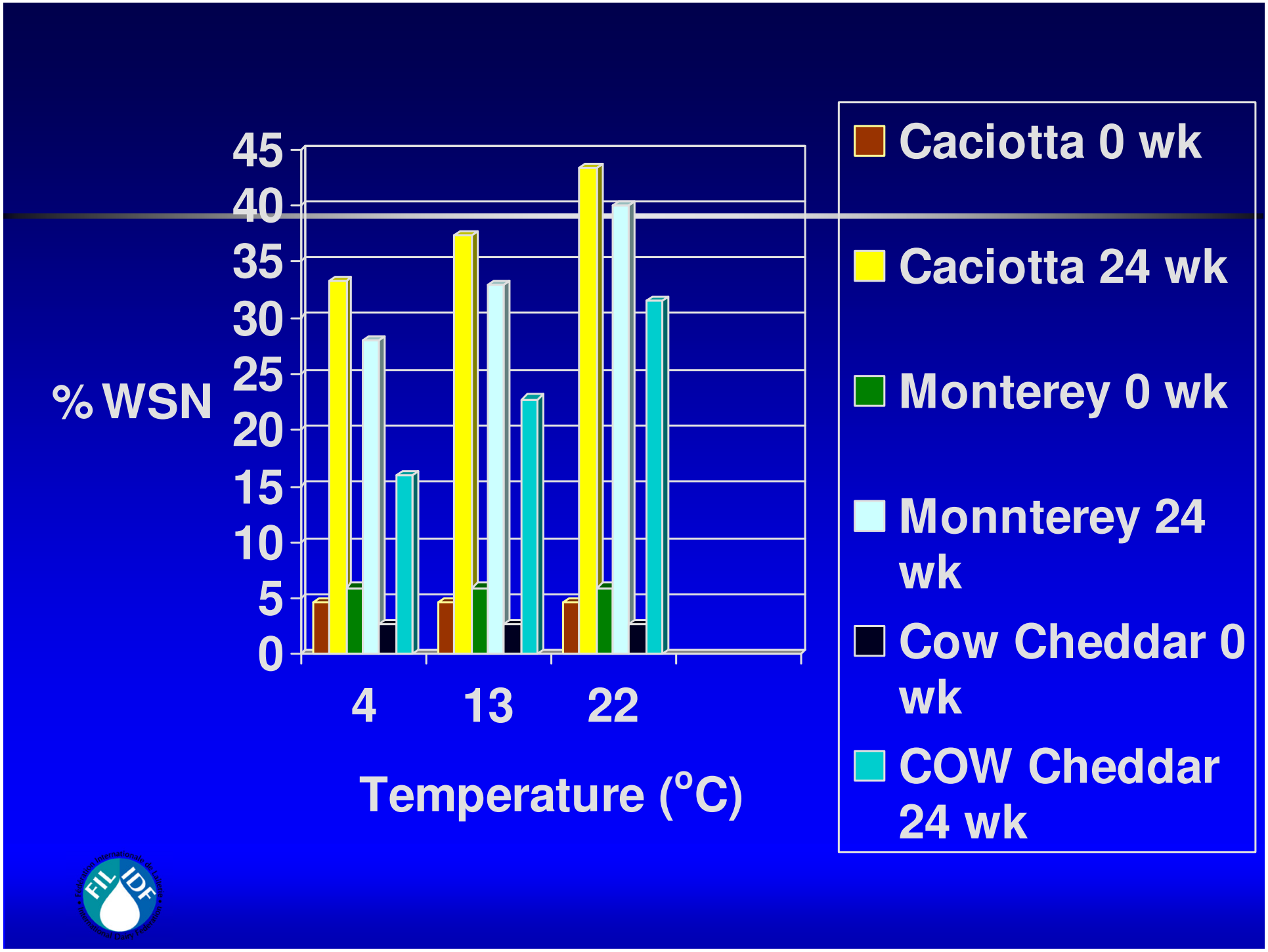
2. Spontaneous Lipolysis:
   a. Milk processing factors; cooling, mixing and separation.
   b. Animal factors; Lactation stage, feed, season, breed, mastitis, milk and fat yield, physiological factor.

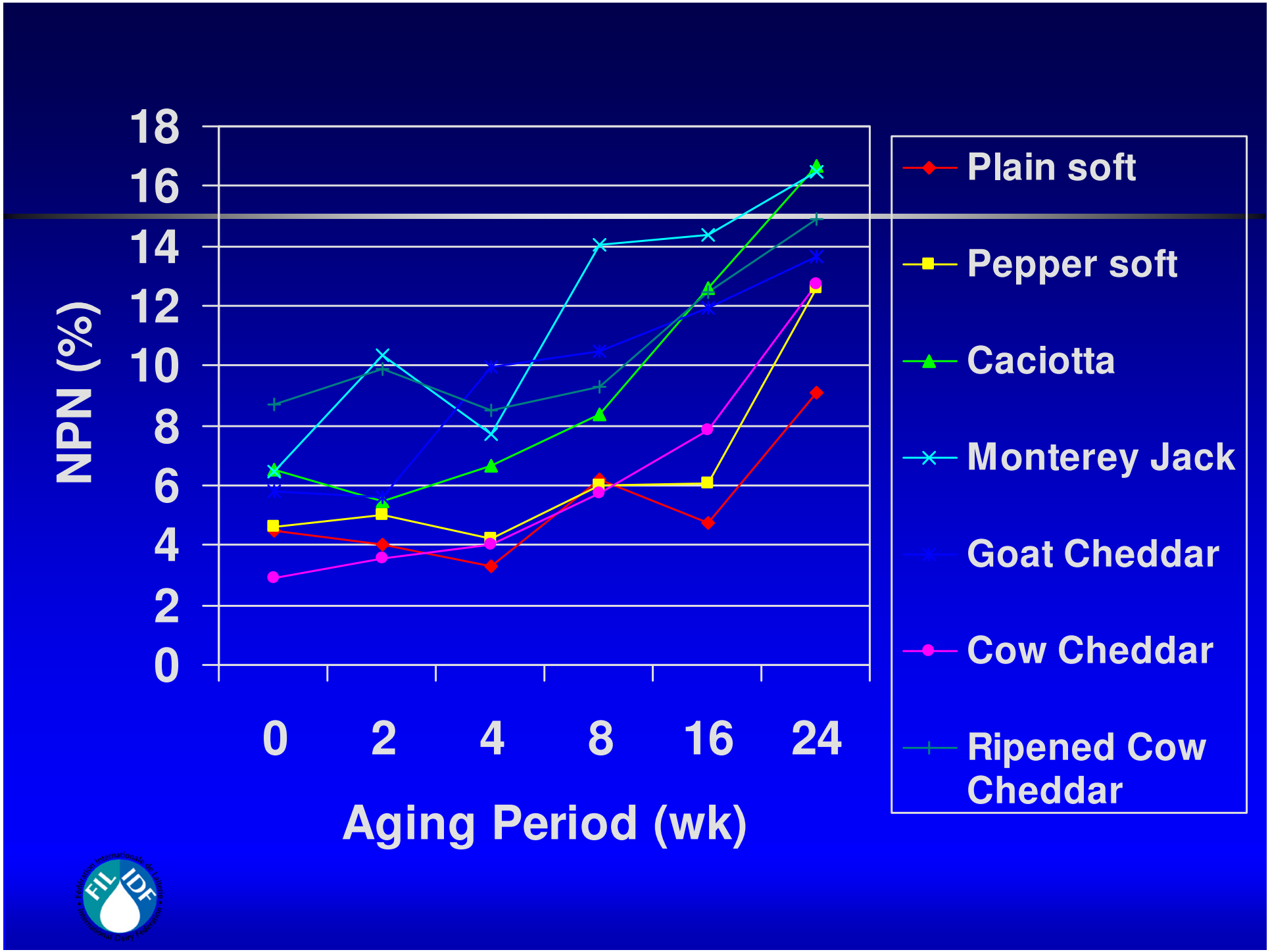
3. Microbial Lipolysis:
   a. Microbial lipases
   b. Psychrotrophic bacterial lipases

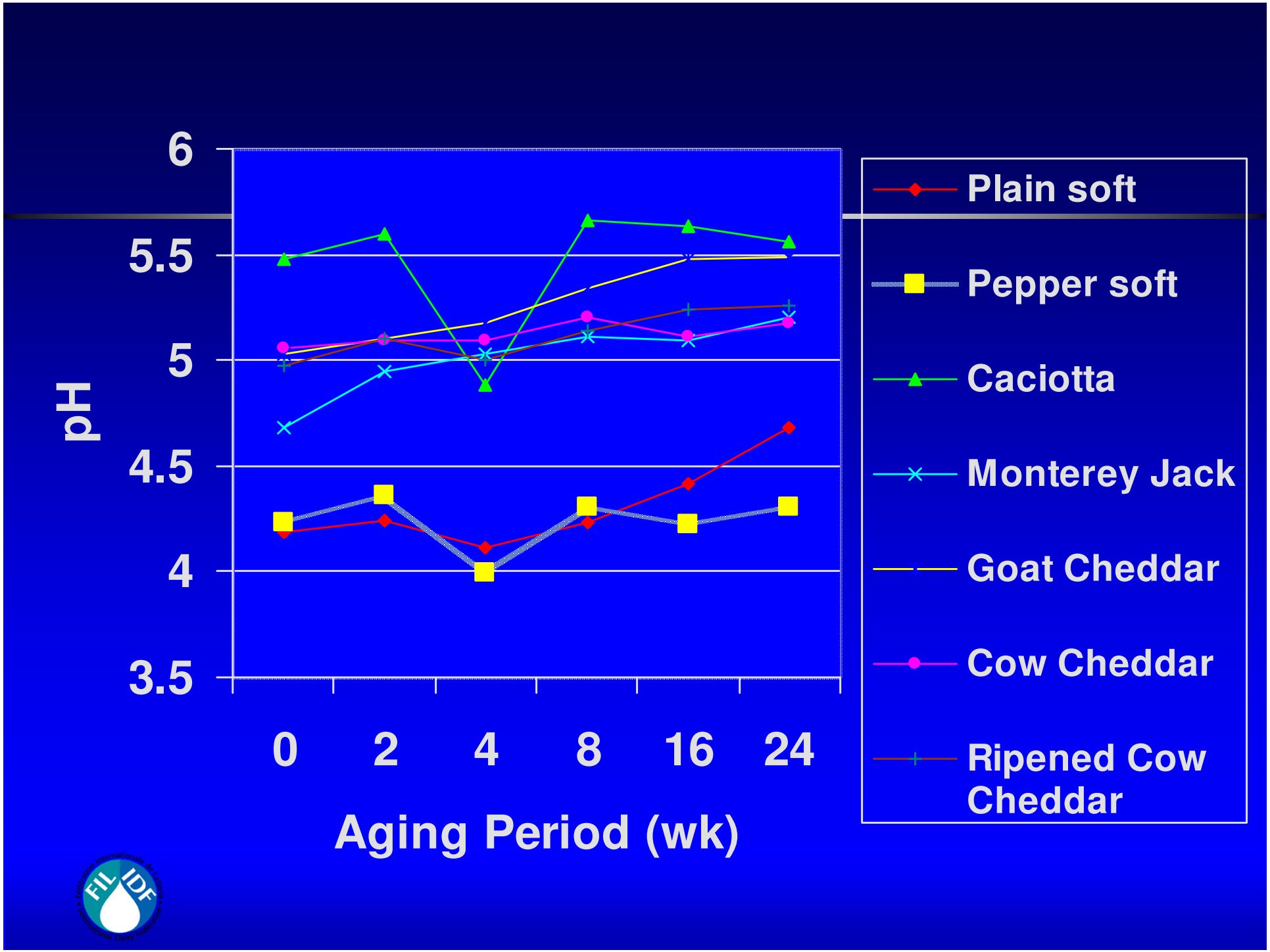
FACTORS AFFECTING LIPOLYSIS IN CHEESE

1. Free fatty acids
2. Lipolytic enzymes
3. Lipolytic microorganisms
4. Temperature
5. Storage time
6. Oxygen concentration
7. Moisture content
8. Presence of Antioxidant and pro-oxidant

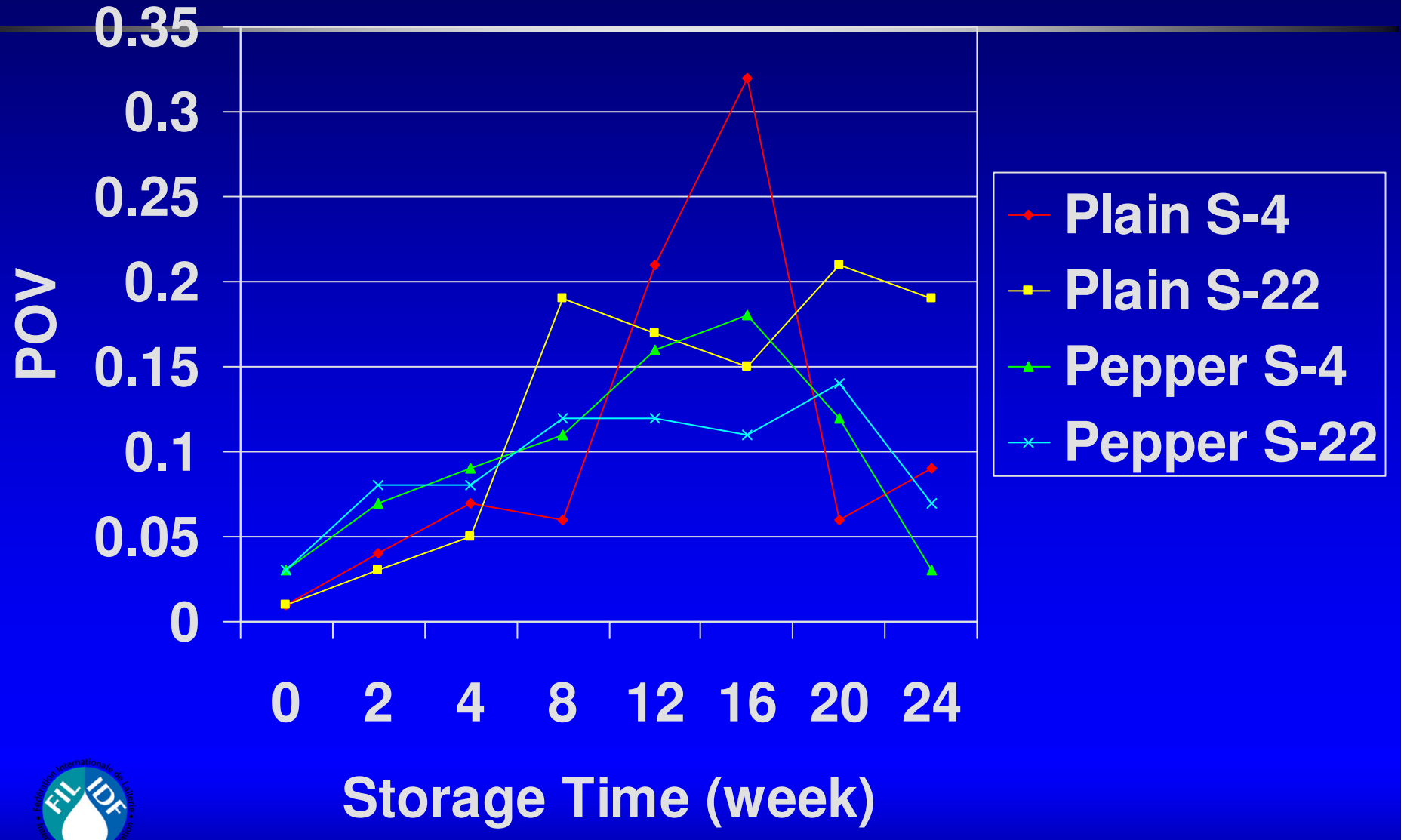

35
30
25
20
15
10
5
0
WSN (%)
0
2
4
8
16
24
Aging period (wk)
Plain soft
Pepper soft
Caciotta
Monterey Jack
Goat Cheddar
Cow Cheddar
Ripened Cow Cheddar


% WSN
45
40
35
30
25
20
15
10
5
0
4
13
22
Temperature (°C)
Caciotta 0 wk
Caciotta 24 wk
Monterey 0 wk
Monnterey 24 wk
Cow Cheddar 0 wk
COW Cheddar 24 wk


NPN (%)
18
16
14
12
10
8
6
4
2
0
0
2
4
8
16
24
Aging Period (wk)
Plain soft
Pepper soft
Caciotta
Monterey Jack
Goat Cheddar
Cow Cheddar
Ripened Cow Cheddar


6
5.5
5
4.5
4
3.5
pH
0
2
4
8
16
24
Aging Period (wk)
Plain soft
Pepper soft
Caciotta
Monterey Jack
Goat Cheddar
Cow Cheddar
Ripened Cow Cheddar

POV of soft goat milk cheeses



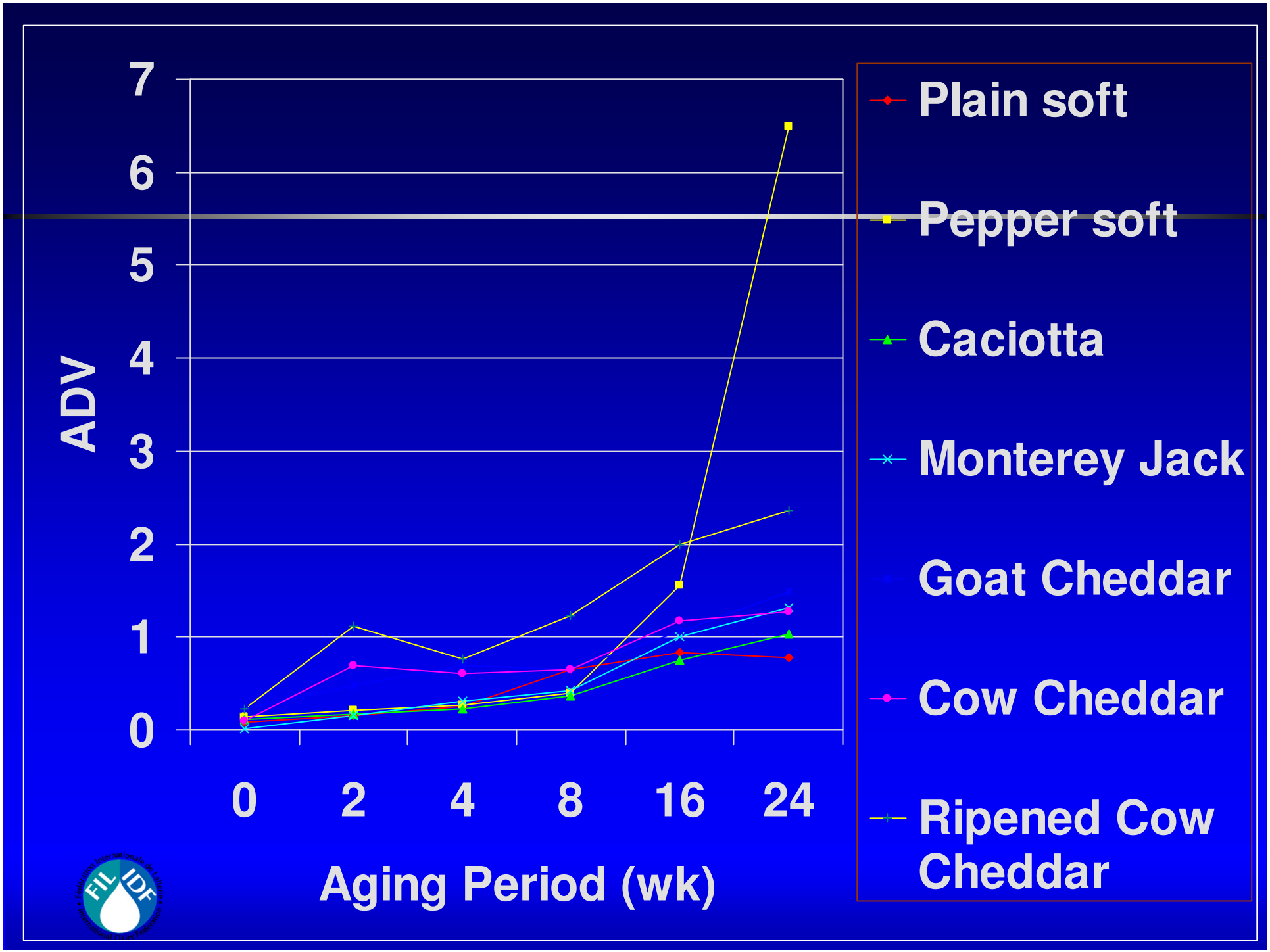
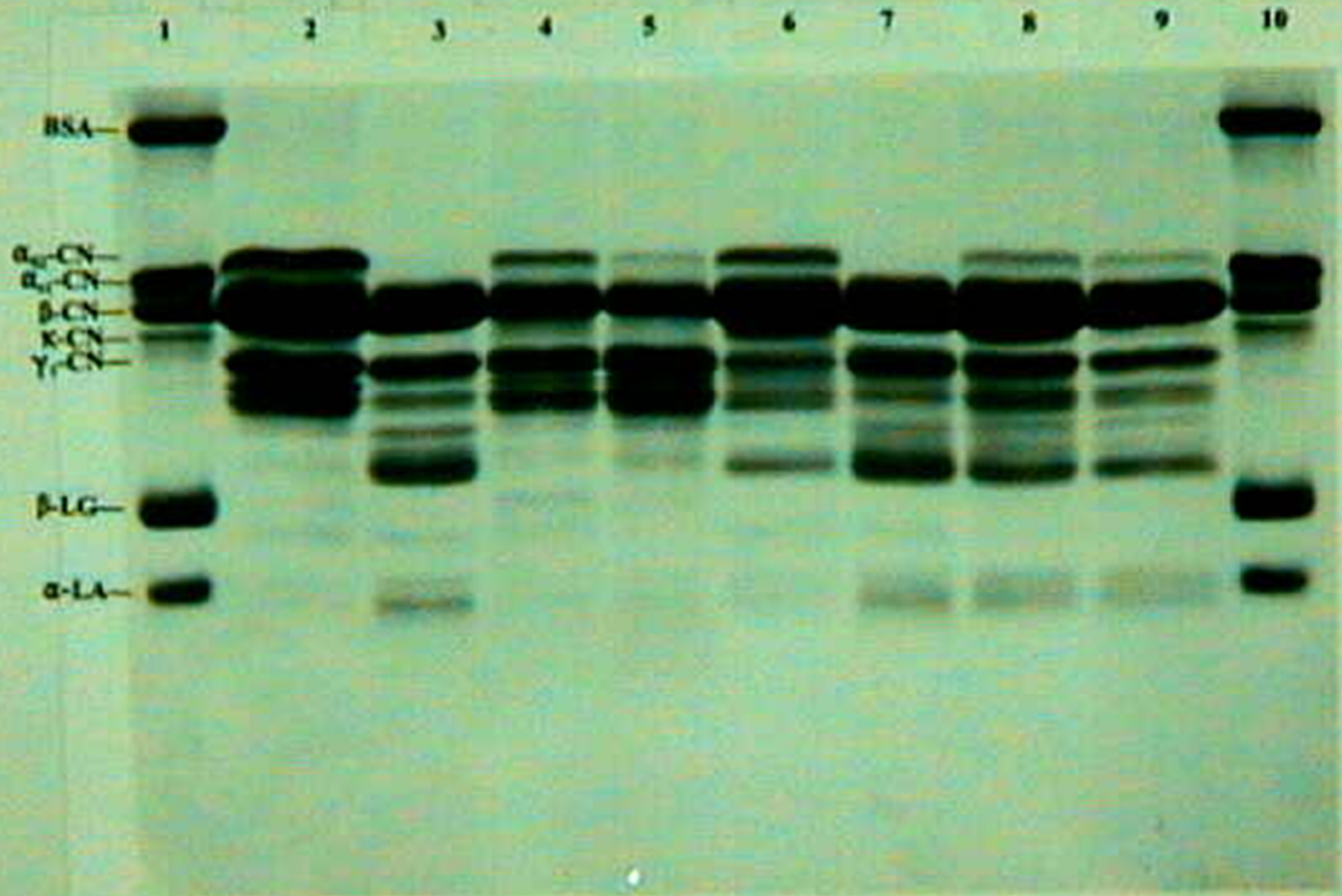

ADV
7
6
5
4
3
2
1
0
0
2
4
8
16
24
Aging Period (wk)
Plain soft
Pepper soft
Caciotta
Monterey Jack
Goat Cheddar
Cow Cheddar
Ripened Cow Cheddar

Figure 1. The SDS-PAGE of proteins in Caciotta and Monterey Jack goat milk cheeses aged at 4°C for 6 months experimental period.



Densitometric values of SDS-PAGE bands of goat milk Monterey Jack Cheese aged at 4°C for 0 and 8 weeks.

Band No.	0 wk	0 wk	8 wk	8 wk
	I.I x Area	% I.I	I.I x Area	% I.I
1. α_{s2}-CN	114.7	6.1	-a	-
2. α_{s1}-CN	-	-	-	-
3. β-CN	6832	74.4	4277	59.9
4. κ-CN	-	-	-	-
5. γ_1-CN	190.6	6.8	423.3	15.3
6.	7.61	1.0	25.9	2.2
7.	131.8	6.0	17.8	1.3
8.	114.5	4.7	542.7	15.5
9. β -LG	14.6	0.5	36.2	1.7
10. α-LA	7.48	0.3	97.2	3.8

Densitometric values of SDS-PAGE bands of goat milk Monterey Jack Cheese aged at 4°C for 16 and 24 wks.

Band No.	16 wk	16 wk	24 wk	24 wk
	I.I x Area	% I.I	I.I x Area	% I.I
1. α_{s2}-CN	13.5	1.1	10.5	0.9
2. α_{s1}-CN	-	-	-	-
3. β-CN	7133	70.8	4378	73.0
4. κ-CN	-	-	-	-
5. γ_1-CN	283.8	10.2	176.3	10.0
6.	172.2	6.0	113.9	3.9
7.	6.04	0.4	8.04	0.5
8.	177.0	7.0	144.7	7.4
9. β-LG	33.6	1.6	37.5	1.9
10. α-LA	84.8	2.9	53.1	2.4

Figure 2. The SDS-PAGE of proteins in Caciotta and Monterey Jack goat milk cheeses aged at 13°C for 6 months experimental period.

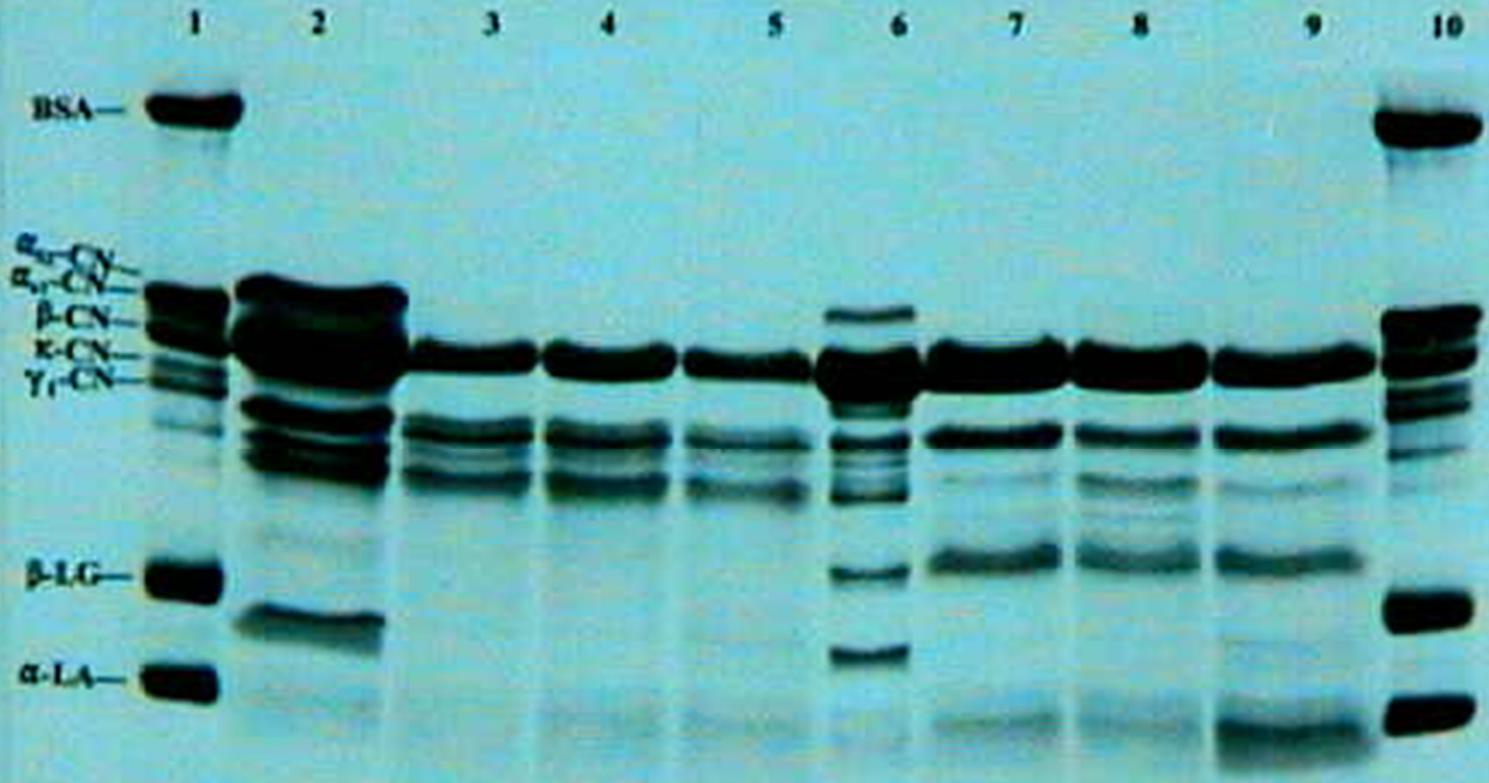
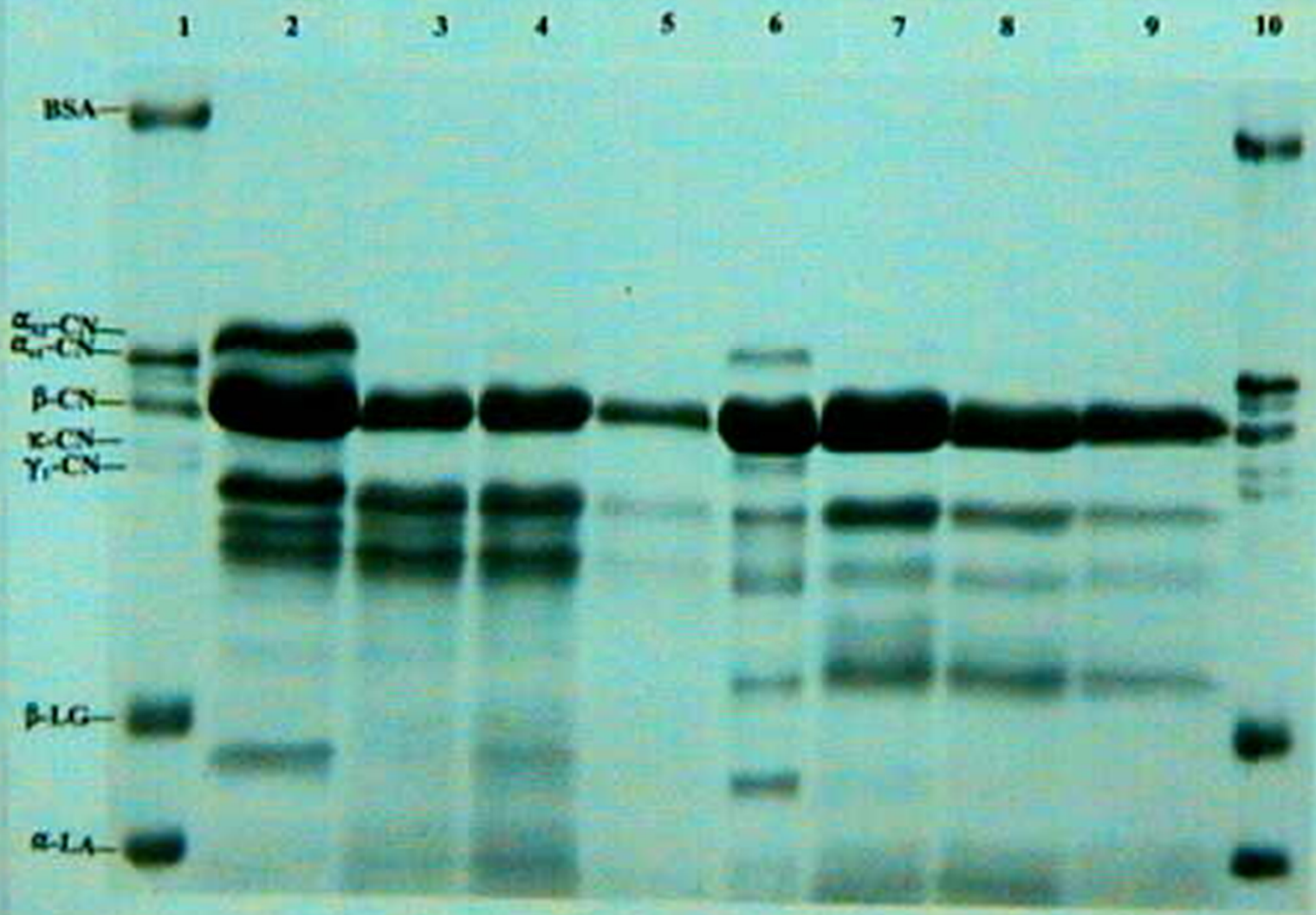


Figure 3. The SDS-PAGE of proteins in Caciotta and Monterey Jack goat milk cheeses aged at 22°C for 6 months experimental period.



Changes in pH 4.6 soluble nitrogen content of bovine and caprine Mozzarella cheese during storage (Imm et al., J. Dairy Sci. 86:2790-2798, 2003)

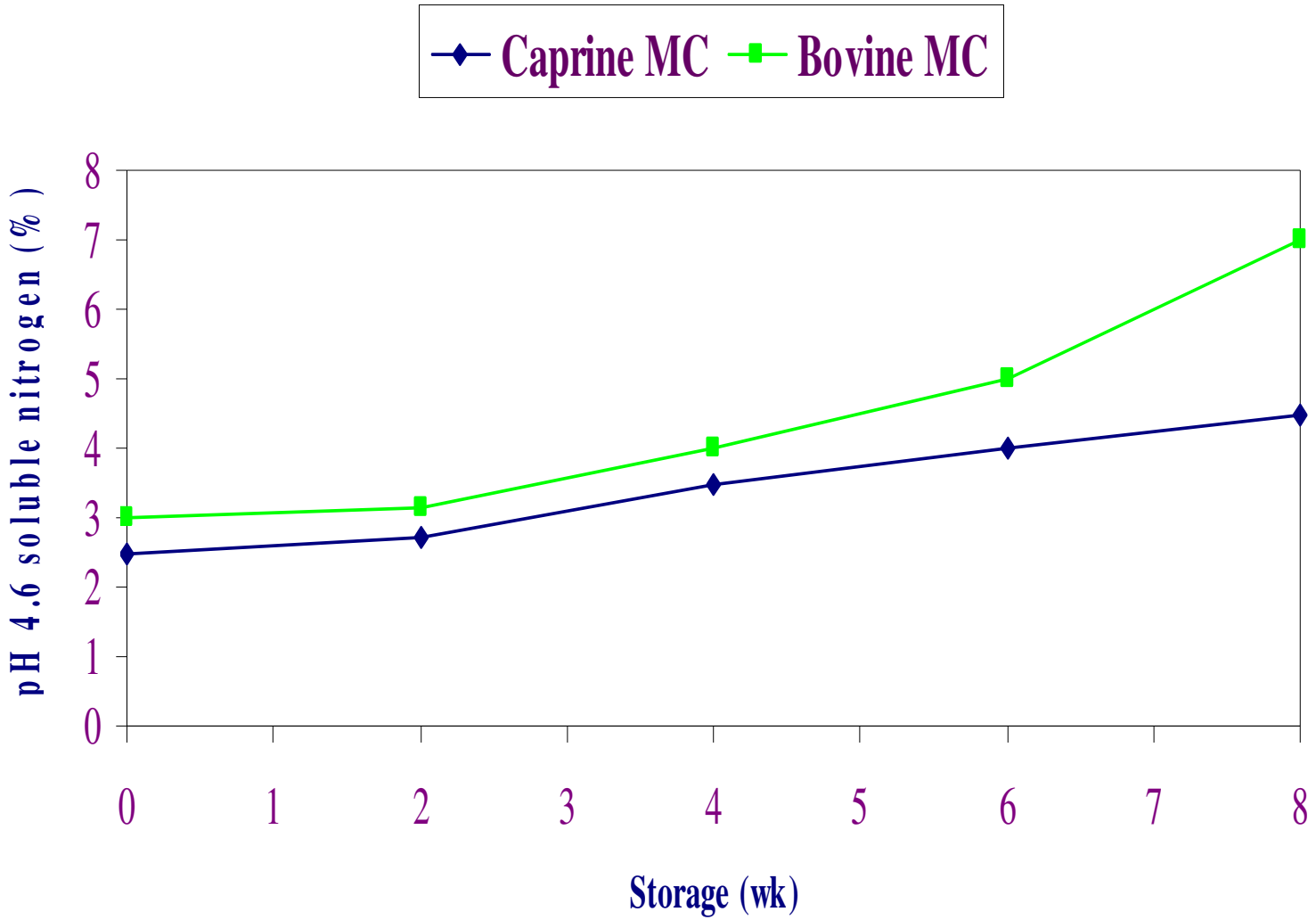


Table 1. Changes in β-CN and water soluble nitrogen (WSN) concentrations of Caciotta, Monterey Jack, Cow and Cheddar cheeses aged at 4°C for 6 months[1]

β -CN[2] & WSN[3]	Aging Period (weeks)			
	0	8	16	24
Caciotta				
β -CN	12,991	4,629	2,217	1,511
WSN	4.61	21.9	29.4	33.3
Monterey Jack				
β -CN	6,832	4,277	7,133	4,378
WSN	5.97	19.3	25.9	28.0
Cow Cheddar				
β -CN	2,447	2,870	1,357	1,140
WSN	2.70	9.92	12.9	15.9

[1]Values are listed only for β -casein at 4oC and all other values for 13oC and 22oC were not included due to space limitations; [2]β -CN: Densitometric values; 3 WSN: Water soluble N.

Correlation coefficients (r) between levels of measured proteolytic indices in cheeses for 6 months of storage period[1].

	NPN	pH	ADV
WSN	0.831**	0.366	0.354
	0.782**	0.840**	-0.444
	0.987**	0.899**	-.556*
	0.984**	0.971**	-0.492
NPN		0.291	0.384
		0.607*	-0.068
		0.932**	-0.522
		0.946**	-0.398
pH			0.218
			690**
			-0.322
			-0.423

[1]r values for the 1st, 2nd, 3rd, and 4th rows represents for the initial, 4°C, 13°C, and 22°C treated groups across all variety and storage periods.
Number of observation was 84.
* significant at 5% level ** significant at 1% level

Mean free fatty acid content (mg/kg) in Majorero goat milk cheese during 90 days ripening period.

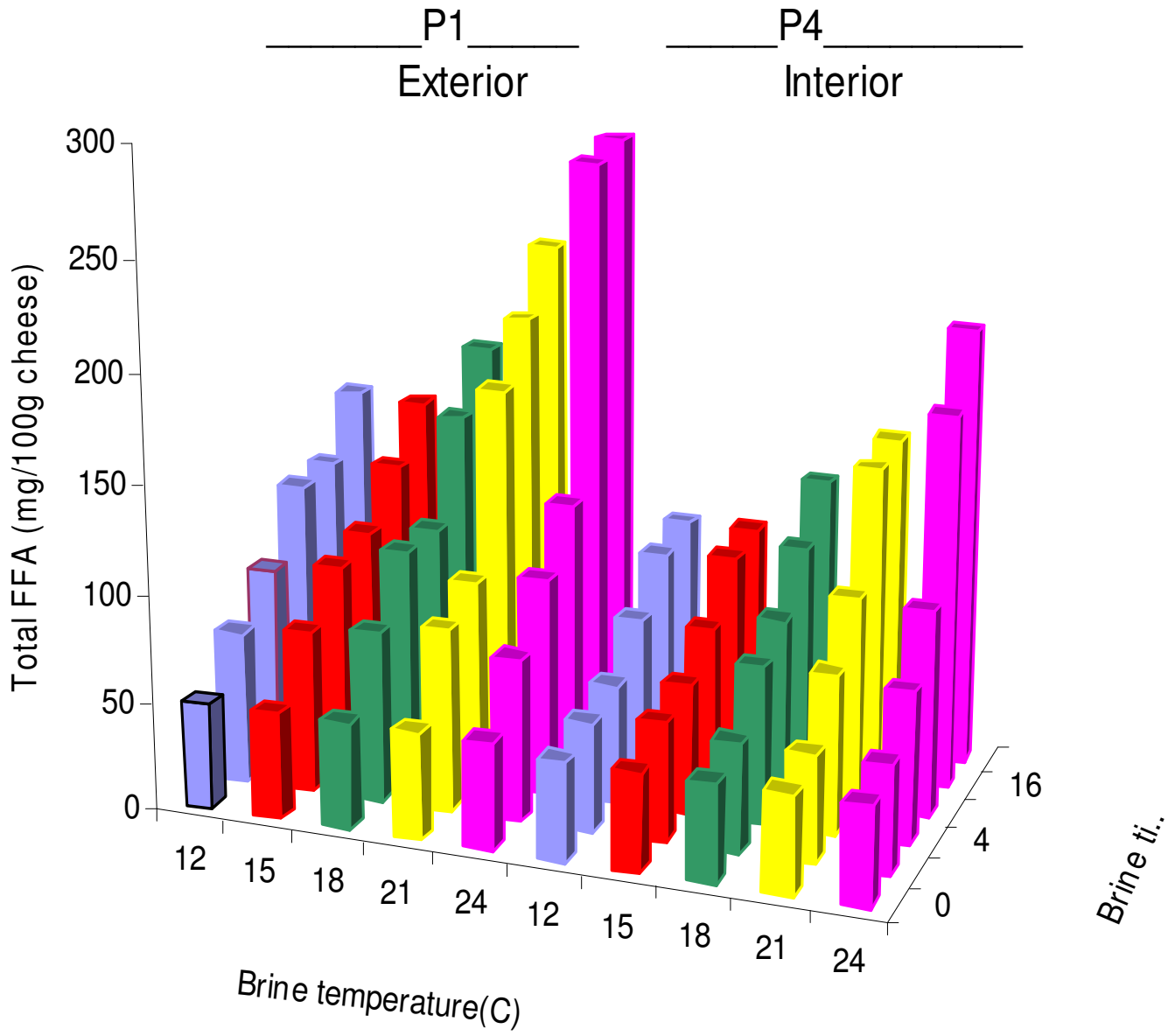
Fatty acid	Curd	2 d	15 d	30 d	60 d	90 d
C_4	153	171	161	343	434	744
C_6	273	305	248	371	495	709
C_8	194	189	196	430	646	1134
C_{10}	576	614	672	1608	2433	4187
C_{12}	249	300	400	913	1671	2399
C_{14}	388	554	986	1720	3256	4045
C_{16}	890	1439	1751	4346	7664	9507
C_{18}	252	403	834	1237	1806	2206
$C_{18:1}$	689	1115	1472	3559	5542	7053
Total	3664	5090	6720	14527	23947	31984

Fontecha et. al. (J. Dairy Sci. 73:1150-57, 1990)

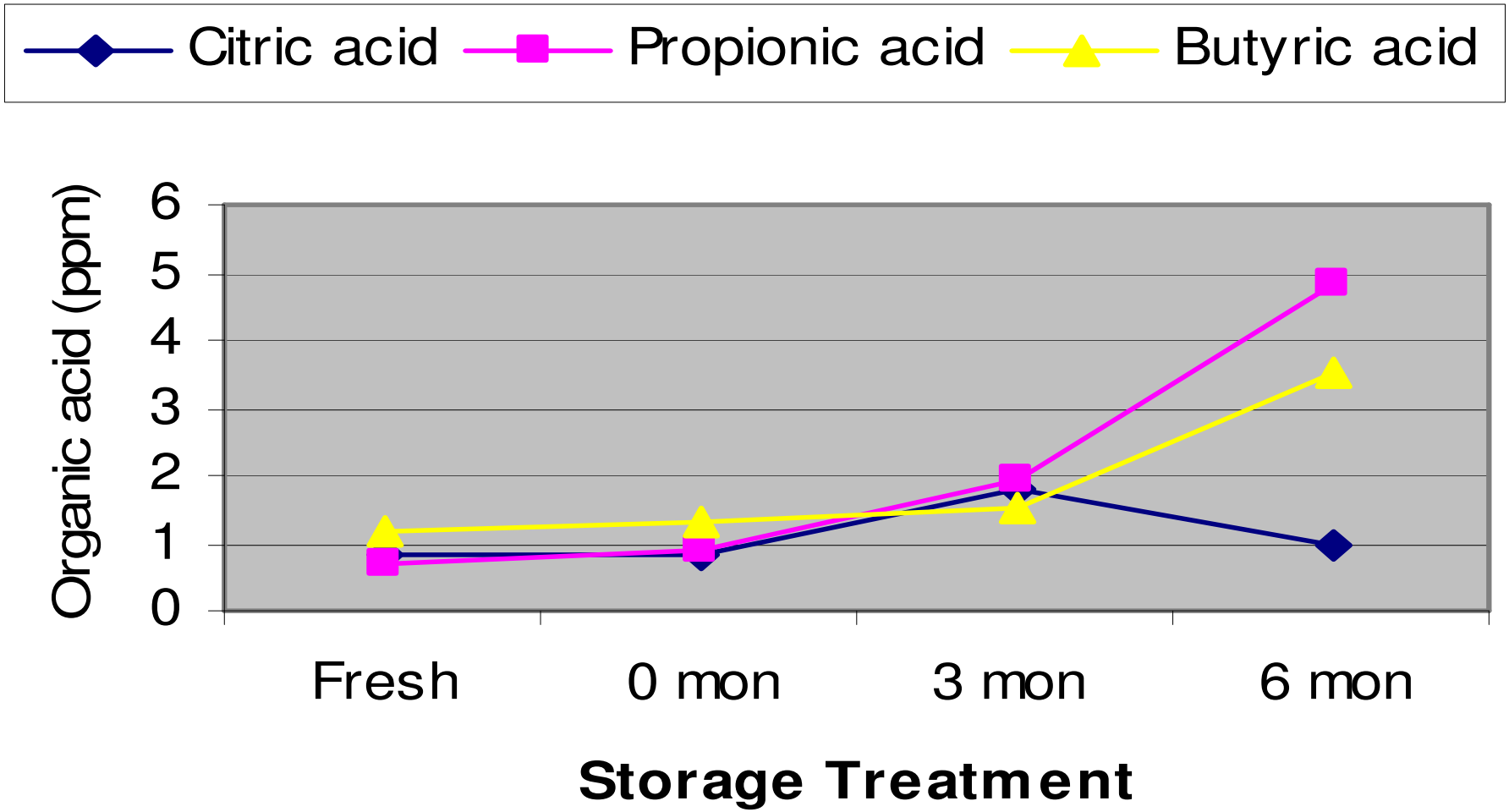
Concentrations (ug/g) of short chain free fatty acids in Cheddar goat milk cheese aged for 6 months period

Free fatty acid	0 wk	6 wk	12 wk	18 wk	24 wk
C_4	19.7	30.4	41.8	34.9	34.8
C_6	38.6	60.5	80.4	70.1	81.0
C_8	75.6	106.9	135.7	128.7	127.5
C_{10}	349.8	432.7	593.8	528.6	539.3
C_{12}	180.1	241.3	299.3	277.6	297.3

Attaie and Richter (J. Dairy Sci. 79:717-24, 1996)


P1
P4
Exterior
Interior
300
250
200
150
100
50
0
Total FFA (mg/100g cheese)
12
15
18
21
24
12
15
18
21
24
Brine temperature(C)
16
4
0
Brine ti..

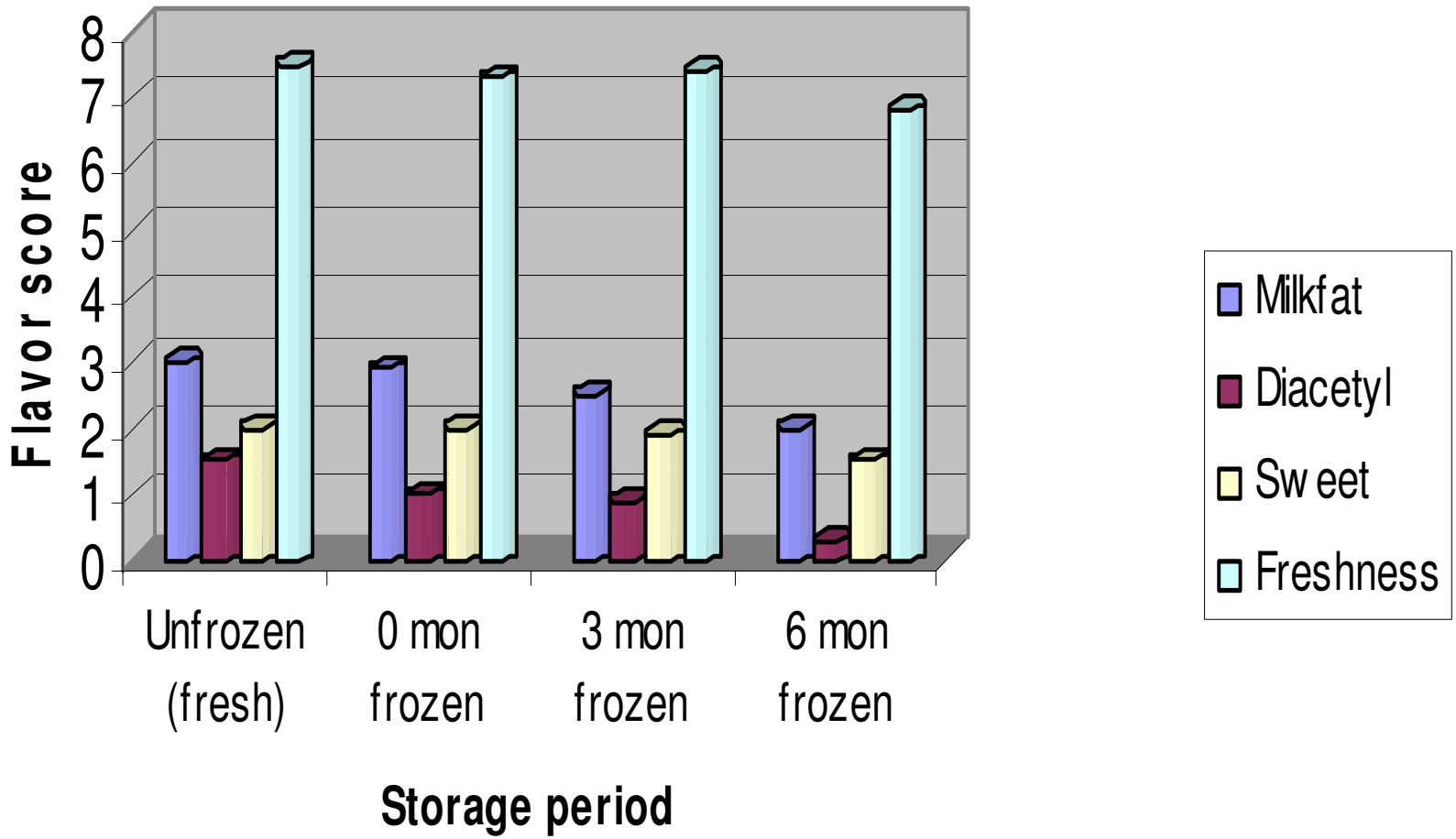
Changes in organic acids (pooled data) of soft goat cheese during storage treatment



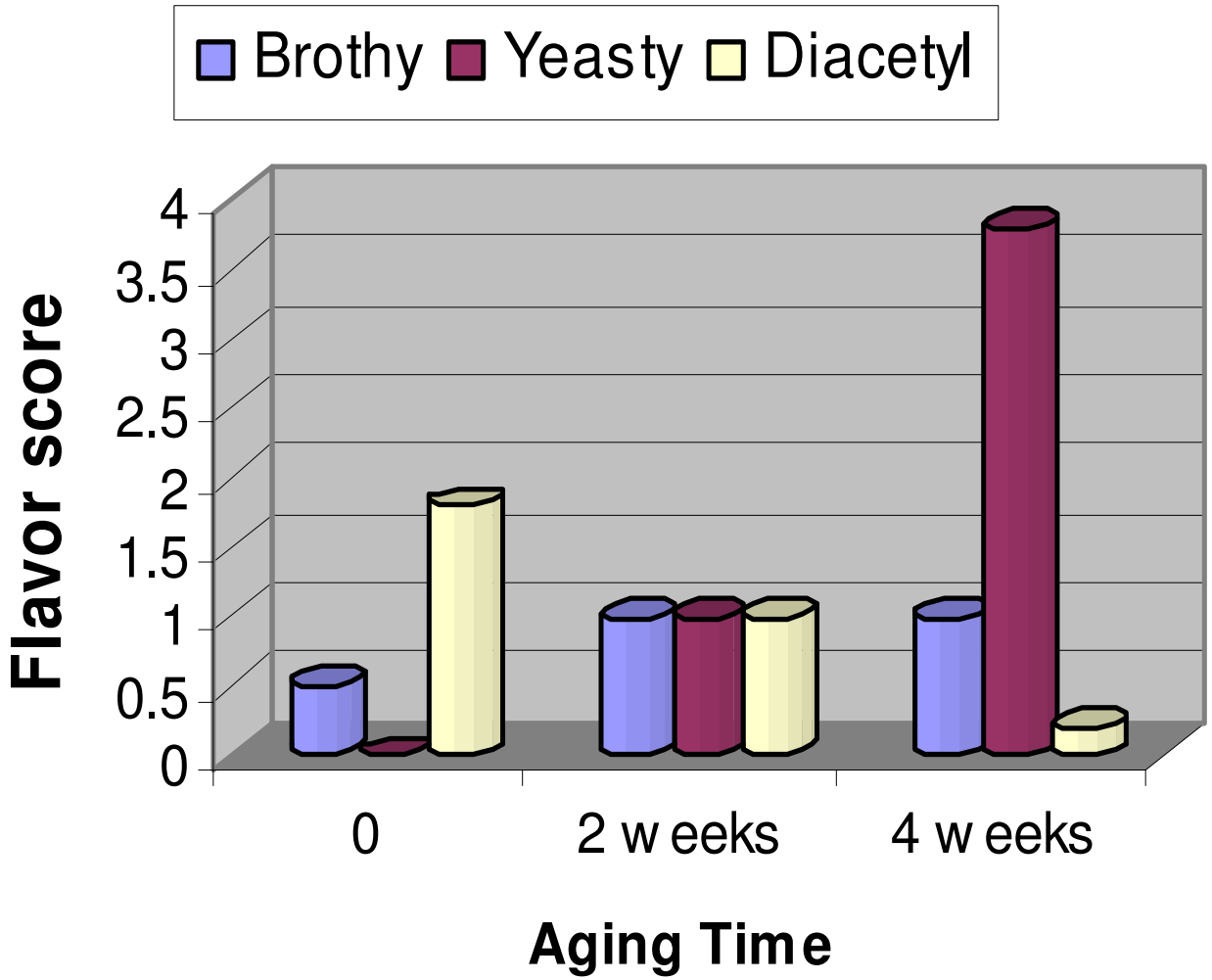
Analysis of variance on effects of treatments and interactions on different organic acid contents.

Effect	Acetic Acid	Butyric Acid	Citric Acid	Formic Acid	Lactic Acid
Cheese type	15.7***	**164.8***	0.03	4.21*	3.33
Storage	5.36**	20.2**	4.86**	2.59	8.5***
Aging	1.46	1.18	0.24	0.27	0.31
Type x storage	7.67**	16.2***	6.02**	2.10	2.46
Type x age	0.29	1.50	1.26	0.85	0.29
Storage x age	1.53	0.44	0.51	0.07	0.85
Type x Storage x age	1.14	0.71	1.48	0.37	1.41

Comparison of flavor scores after frozen-storage treatments



Effect of storage at 4oC on flavor score in FS cheese



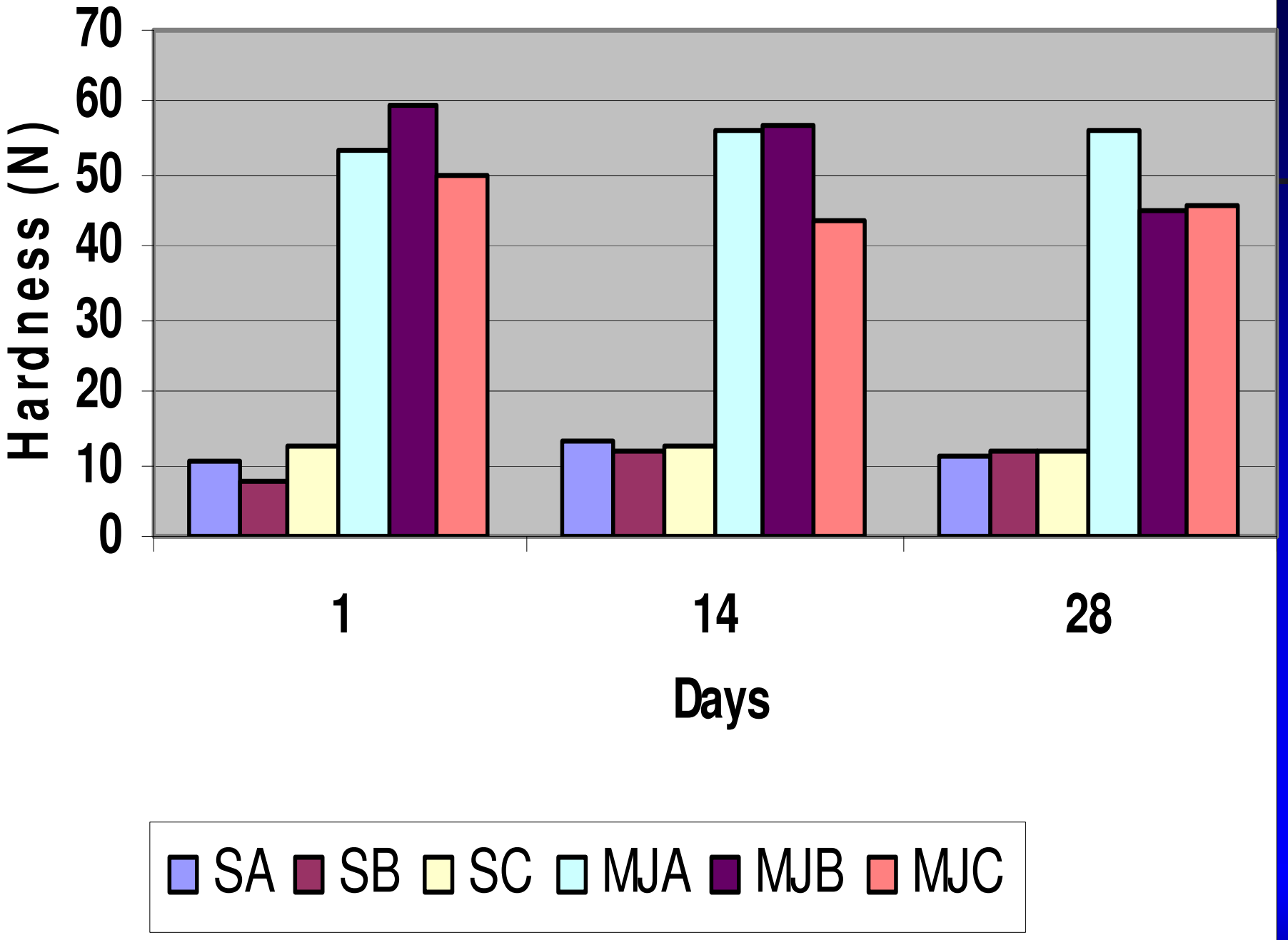
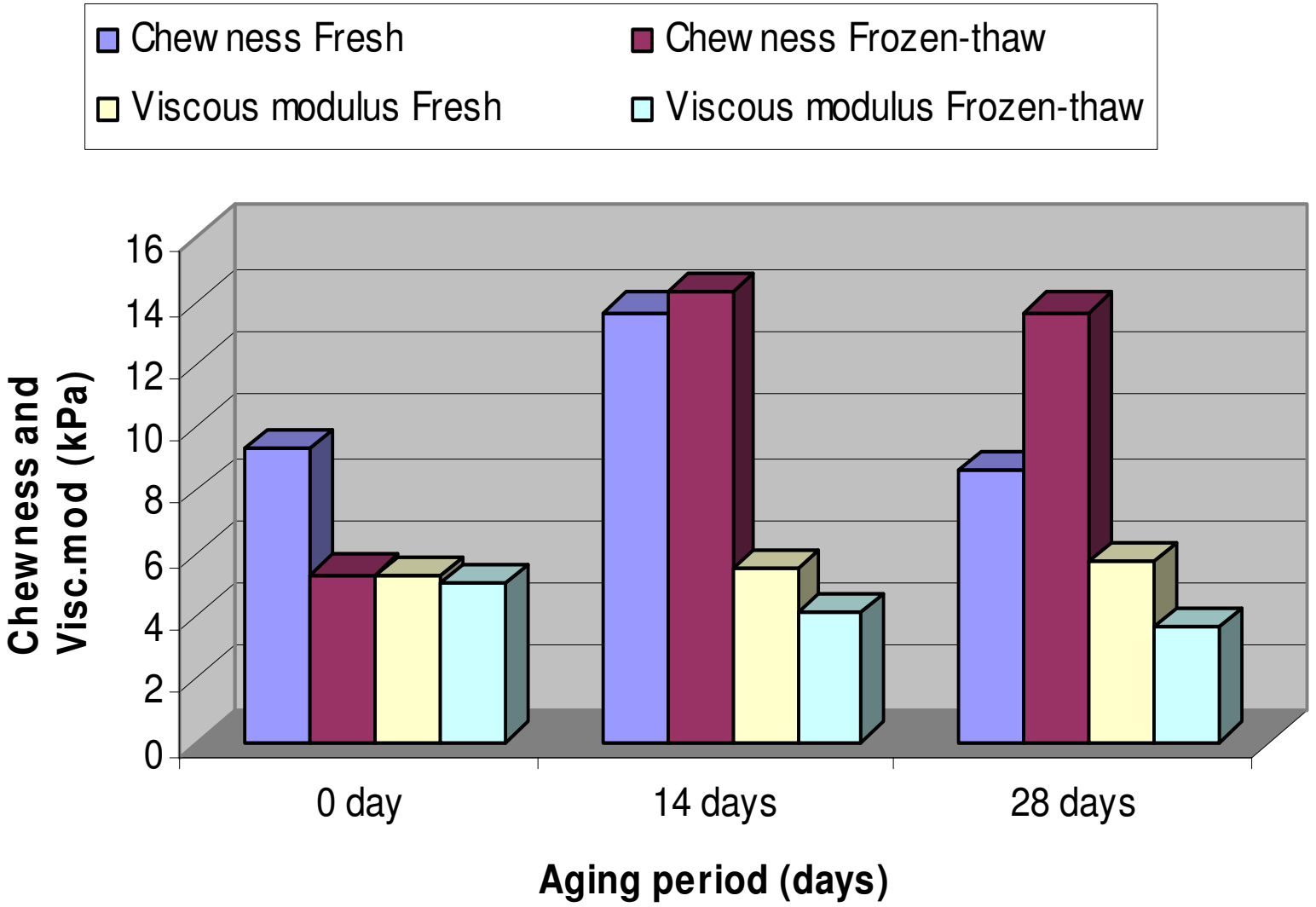

70
60
50
40
30
20
10
0
Hardness (N)
1
14
28
Days
SA
SB
SC
MJA
MJB
MJC

Fig 1c. Comparison of chewness and viscous modulus of fresh soft goat cheese with those of frozen-thawed ones during 4 wks aging at 4oC.



SUMMARY

1. Proteolysis, lipolysis and glycolysis are the three major biochemical events, which are responsible for a variety of chemical, textural, rheological and flavor changes occurring during storage of goat milk cheeses.

2. Prolonged storage of goat cheeses caused elevation of proteolytic and lipolytic indices such as WSN, TCA-SN, low-molecular-weight peptides and free fatty acids.

3. Strong positive correlations were observed between increased levels of WSN, AB-SN, TCA-SN, and extent of proteolysis in goat milk cheeses.

4. Increased degradation of β-casein of goat milk cheeses resulted in a concomitant increase in many degraded low-molecular-weight protein bands.

SUMMARY-Cont'd

5. Storage temperature and time had a synergistic effect on elevation of WSN, TCA-SN, FFA, and ADV in goat cheese.

6. Degradation patterns of electrophoretic protein bands of goat cheeses during storage showed a strong inverse correlation between increase in soluble N and disappearance of large molecular casein fractions.

7. Our studies confirmed that goat milk cheeses contained much higher α_{s2}-CN and β-CN than cow milk Cheddar cheese, while the latter showed a distinctive α_{s1}-CN band.

8. Lipolytic parameters (i.e., FFA and ADV) in goat cheeses also increased with advanced storage period and temperature, as did in proteolytic indices.

SUMMARY-Cont'd

9. Goat cheeses showed larger increases in WSN compared to cow milk Cheddar as aging progressed, indicating that goat cheeses may undergo more proteolysis, probably due to the higher moisture contents and soft cheese protein matrices.

10. The frozen-control (0 mon) goat cheese had lower rheological and textural values compared to the unfrozen fresh control.

11. As storage time advanced, the internal structure matrix of Monterey Jack goat cheese became less rigid with increased meltability and decreased hardness, shear stress and rigidity.

12. Although several organic acid levels were changed in goat cheeses, prolonged frozen-storage up to 6 months appeared to be feasible due to its minimal impact on sensory scores.