Milk Coagulants

Cheese & Fromage: Common Cultures

MONTRÉAL

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Agenda

- Review of history and chemistry of coagulation
- Properties of coagulants
- Use of coagulants
- So.. What kind of variety of cheese do you want to make and what make procedure are you going to use to make it?

History

- The first commercially available, standardized calf rennet was produced by Christian D.A. Hansen in 1874.
- In 1972, the FDA permitted the use of microbial coagulants as availability of calf abomasums became limited.
- In 1980's, Pfizer, Hansen/Genencor, and DSM developed Fermentation Produced Chymosin (FPC).
- Between 2000 and 2002, FPC became increasing popular due to mad cow and hoof-and-mouth concerns.
- FPC is currently used in over 90 % of the cheese made in the U.S.
- 2008 – Introduction of ChyMAX M non-bovine chymosin

Nomenclature

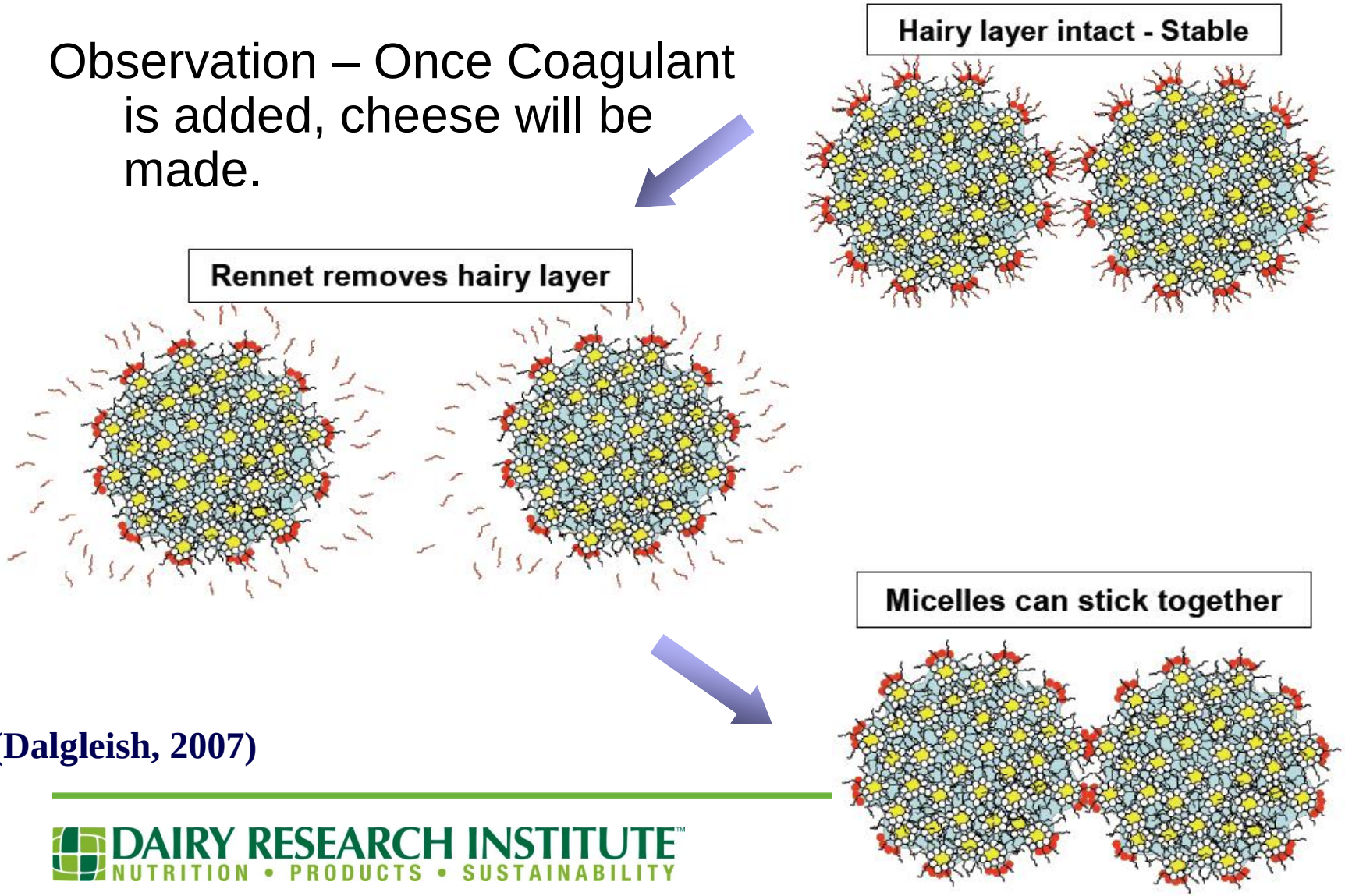
- **“Rennet” – Generic term for animal derived coagulant. A mixture of chymosin and pepsin.**
- **“Rennet Paste” – Traditionally made from calves that had just suckled so had both chymosin and pregastric lipase.**
- **Chymosin – The main gastric protease in young ruminants. (E.C. 3.4.23.4)(M.W.= 35,600)**
- **FPC – Fermentation Produced Chymosin – Chymosin produced in either yeast or fungi instead of calves.**

Nomenclature

- **Pepsin – The main gastric protease in adult animals including pigs, cattle, and chickens.**
- **Microbial –(Mucor types) from *Rhizomucor miehei* or *pusillus***
- **Microbial –(Endotheia) – from *Cryphonectria parasitica***
- **Cardoon (Vegetable) from Cynara (thistle)**

Enzymatic destabilizing of casein micelles

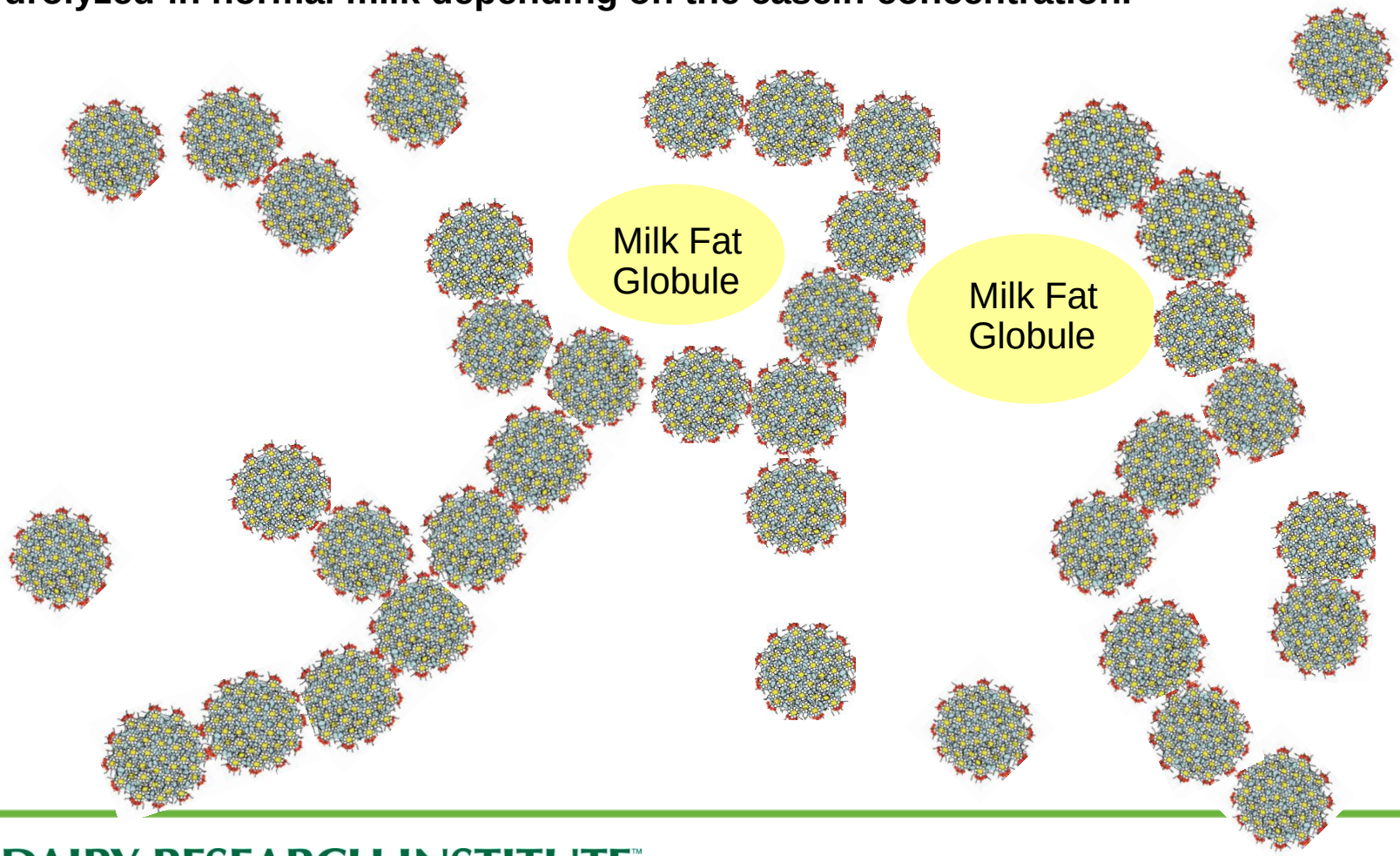
Observation – Once Coagulant is added, cheese will be made.



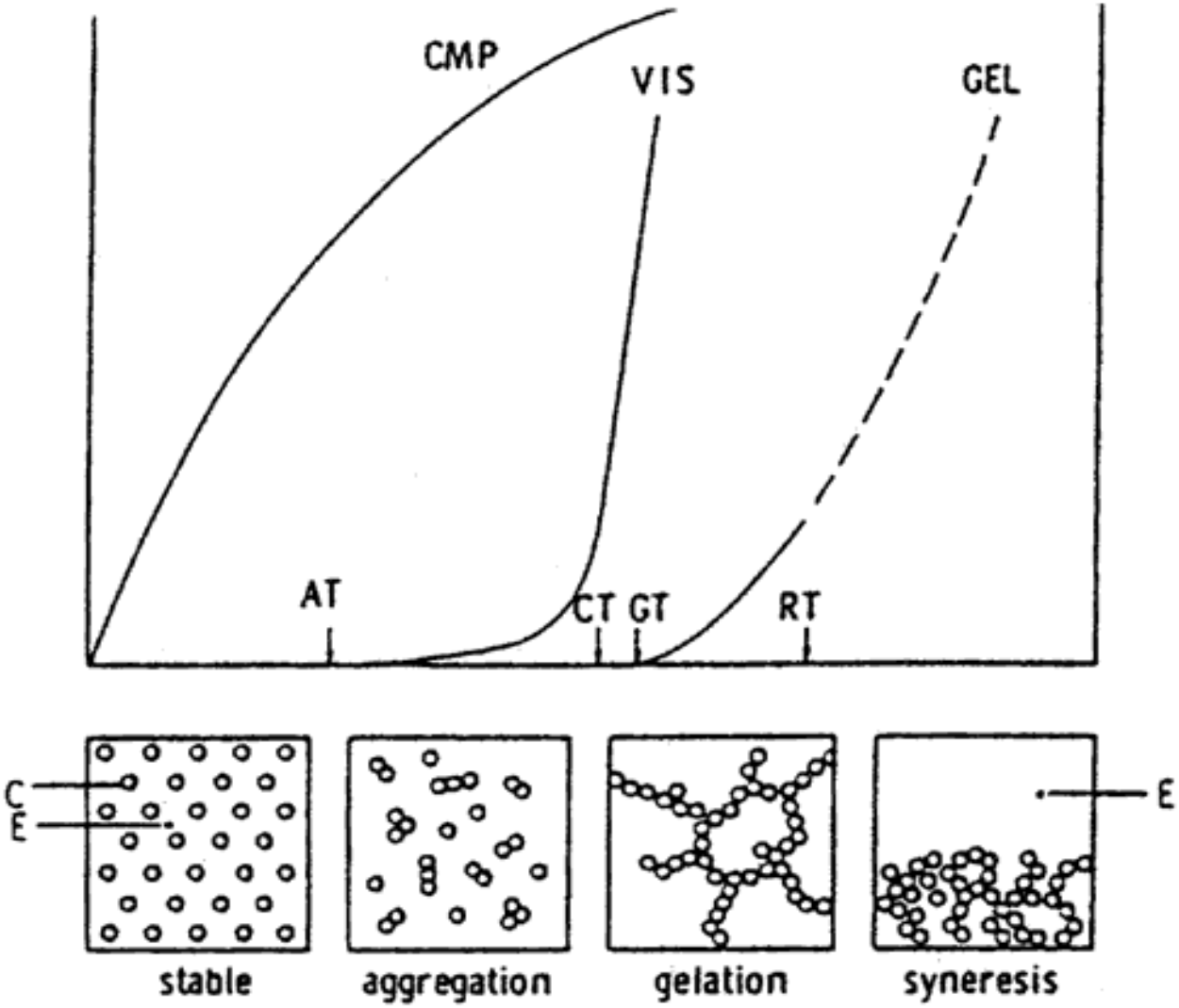
(Dalgleish, 2007)

3 D aggregation of casein micelles ➔ curd

The coagulation reaction is delayed until 60 to 75 % of surface k-casein is hydrolyzed in normal milk depending on the casein concentration.



Reactions Occurring During Renneting



Coagulant Comparison Chart

Coagulant Type	Calf Rennet & Bovine Rennet	Fermentation Produced Chymosin	ChyMAX M	Microbial (Rhizomucor)	Microbial (Cryphonectria)
Milk Clotting Enzyme	Chymosin & Pepsin	Chymosin	Chymosin, non-bovine	Mucorpepsin	Endothia-pepsin
Certification	Limited Halal	Halal, Kosher, Vegetarian	Halal, Kosher, Vegetarian	Halal, Kosher, Vegetarian	Halal, Kosher, Vegetarian
Casein Specific Activity	+++ depending on enzyme ratio	++++	+++++	++	+
Proteolytic Activity	+++ depending on enzyme ratio	++	+	++++ (depending on type)	++++++
Concerns	Animal Product	GM Host	GM Host	Reduced Yield / Bitterness	Reduced Yield / Bitterness (Swiss OK)

Effect of Coagulant on Mozzarella

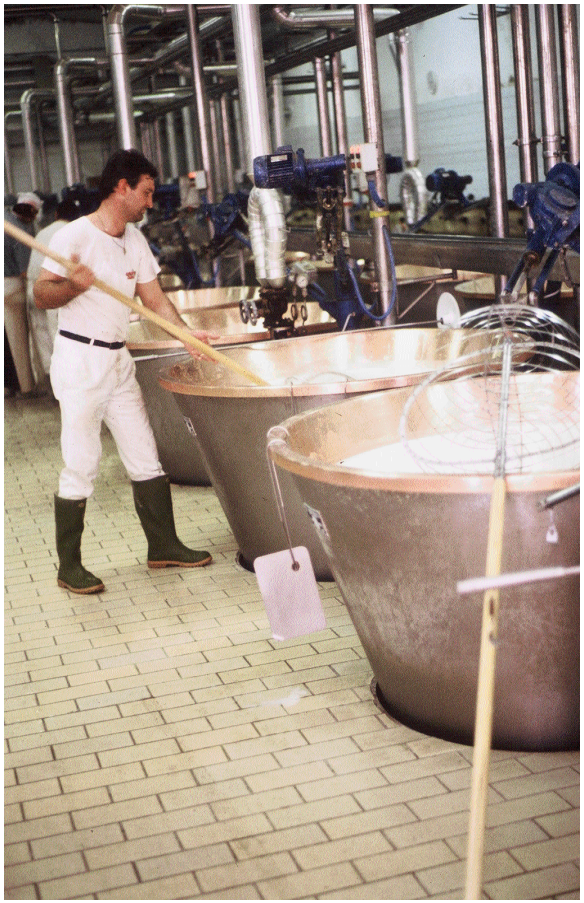
	Chymosin	Mucor	Endothia
α Casein	+	+	+
β Casein	-	-	+
Proteolysis (12% TCA)	8%	7%	14%
Melt Diameter	50mm	50mm	57mm
Free Oil	27%	27%	35%

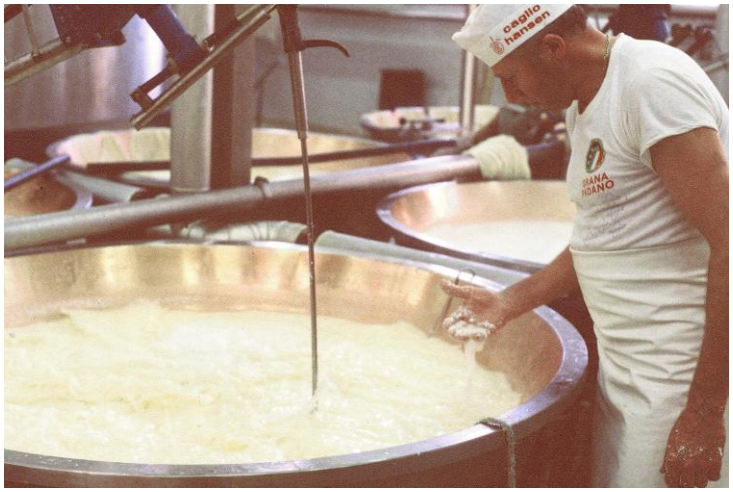
* Drain pH = 6.40 ; Stretch Temperature - Curd = 55°C

Yield Loss Compared to FPC

Coagulant	Difference (kg cheese / 100 kg milk)	Kg Cheese / M kg milk	Difference in Cheese (kg)
Fermentation Chymosin	0.00	100,000	0
Bovine Rennet	- 0.08	99,200	- 800
R. miehei	- 0.25	97,500	- 2,500
R. pusillus	- 0.26	97,400	- 2,600
C. parasitica	- 0.52	94,800	- 5,200

Use considerations





Coagulant Use Instructions - Dilution

- **Dilute 1:20 to 1:40 with water in a clean, sanitized container.**
  - **Prefer distilled or demineralized water**
  - **1 quart of clean water per 1000 lb. of milk**
- **Chlorine**
  - **Rennet - 2 PPM chlorine - 40 % lost in 3 min.**
  - **Rennet - 5 PPM chlorine - 60 % lost in 3 min.**
- **pH of Hard Water > 7.00**
  - **Coagulants are unstable above pH 6.5.**
  - **$CaCl_2$ Solutions and Annatto are above pH 7**
- **Add enzyme to water, minimally but completely mix, add immediately to milk**

Coagulant Use Instructions – Addition to the vat

- Add diluted coagulant to the vat with sufficient agitation to ensure proper distribution.
  - Typically 5 minutes
- Stop all agitation and let vat set.
- Test vat before cutting.

Coagulant Use Instructions – Cutting the vat

- **Methods of Testing**
  - **Time**
  - **Finger or Knife Method**
  - **Rennet Cup**

Factors that Effect Rate of the Reactions

- Use level
- Temperature
- pH
- Calcium Concentration-Summer milk slower firming
- Casein Concentration
- Inhibitors to coagulation – Heat Treatment & Calcium

Use considerations -coagulant strength

- FPC is typically packaged at 600 IMCU / gm. (3 mg protein / gm.). Double the strength of calf rennet.
- Coagulants are typically used at:
- ~ 1.5 to 3 oz. / 1000 lbs. Milk (45 to 90 gm. / 500 kg milk) @ single strength equivalent.

Temperature of Storage

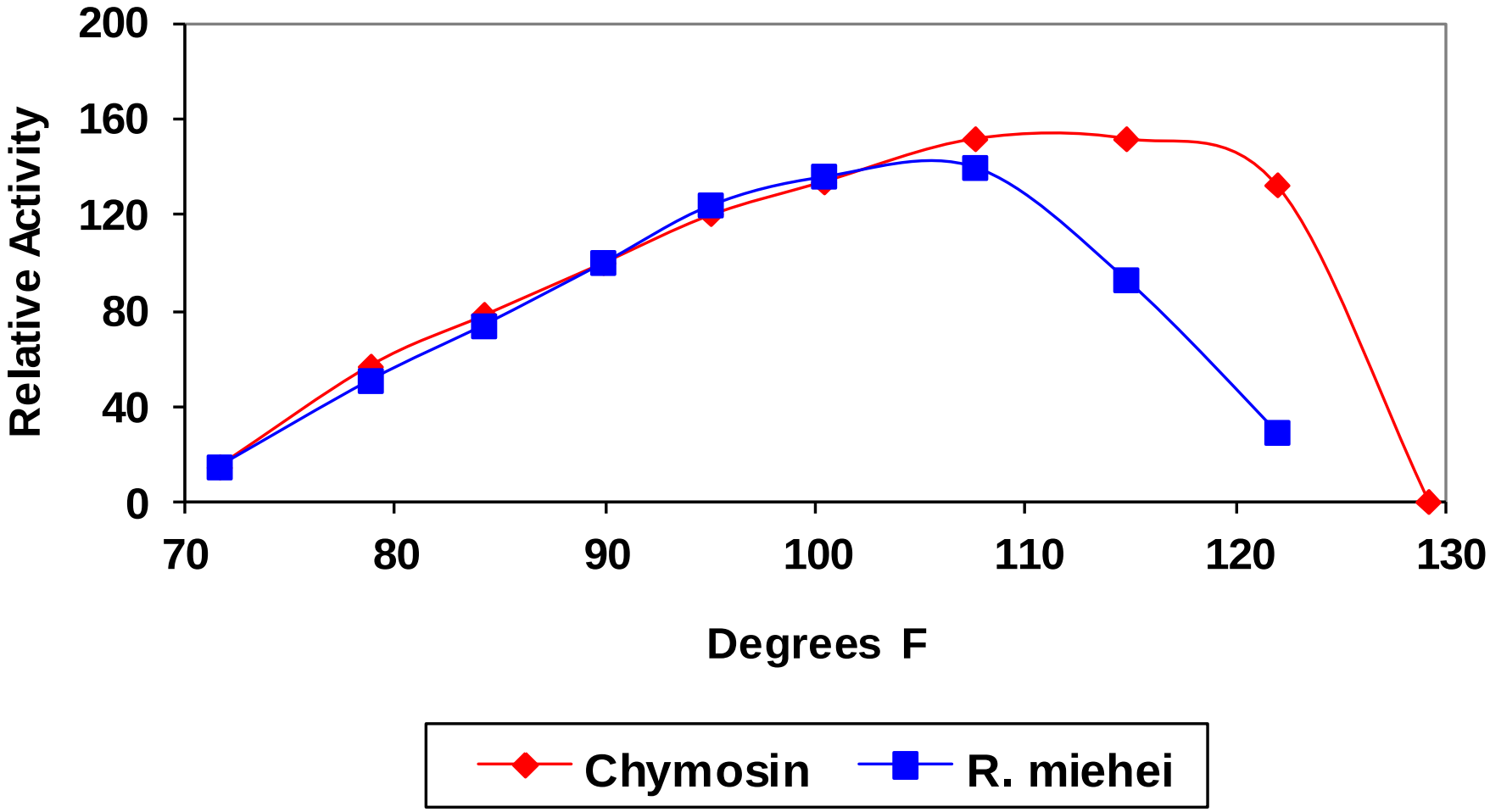
- Recommended 40 - 45 ^{o} F in package
  - Contamination risk on re-use

- % Loss in One Month

Temp.	Rennet	Microbial
50 ^{o} F	0 %	0 %
70 ^{o} F	1 %	1 %
85 ^{o} F	3 %	2 %

Barbano, 1986

Effect of Temperature on Coagulant Activity



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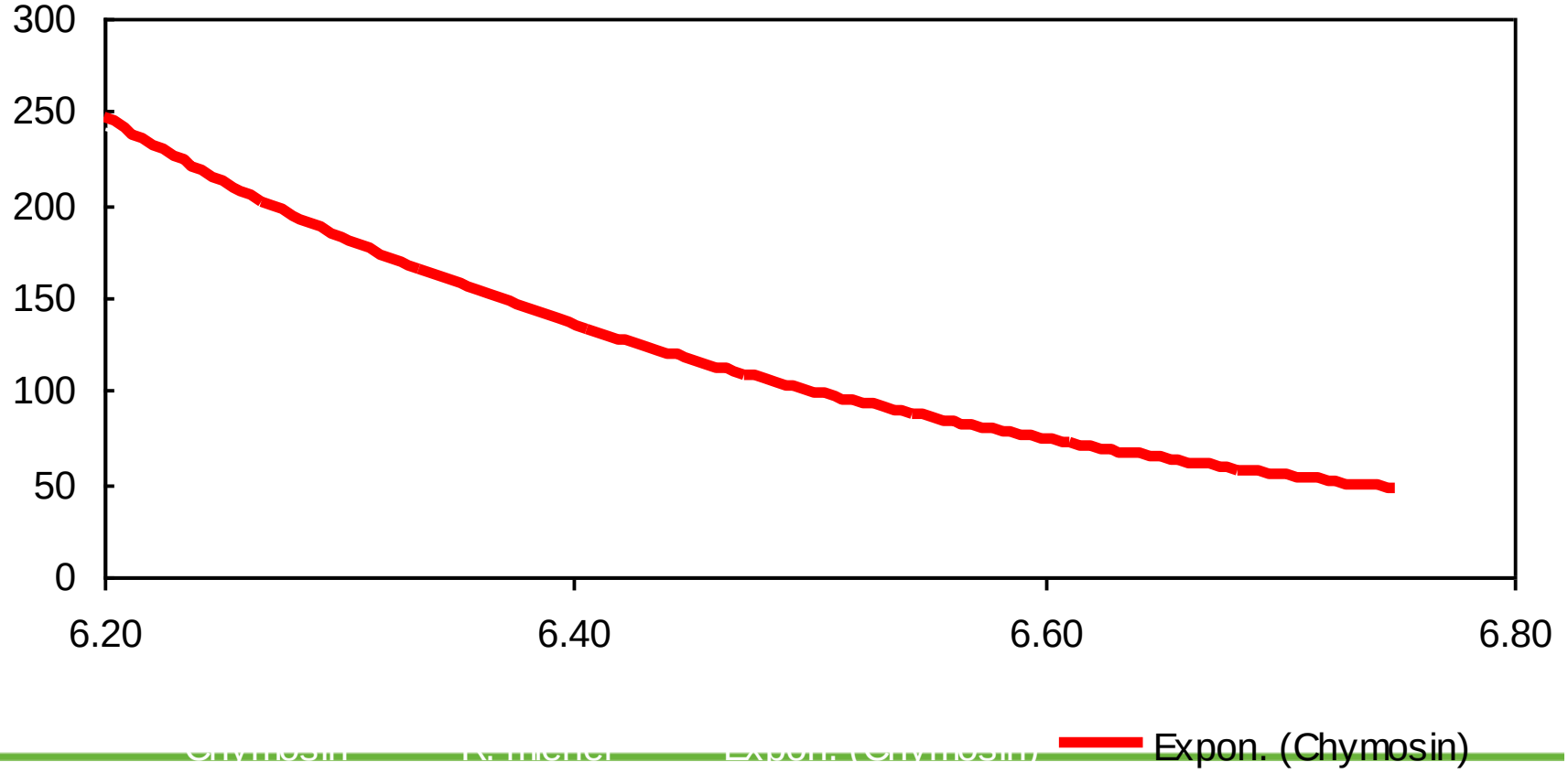
Chymosin

- Minimum Temperature for Coagulation = 50° F
- Optimum** Temperature for Coagulation = 112° F
- Maximum Temperature for Coagulation = 122° F
- 99+% inactivated in whey/ 162° F/15 sec./ pH >6.0

Effect of pH on Enzyme Activity



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High Solids Milk

- All protease reactions are dependent on the enzyme to substrate ratio.
  - In high solids milk (UF / MPC / Condensed) the hydrolysis of kappa casein and the coagulation reaction happen much quicker than with normal solids milk.
  - In ageing of cheese made with high solids milk, the hydrolysis reactions are often slowed since the protease to casein ratio is lower than normal cheese.

The effect of breed on protein in milk

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Table 1. The effects of breed on the content and yield of milkfat and milk protein.*

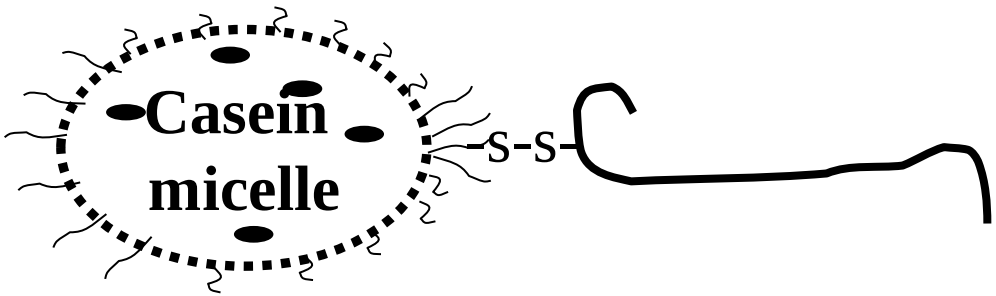
Breed	Milk Content (%)		Milk Yield (lb/lactation)	
	Fat	Protein	Fat	Protein
Ayrshire	3.99	3.34	464	389
Brown Swiss	4.16	3.53	537	462
Holstein	3.40	3.32	581	497
Guernsey	4.87	3.62	519	389
Jersey	5.13	3.80	506	385
Milking Shorthorn	3.60	3.20	-	-

Wisconsin CDR Pipeline vol. 23 # 1, 2011

Heat-Induced Changes in Milk Proteins

Native whey Protein (folded)

Denatured whey protein (unfolded)



Disulfide bond -S-S-

Colloidal calcium phosphate (CCP)

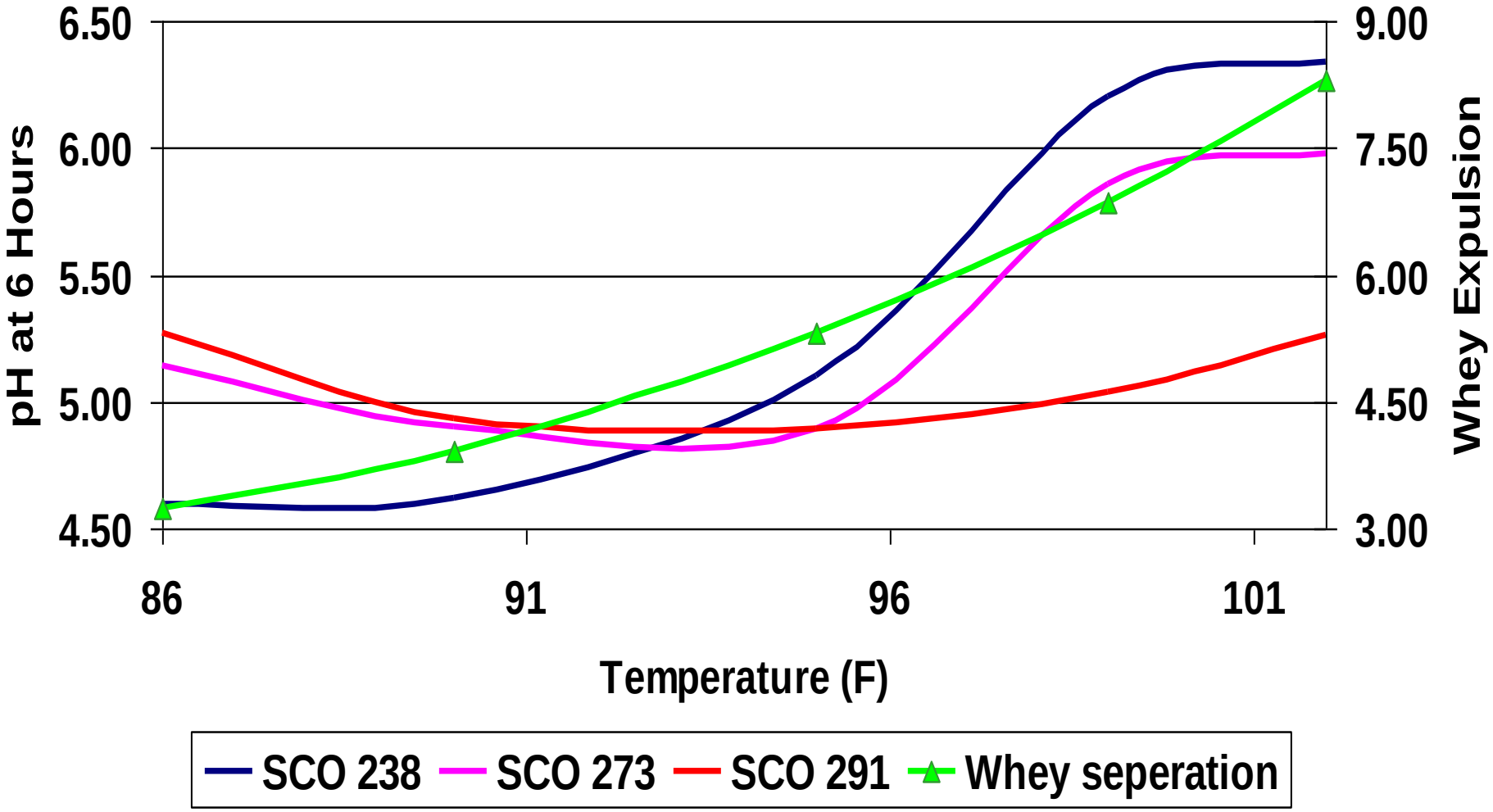
Syneresis

- Weaker set = faster shrink
- Cutting - Smaller curd = faster whey expulsion
- Acidification - Lower pH = faster shrink
- Cook - Higher temperature = faster shrink

Questions?

Much of the data presented was courtesy of Chr. Hansen, Inc.

Acid Formation & Whey Expulsion vs. Temperature



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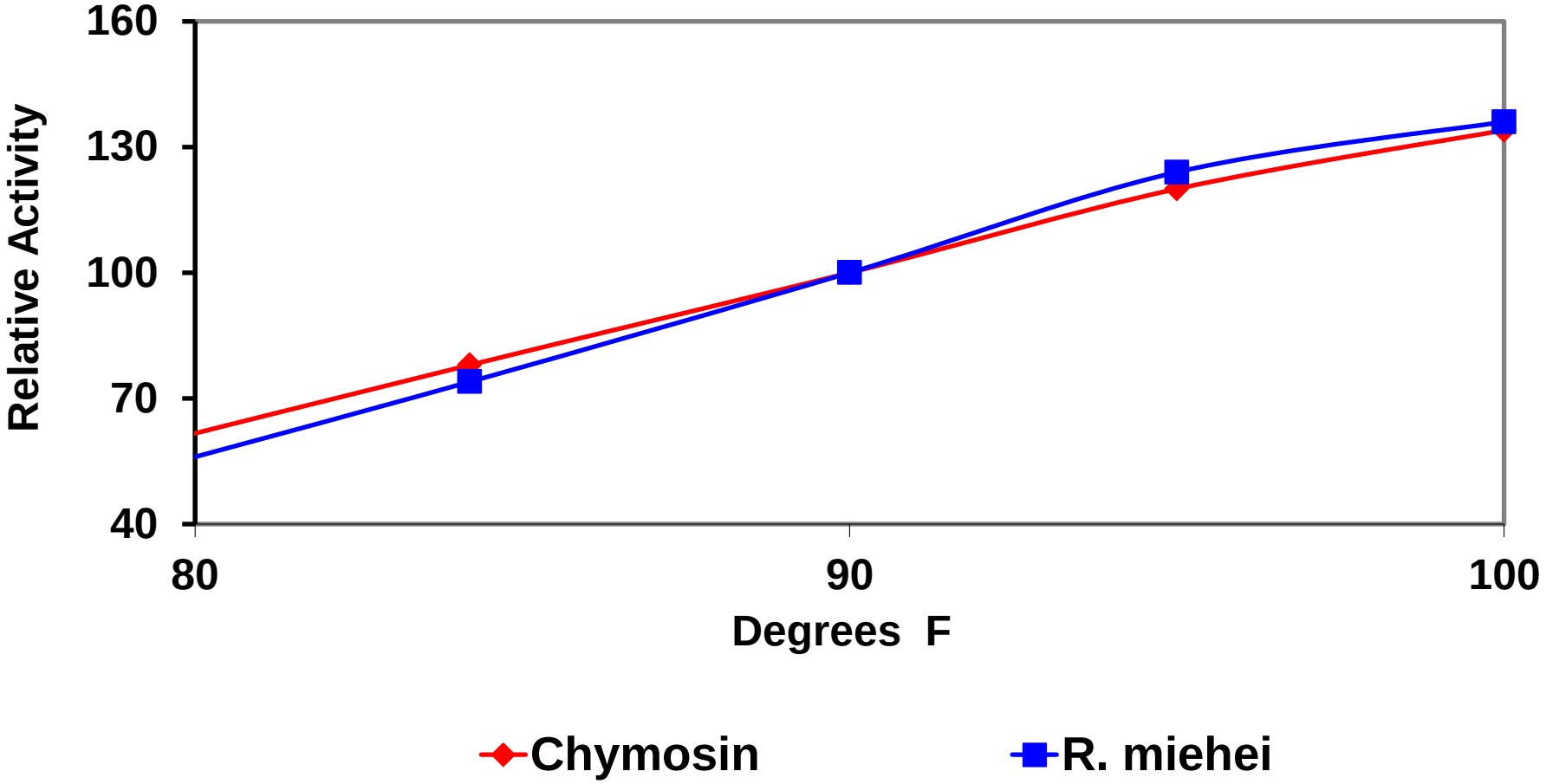
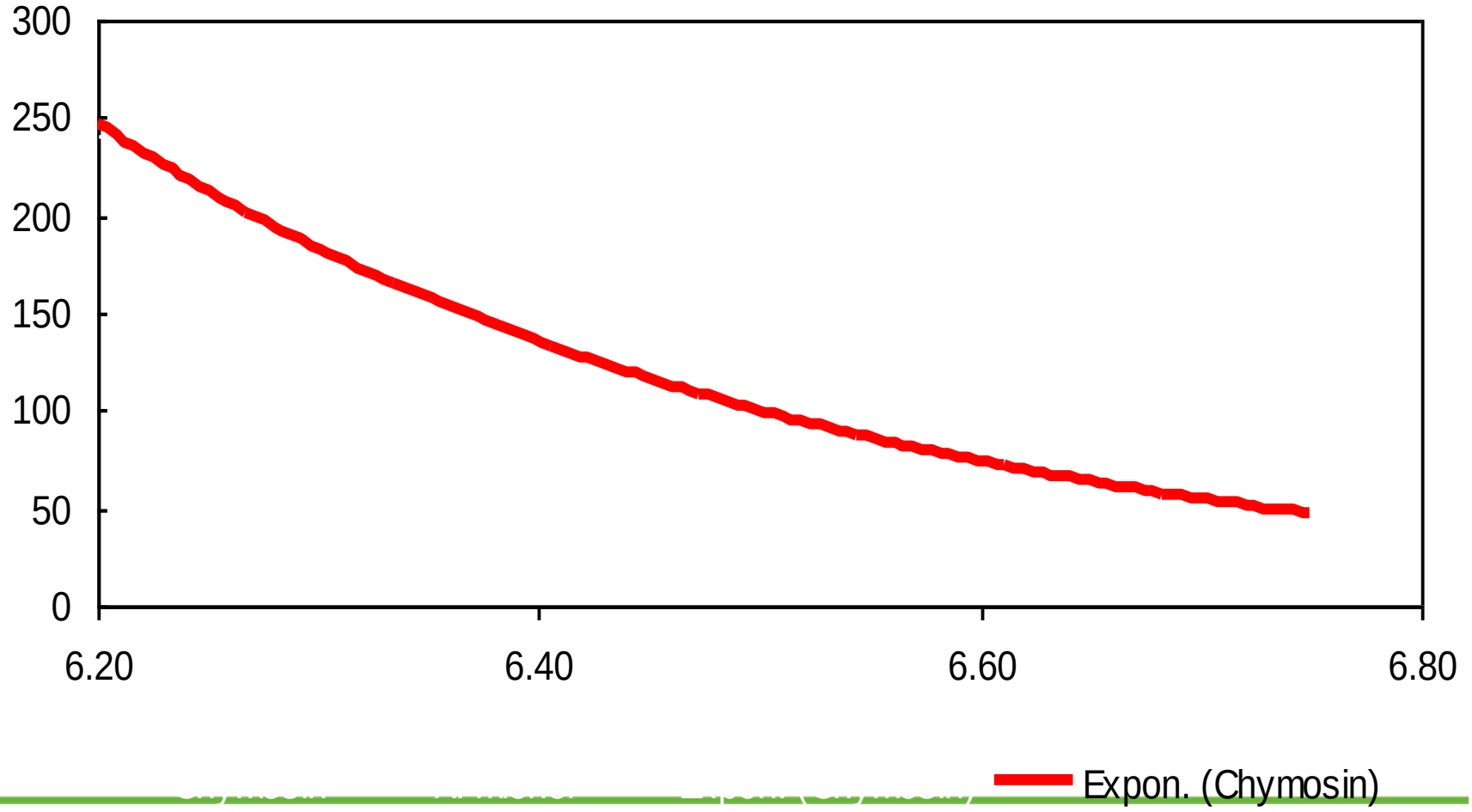
Effect of Temperature on Coagulant Activity



Effect of pH on Enzyme Activity



Thank you