# BIOTECH-UPLB Microbial Rennet for Cheese Production: Technology Demonstration & Business Highlights

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#### **Activities**

- Lecture
- Cheese making demo
- Cheese tasting
- Sensory evaluation





#### **BIOTECH Microbial Rennet**

- an effective milk coagulant produced by solid substrate fermentation using the fungus Rhizopus chinensis BIOTECH 3273
- developed by the National Institute of Molecular Biology and Biotechnology (BIOTECH) through funding from DOST and DA-BIOTECH.
- The product is a better substitute for traditionally produced animal rennet.
- It has been found to be better than imported rennet in terms of quality and volume of cheese produced





#### **BIOTECH Rennet**

Product	Liquid	Granule
Activity	60 IMCU	100 IMCU
Application rate	10 ml/L milk	5 grams/L milk
Price	PhP 480/L	PhP 750/Kg
Shelf life (28°C– 30°C)	5 months	1 year







#### Rennet

- ❖ 3500 B.C.
- An Arabic Nomad poured milk into a leather container made from sheep's stomach.
- When he stopped to drink the milk, it was discovered that the milk had been transformed into curd and whey by heat of the sun and rennet in the sheep stomach
- Led to the development and production of the curds known as cheese, as a way of converting surplus milk into another form in order to conserve and preserve the principal constituent and the nutritious value of milk.

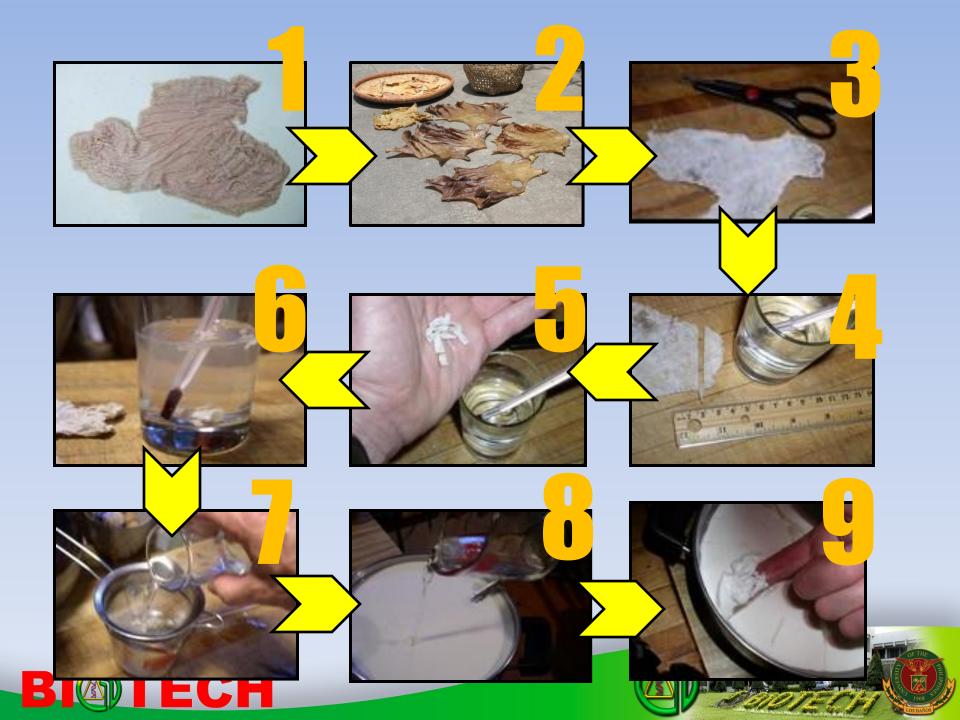


## Rennet; FOUR CHAMBERS OF THE RUMINANT STOMACH







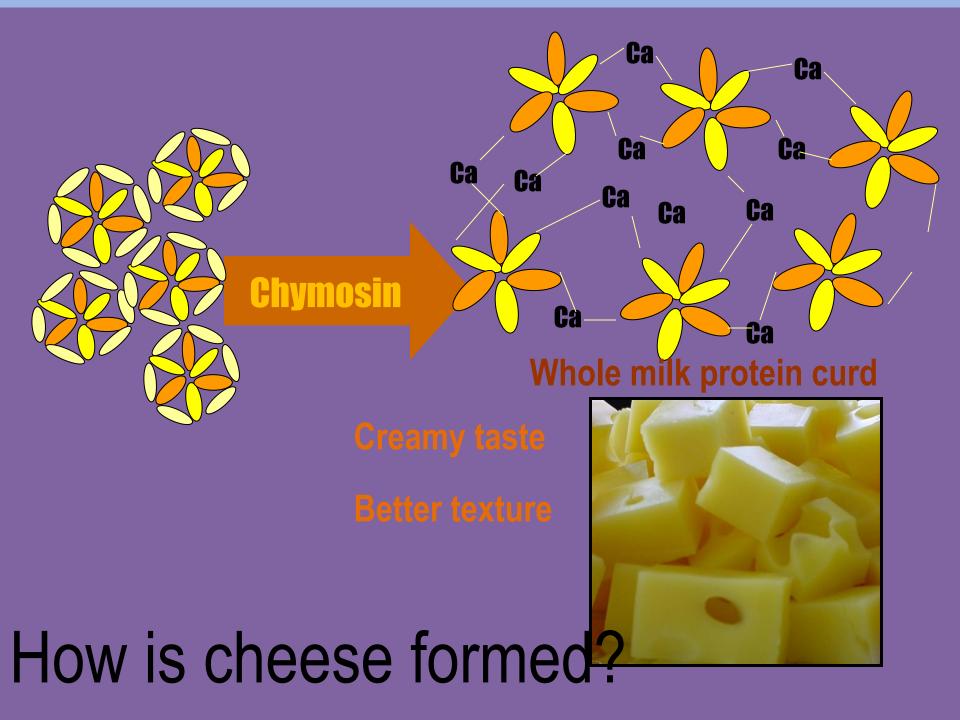


# Rennet Composition

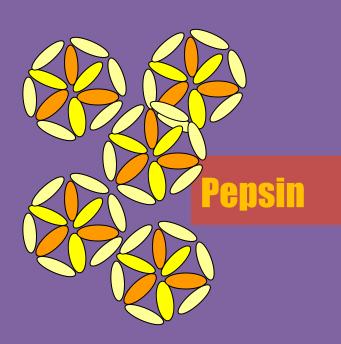
- Rennet from milk fed calf
  - >Chymosin or rennin 95%
  - >Pepsin 5%
- •Rennet from older cows, sheep, carabao, etc
  - >Chymosin 5%
  - >Pepsin 95%



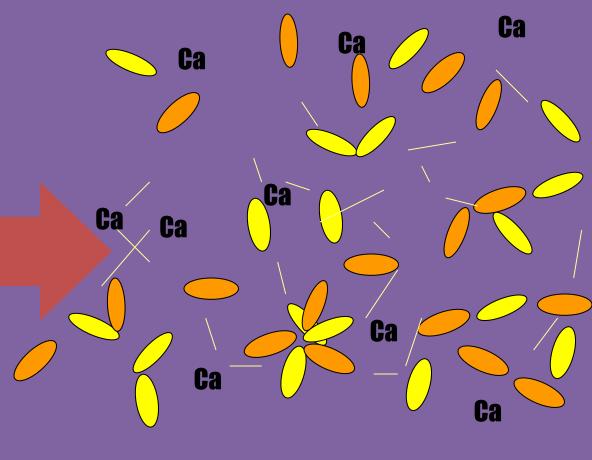




# RENNET from Adult Animals



MIIIK proteins



Digested milk protein

=Bitter taste

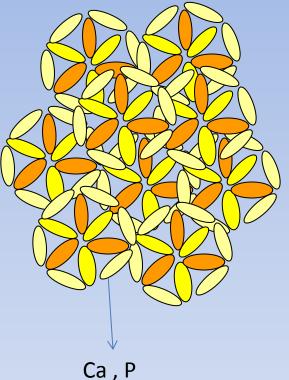
=Low cheese





Coagulation by Acid

- Coagulation of casein by acid result from loss if electric charge
- Lowering of pH neutralizes the negative ions of casein micelles
- Micelles become unstable & begin to agglomerate
- Acidity increase the solubility of minerals such as calcium and phosphorus
- Result into demineralized curd
- Resulting curd is porous, brittle and unable to contract



Ca , P (Whey)





# Plant sources of milk coagulant



Cynara cardunculus (thisle plant)



Mallow plant



Fig tree bark



**Nettles** 



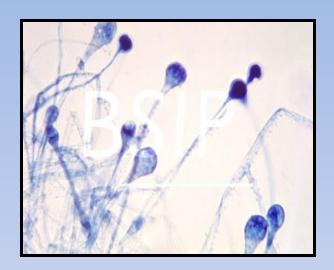






- Wild type from fungi, also classified as vegetable rennet
  - Mucor pussilus
  - Mucor miehei
  - Endothia parasitica
- Recombinant

# Microbial Rennet









#### **Commercially Available Microbial Rennet**

(Sternberg, 1976)

Microorganism	Trade Name	Supplier	
Endothia parasitica	Suparen Sure Curd	Pfizer International	
Mucor miehei	Fromase	Wallerstein Co.	
Mucor pusillus	Hannilase Emporase	Christian Hansen  Dairyland Fd.	
γιασοι μασιπασ	Meito MR	Meito Sangyo	
	Noury Rennet	Vitex	





# Recombinant rennet

- Use of genetically modified organism
  - Aspergillus niger (extracellular)
  - Kluyveromyces lactis (extracellular)
  - Escherichia coli (intracellular)
- Produced by fermentation
- Milk clotting enzyme produced has the same amino acid sequence as chymosin found in calf stomach
- 1990 approved fo human consumption and given marked as GRAS
- Tradename : CHyMax (Pfizer) & Maxiren (Gist Brocades)





# Milk coagulant being used by local dairy processors and multinational companies compared to BIOTECH microbial rennet

	Cost of coagulant per 100 L milk	% Cheese Yield	Production Cost of Cheese/100 grams	Net benefit /100L milk
Animal Rennet*	700.00	18	20.53	5,305.00
Chymax**	56.00	18	16.95	5,949.00
Vinegar***	250.00	10	32.86	1,714.38
BIOTECH rennet Granules	350.00	23	14.54	8,155.00
BIOTECH Liquid rennet	480.00	23	15.11	8,025.00

<sup>\*</sup>Rennet extracted from 4<sup>th</sup> stomach of ruminant (PhP 70/L)

<sup>\*\*</sup>Commercial rennet produced by Pizer and distributed by Christian Hansen, recombinant rennet, (PhP 21,000 per Kilogram, 2080 IMCU/gram)



#### Market



The market for microbial rennet is attractive because there is still no local producer or dominant distributor serving the small to medium cheese manufacturers.

Positioning: The "best quality and most efficient cheese coagulant" in the market.

Price: P 480.00 per liter and P 750.00 per Kg – similar price as the imported rennet to emphasize the best product image, better quality of the product and its efficiency as cheese coagulant.





# **Profitability Analysis**

	Liquid	Rennet
	Concentrate	Granules
Net Present Value (15% discount		
rate)	1,624,560.92	1,534,580.72
Total Investment	1,702,825.79	4,492,624.72
Selling Price	480.00/ Li	750.00/ kg
Break-even point	8,770.84 Li	11,845.88, kg
Profit margin	194.15/Li	379.26/kg
Break-even period (in year)	1.5 years	1.5 years
Average net income/year	1,072,168.85	1,916,675.97
Average return of		
Investment/year (%)	62.96	42.66





# **Cheese Manufacturing**

- Ingredients
  - -Milk
  - -Milk coagulant
  - -Starter culture
  - -Salt
  - -Cheese color





#### Milk

- Raw milk
  - Cow
  - Carabao or water buffalo
  - Goat
  - Sheep
- Processed milk
  - Full cream powdered milk (Anchor)
  - UHT milk (for cream cheese)





## Cheese Manufacturing Process

- Pasteurization
- Addition of starter
- Coagulation
- Cutting
- Draining or whey separation
- Salting







#### Use of BIOTECH Rennet

- Liquid Rennet
  - 10 milliliters per 1 liter of milk dilute with equal volume of water

- Granulated Rennet
  - 5 grams per 1 liter of milk
  - Suspend in 10 ml warm (50°C) water + 10 ml vinegar





#### PREPARATION OF WHITE CHEESE

- Ingredients:
  - 1 liter Raw milk
  - BIOTECH Microbial Rennet
  - -17-20 grams rock salt





#### White Cheese

#### Procedure

- Add salt to raw milk and pasteurize at 72 ° C for 15 seconds
- Cool pasteurized milk to 50-52 <sup>o</sup> C) and add microbial rennet. Mix well
- Let stand for 40 to 1 hour. If it takes too long to coagulate, place in 50° C waterbath
- Cut the curd into 1 inch cube and stir the curd carefully
- Transfer the curd into perforated tray lined with cheese cloth
- Drain for two hours, dispense in cups or any other packaging material. Refrigerate



#### Preparation of Soft Cheese using Full Cream Powder Milk

- Ingredient
  - 150 grams full cream powder milk
  - 923 ml water
  - 18 gram salt
  - 10 ml BIOTECH rennet or 5 grams granulated rennet

Yield: 45%

Cost per 100 gram: P 17/100gram

- Procedure
  - Add salt to milk
  - Heat to 50°C
  - Add rennet
  - Let stand until coagulated
  - Transfer the curd in perforated tray lined with cheese cloth
  - Drain
  - Pack







# Average chemical composition of milk of different species

SPECIES	WATER	PROTEINS %	FAT	LACTOSE	MINERAL SALTS
BUFFALO	82.2	4.8	7.5	4.7	0.8
GOAT	86.5	3.9	4.3	5.8	0.8
EWE	80.9	6	7.5	5.4	1.1
COW	87.5	3.2	3.7	4.6	1





# Milk quality testing

- Organoleptic test:
  - smell
  - -appearance
  - taste
- Clot –on boiling test
- Alcohol test





## Clot-On-Boiling

- Materials needed
  - 5 or 10 ml pipette
  - 30 ml test tube
  - Water bath
- Procedure
  - Pipette about 5 ml of milk sample into test tube
  - Place in boiling water bath for 5 minutes
  - Examine milk for the presence or absence of clots or precipitates





## **Alcohol Precipitation Test**

#### Materials

- 5 ml pipette
- 68 % ethyl alcohol (dilute 72ml of 95% alcohol to 100 ml with distilled water)
- Test tube

#### Procedure

- Place equal volume of milk and alcohol at room temperature
- Mix the contents by inverting the tube several times slowly
- Observe whether the milk coagulates



