

The Biochemical, Anatomical, and Operational Methodologies for Harvesting Bovine Chymosin: A Comprehensive Guide to Traditional Rennet Paste Processing

The conversion of liquid milk into a stable, solid matrix is the foundational biochemical event in cheesemaking, a process fundamentally reliant on the enzymatic destabilization of casein micelles¹. Historically and contemporarily, this destabilization is driven by chymosin (EC 3.4.23.4), an aspartic endopeptidase secreted exclusively in the gastric mucosa of unweaned ruminants³. While the modern global dairy industry heavily relies on fermentation-produced chymosin (FPC) or microbial coagulants synthesized from fungi such as *Rhizomucor miehei*, the production of highly specific, authentic traditional cheeses—particularly those bearing Protected Designation of Origin (PDO) status—necessitates the extraction of natural animal rennet⁵.

The extraction of traditional rennet paste from an unweaned calf transcends basic agricultural processing; it represents a highly orchestrated intersection of veterinary neuroanatomy, biomechanical engineering, and enzymatic biochemistry. The procedure requires strict adherence to hygienic slaughter protocols to mitigate enteric pathogen translocation, followed by precise osmotic brining, controlled thermodynamic desiccation, and mechanical pulverization⁸. This exhaustive analysis details the rigorous methodologies required to harvest the bovine abomasum and process it into a highly functional traditional chymosin rennet paste, examining the entire operational continuum from the slaughter floor to the biochemical activation of the prochymosin zymogen.

Neurophysiology and Biomechanical Execution of Humane Bovine Dispatch

The successful and hygienic extraction of the abomasum begins prior to evisceration, relying entirely on the precision of the slaughter methodology. The initial objective is to render the bovine animal irreversibly insensible to pain, a process that requires a catastrophic, short-term disruption of nerve function and a global depolarization of neuronal membranes⁸.

The undisputed gold standard for physical stunning in modern abattoirs is the penetrating captive bolt (PCB)⁸. The architecture of the bovine skull has evolved to withstand massive blunt force, featuring an expansive frontal bone with cavernous, air-filled frontal sinuses that act as effective biomechanical crumple zones⁸. The total tissue thickness—comprising the hide, subcutaneous tissue, the outer bony plate, the sinus cavity, and the inner bony plate—averages 46.7 millimeters at the ideal frontal shot placement in cattle, with the targeted Ascending

Reticular Activating System (ARAS) located within the thalamus at a depth of approximately 92.2 millimeters⁸. To overcome this osteological resistance and ensure the bolt retains enough terminal energy to macerate the thalamus, the PCB device must generate between 300 and 500 Joules of kinetic energy, requiring bolt velocities exceeding 55 meters per second⁸. Clinical validation of insensibility is mandatory prior to hoisting. A properly stunned bovine exhibits immediate collapse, a loss of rhythmic breathing, and the absence of cranial nerve reflexes, including the corneal and palpebral reflexes⁸. Post-stun clonic kicking frequently occurs as an anoxic spinal reflex generated by the sudden removal of inhibitory signals from the destroyed higher brain centers, which must not be confused with consciousness⁸.

The Hemodynamics of Exsanguination and Pre-Flaying Interventions

Exsanguination is the foundational hygienic step following concussive stunning. The physiological goal is the rapid induction of hypovolemic shock, which drops the cerebral perfusion pressure to zero⁸.

The efficiency of exsanguination relies heavily on the residual pumping action of the bovine heart. Prolonged intervals between stunning and sticking risk cardiac arrest prior to venesection, which traps blood within the capillary beds of the musculature and internal organs⁸. Consequently, regulatory frameworks mandate that sticking must occur within a strict stun-to-stick interval of a maximum of 60 seconds⁸.

To execute the stick, operators must circumvent the highly elastic bovine carotid arteries, which feature thick smooth muscle walls that rapidly contract and retract deep into connective tissue sheaths upon severing⁸. This retraction promotes the formation of false aneurysms that physically seal the severed ends. The optimal anatomical approach is the thoracic stick (brachiocephalic transection), wherein a specialized knife is inserted at the cranial base of the sternum (the thoracic inlet) and directed inward toward the heart, severing the massive brachiocephalic trunk and the bicarotid trunk directly at the aortic arch⁸. This methodology precipitates total brain death within 10 to 20 seconds by rapidly draining both the carotid and vertebral networks⁸.

Hygienic Pathogen Mitigation: The Two-Knife System

Because the external hide of the neck is heavily contaminated with soil, feces, and environmental bacteria, utilizing a single knife to incise the hide and sever the arteries acts as a vector, injecting hide-borne pathogens directly into the sterile bloodstream⁸. To prevent this cross-contamination, a "two-knife system" is universally mandated. A primary sterilized knife is used solely to incise and reflect the contaminated hide overlying the thoracic inlet, and a secondary, sterile sticking knife is inserted into the exposed subcutaneous tissue to sever the vasculature⁸. Between uses, knives must be sterilized in water maintained at no less than 82°C to destroy vegetative bacteria⁸.

High-volume facilities further implement localized chemical decontamination systems applied directly to the pattern lines (the lines where opening cuts are made). The application of organic acids, such as 6.0% lactic acid or 5.0% acetic acid, severely alters the surface pH, disrupting

the proton motive force of bacterial cell membranes and reducing Aerobic Plate Counts (APC) on the hide by up to 2.62 log CFU/400 cm², massively reducing the prevalence of *Escherichia coli* O157:H7⁸.

Exsanguination & Pre-Flaying Parameter	Optimal Protocol	Physiological and Hygienic Rationale
Stun-to-Stick Interval	Maximum 60 seconds	Utilizes residual cardiac pumping to maximize blood evacuation from the viscera before cardiac arrest occurs ⁸ .
Anatomical Target	Brachiocephalic and Bicarotid Trunks	The thoracic stick prevents false aneurysm formation and rapidly drains vertebral anastomoses ⁸ .
Hygienic Cutting Protocol	Two-Knife System	Prevents pathogen translocation from the contaminated hide directly into the sterile vascular bed ⁸ .
Tool Sterilization	Water maintained at $\geq 82^{\circ}\text{C}$	EU Regulation (EC) 853/2004 mandate to destroy vegetative bacteria between individual carcasses ⁸ .
Chemical Decontamination	6.0% Lactic Acid / 5.0% Acetic Acid	Disrupts the proton motive force of bacterial cell membranes, yielding a 2.62 log CFU reduction of <i>E. coli</i> ⁸ .

Suspension, Flaying Kinematics, and Spinal Stress Mitigation

Once exsanguinated, the carcass is vertically suspended to prevent the exposed subcutaneous tissues from contacting the contaminated outer surface of the hide or the floor⁸. Vertical dressing commences with legging, where the hoof is amputated at the break joint and a sterile

shackle is inserted beneath the Achilles tendon to elevate the carcass via an overhead rail⁸. To strip the hide from the back and shoulders, operators utilize mechanical downward hide pullers (DHP). Unlike upward hide pulling (UHP) systems—which are hygienically flawed because soil, hair, and aerosolized particulate matter rain down onto the freshly exposed lower carcass—DHP systems pull the hide from the hindquarters downward over the head, ensuring dislodged debris falls away from the sterile meat⁸. However, DHP systems exert massive, unyielding downward kinetic tension on the bovine spinal column, which can cause severe vertebral fracturing (broken backs)⁸. To counteract this structural failure, rigid electrical stimulation (ES) probes are applied, inducing violent, tetanic muscle contractions. This artificial stimulation splints the spinal column with the massive *longissimus dorsi* muscles, reinforcing it against the pull of the machine⁸.

While necessary for skeletal integrity, electrical stimulation drastically alters the thermodynamic and biochemical conversion of muscle to meat by accelerating the depletion of adenosine triphosphate (ATP) and driving the anaerobic glycolysis of muscle glycogen, leading to a rapid accumulation of lactic acid⁸. Operators must carefully calibrate ES voltages (e.g., reducing from 150V to 85V) to balance the prevention of spinal fractures with the optimization of tissue pH⁸.

Prophylactic Sealing and the Dynamics of Evisceration

The absolute most critical sanitary intervention prior to extracting the abomasum is the hermetic sealing of the gastrointestinal extremities. The bovine rumen contains up to 100 liters of highly acidic, bacteria-laden ingesta, while the lower intestines hold significant volumes of concentrated fecal matter⁸. If the esophagus (weasand) and the rectum (bung) are not completely occluded, the mechanical pressures exerted during handling will force these fluids out onto the sterile abdominal cavity, catastrophically contaminating the abomasum and necessitating severe trimming protocols⁸.

Weasand Rodding and Rectal Ligation

1. **Esophageal Occlusion:** The operator manually isolates the muscular tube of the esophagus from the cartilaginous rings of the trachea⁸. A specialized metallic tool, known as a weasand rodder, is threaded over the free end of the esophagus. As the rodder is pushed cranially deep into the thoracic cavity toward the diaphragm, it physically strips the connective tissue away while simultaneously forcing residual ingesta backward into the paunch (rumen)⁸. Once the rodder reaches the diaphragmatic hiatus, a high-density polymer clip (e.g., Adept clip) or a heavy-duty latex Elastrator ring is applied via a pneumatic dispenser, locking via barbed recesses to form a leak-proof seal⁸.
2. **Bung Bagging:** Working at the posterior aspect of the suspended carcass, a careful circumferential incision is made around the anal sphincter, traversing the muscular attachments of the pelvic floor without penetrating the rectal wall⁸. The rectum is pulled outward, and a sterile plastic bag is inverted over the exposed bung. A tight elastic ring or heavy twine is applied around the neck of the bag, capturing residual fecal material. Performing this bagging *prior* to any high-pressure carcass washing is highly critical;

washing an unsealed rectum flushes fecal bacteria outward, massively increasing pathogen incidence⁸.

Pelvic Osteology and Paunch Extraction

Before the abdominal cavity is fully opened, the pelvic girdle is split to allow the safe extraction of the lower digestive tract. Modern abattoirs utilize specialized, hydraulically powered aitch bone cutters (e.g., Jarvis Model 30CL-ABC) operating at pressures up to 1,500 psi⁸. These tools feature a guarded, dual-action scissor mechanism that slides seamlessly into the ischiatic notch, cleanly snapping the pubic symphysis apart without projecting sharp bone edges into the pelvic cavity, thereby virtually eliminating the risk of puncturing the bladder or shearing the bagged rectum⁸.

Evisceration commences with a mid-ventral incision moving downward along the linea alba toward the sternum⁸. To physically shield the massive underlying rumen from the sharp edge of the skinning knife, the operator inserts two fingers into the initial incision, pulling the abdominal wall outward and guiding the blade between the fingers⁸. Once the abdominal wall is fully opened, the immense weight of the bovine gastrointestinal tract causes it to bulge forward. The operator reaches into the pelvic cavity to grasp the previously bagged rectum and applies gentle downward traction⁸. Because the weasand was clipped and the bung bagged, the entire alimentary canal acts as a sealed, impermeable unit⁸. Gravity assists the operator in rolling the entire paunch—consisting of the rumen, reticulum, omasum, and abomasum—out of the abdominal cavity, allowing it to drop cleanly onto a sanitized viscera table⁸.

SRM Regulatory Frameworks and BSE Mitigation

Following evisceration, the carcass is split longitudinally. The brain and spinal cord are the primary reservoirs for the misfolded prion proteins responsible for Bovine Spongiform Encephalopathy (BSE)⁸. During the splitting process, high-speed band saws can shred the spinal cord, generating an infectious aerosol of bone dust and central nervous system (CNS) tissue⁸. To mitigate this severe public health hazard, international bodies strictly enforce the removal of Specified Risk Material (SRM)⁸.

The spinal cord is extracted utilizing pneumatic Spinal Cord Remover (SCR) vacuum systems, which trace the spinal cavity, cleanly severing the spinal ganglia and vacuuming the delicate dura mater and cord tissue directly into a sealed containment vessel⁸. This tissue is classified as Category 1 Animal By-Product under EU Regulation (EC) No 1069/2009, mandating immediate staining with a visual dye (e.g., Brilliant Blue FCF) and complete destruction via high-temperature incineration⁸. Conversely, the abomasum—provided it is free of contamination—is classified as a low-risk, edible byproduct suitable for further processing.

Anatomical Identification and the Physiology of the Neonatal Abomasum

The ruminant stomach is a highly complex, multi-chambered organ. In mature cattle, the rumen operates as a massive fermentation vat responsible for the microbial degradation of cellulose⁸. However, in the unweaned calf, the architectural and physiological dynamics are entirely

different. When a neonatal calf consumes its mother's milk, a specialized muscular fold known as the esophageal groove closes, forming a temporary tube that bypasses the largely underdeveloped rumen, reticulum, and omasum⁵. The milk is channeled directly into the fourth compartment: the abomasum⁵.

The abomasum is the only truly glandular compartment of the bovine stomach, analogous to the monogastric stomach in humans. Its mucosal lining is densely populated with gastric chief cells (peptic cells), which are responsible for the secretion of the zymogen preprochymosin and various gastric lipases⁵. The presence of these enzymes is an ancient evolutionary trait designed specifically to coagulate milk in the acidic environment of the stomach, slowing its passage into the duodenum to maximize nutrient extraction⁵.

Age Constraints and Enzymatic Shifts

To harvest a highly functional traditional rennet, the abomasum must be extracted from a neonatal animal strictly under two weeks of age, prior to the introduction of forage or the commencement of rumination (chewing the cud)⁹. The age of the animal dictates the enzymatic ratio present within the gastric mucosa:

- **Optimal Neonates (< 2 weeks old):** The enzymatic profile is heavily dominated by chymosin, typically representing 90% to 95% of the total proteolytic enzymes, with pepsin comprising the remaining 5% to 10%¹³. Chymosin possesses a uniquely high specificity for milk coagulation relative to general proteolysis.
- **Weaned or Mature Cattle:** As the calf begins to ingest grass and solid feed, the physiological requirements of the stomach shift. The expression of the chymosin gene is downregulated, and the secretion of pepsin (a highly aggressive general protease) increases sharply, eventually reaching ratios of 20% chymosin to 80% pepsin¹³.

Utilizing an older calf's abomasum for rennet paste production is industrially detrimental. High

concentrations of pepsin induce excessive, non-specific hydrolysis of α_s - and β -caseins during cheese maturation, generating vast quantities of bitter peptides that ruin the organoleptic profile of the final product¹³. Therefore, strict age verification is the first critical control point in rennet manufacturing.

Excision, Isolation, and Internal Cleaning

On the viscera table, the operator locates the abomasum, which is easily identifiable as the largest and most distinct of the stomach compartments in the neonate⁹. It is characterized internally by the *plicae spirales abomasi*, which are gentle, spiraling folds of mucosal skin that drastically increase the internal surface area for enzyme secretion¹¹.

The operator utilizes a sterilized knife to carefully sever the abomasum at its cranial junction with the omasum and its caudal terminus at the pyloric sphincter⁵. The exterior serosal layer of the organ is frequently lined with visceral adipose tissue (fatty deposits); these ends must be meticulously trimmed away, as extraneous fat can oxidize and cause rancidity during long-term storage⁶.

The internal handling of the abomasum depends entirely on the desired sensory profile of the final rennet paste:

1. **For Standard Rennet Paste:** The operator cuts the abomasum open and manually extracts the internal contents, which typically consist of coagulated milk clots along with potential foreign impurities (grass, hair, or soil)⁶. The mucosal lining is gently rinsed with cool, non-chlorinated water¹³. It is absolutely paramount that the operator does not aggressively scrub the tissue. The chief cells embedded in the delicate mucosal folds house the prochymosin zymogen granules; scrubbing will physically sheer these cells away, drastically reducing the coagulating power of the organ¹³. The tissue is then patted completely dry using sterile paper towels⁹.
2. **For Piccante (Piquant) Rennet Paste:** In highly traditional European methodologies—such as those utilized in the production of *Provolone*, *Pecorino Romano*, and specific Spanish cheeses like *Queso Palmero*—the interior coagulated milk is deliberately retained⁵. This milk acts as a sponge, trapping high concentrations of pregastric esterases and lipases that were secreted by the calf during suckling¹⁷. Retaining these clots infuses the final paste with massive lipolytic potential, yielding a highly pungent cheese⁵.

The Physicochemical Dynamics of Brining

Following initial cleaning, the raw abomasum tissue must be chemically preserved and its enzymatic contents mobilized. The fundamental mechanism for this extraction is osmotic dehydration via a hypertonic sodium chloride (NaCl) solution, a procedure known as the stomach paste method⁹.

The 15% Salt Ratio Formulation

To achieve rapid enzymatic extraction without causing irreversible protein denaturation, the operator prepares a highly precise brine based on the exact raw weight of the freshly cleaned abomasum:

1. **Tissue Weighing:** The patted-dry abomasum is weighed precisely in grams⁹.
2. **Salt Measurement:** Coarse, non-iodized sea salt is measured at exactly 15% of the abomasum's weight⁹.
3. **Aqueous Solvent:** Non-chlorinated, room-temperature water is measured at exactly 50% of the abomasum's weight⁹.

Practical Example Calculation: If the freshly harvested abomasum weighs 174 grams, the mathematical formulation dictates the addition of 26.1 grams of NaCl (15%) and 87 grams of water (50%)⁹. The salt is vigorously stirred into the water. Due to the minimal water volume and high salt concentration, partial supersaturation may occur, leaving a fraction of the salt undissolved at the bottom of the vessel; this is an acceptable and intended state, ensuring the brine remains at maximum osmotic potential⁹.

Osmotic Plasmolysis and Pathogen Inhibition

The abomasum is completely submerged in the 15% brine solution, sealed in a glass or food-grade vessel, and allowed to cure at a rigorously controlled ambient temperature of 18°C to 21°C (65°F to 70°F) for a duration of 3 to 7 days⁹. (While historical texts occasionally reference curing periods lasting months, contemporary rapid extraction protocols demonstrate high

efficacy within this shorter window)⁹.

This hypertonic brining protocol executes three simultaneous and essential biochemical functions:

1. **Enzymatic Extraction via Plasmolysis:** The extreme salinity of the 15% brine creates a massive osmotic gradient across the cell membranes of the gastric mucosa⁹. Water rapidly exits the chief cells, inducing cellular plasmolysis and causing the membranes to rupture. This action actively flushes the sequestered prochymosin and lipases out of the intracellular space and into the surrounding interstitial fluid and brine matrix¹⁰. Furthermore, the high ionic strength of the NaCl solution increases the solubility of these proteins (a phenomenon known as "salting in"), preventing them from precipitating prematurely²⁰.
2. **Inhibition of Endogenous Proteolysis:** The abomasum is a protein-dense, highly active biological tissue. If left in a low-salt, hydrated environment, endogenous autolytic enzymes would rapidly degrade the structural integrity of the chymosin molecule. The hypertonic salt environment immediately denatures these destructive proteases, stabilizing the target zymogen²².
3. **Reduction of Water Activity (a_w) and Microbiological Safety:** Raw gastric tissue represents a significant microbiological hazard. The integration of 15% NaCl sharply decreases the water activity (a_w) of the tissue²². Pathogenic enteric bacteria such as *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are highly susceptible to osmotic shock and cannot proliferate in hyper-saline environments²³. While certain halotolerant microflora (e.g., *Debaryomyces hansenii* or specific lactic acid bacteria) may survive and subsequently contribute to cheese ripening, the rapid drop in water activity effectively halts the exponential growth of dangerous mesophilic pathogens, establishing a critical control point (CCP) in the HACCP framework of traditional rennet processing²³.

Brining Parameter	Required Metric	Physicochemical / Microbiological Rationale
Salt Concentration	15% of abomasum weight	Drastically reduces water activity (a_w); induces cellular plasmolysis to extract sequestered prochymosin ⁹ .
Aqueous Carrier	50% of abomasum weight	Provides sufficient fluid volume to form a hypertonic gradient without

		over-diluting the extracted enzymes ⁹ .
Incubation Temperature	18°C to 21°C (65°F to 70°F)	Facilitates rapid osmotic diffusion kinetics while remaining safely below the optimal proliferation zone for mesophilic pathogens ¹³ .
Incubation Duration	24 - 36 hours up to 3 - 7 days	Allows for complete osmotic equilibrium between the deep mucosal tissues and the surrounding brine ⁹ .

Thermodynamic Desiccation and Tissue Dehydration

Upon the conclusion of the 3- to 7-day brining cycle, the extracted enzymes are largely suspended within the saturated tissues and the surrounding fluid. To ensure long-term viability and prevent eventual spoilage, the abomasum must be completely dehydrated.

The operator removes the abomasum from the brine, gently shaking off the excess liquid¹³. The residual brine—now rich in soluble enzymes—can be reserved and refrigerated for immediate cheesemaking applications, though the primary yield remains trapped within the physical matrix of the mucosal folds⁹. To expedite the desiccation process, the pouch-like structure of the stomach is sliced open and laid flat in a single layer, maximizing surface area exposure⁹.

Thermal Lability and Drying Environments

The abomasum is transferred to a cooling rack or suspended in a well-ventilated, highly dry environment⁹. The ambient temperature during this phase must be strictly monitored; it must never exceed 21°C (70°F)¹³.

The structural integrity of the chymosin enzyme is highly heat-labile. Exposing the tissue to temperatures above 30°C to 35°C during the extended drying phase risks initiating the thermal denaturation of the enzyme's delicate bilobed tertiary structure, leading to an irreversible loss of coagulating power³. While modern mechanical food dehydrators can be employed to accelerate the process to approximately 24 hours, operators must strictly avoid standard "jerky" settings (which frequently exceed 60°C), opting instead for low-heat or ambient-air circulation modes⁹.

In a traditional, passive ambient setting, the drying process requires one to two weeks, heavily dependent on the ambient relative humidity¹³. During this time, the tissue must be covered with protective netting to prevent contamination by insect vectors (e.g., flies)⁹.

The desiccation phase concludes when the soft biological tissue transforms into a hard, brittle, parchment-like material that snaps cleanly under mechanical pressure⁹. At this stage, the biological water has been entirely evaporated. The extracted enzymes are now suspended in a

crystallized matrix of sodium chloride within the dried connective tissue. In this highly stable, desiccated state, the abomasum can be stored in a frozen or refrigerated environment for over a year without suffering a loss of enzymatic potency⁵.

Mechanical Pulverization, Reconstitution, and Paste Extraction

The final manufacturing stage transforms the stable, desiccated tissue back into a highly active, liquid or paste-based enzymatic suspension ready for inoculation into the cheese vat.

High-Shear Pulverization

The brittle, dried abomasum is weighed and cut into thin, manageable strips¹³. Operators utilize industrial food processors, heavy-duty mechanical meat grinders, or traditional large-scale mortars to mill these strips into a fine, homogenous powder⁹. This pulverization is critical: it drastically increases the total surface area of the tissue, destroying the remaining fibrous architecture and exposing the crystallized enzymes, facilitating rapid and total solubilization upon rehydration⁹.

The 1:8 Reconstitution Ratio and Slurry Generation

The powdered abomasum is reconstituted utilizing an acidic liquid carrier—traditionally non-chlorinated water, or preferably, highly acidic cheese whey (such as whey from a previous cheesemaking cycle or raw milk left to clabber)⁹. The reconstitution formulation requires precise volumetric adherence:

- **The 1:8 Ratio:** For every 1 part (by weight) of pulverized abomasum powder, 8 parts of liquid whey or water are slowly incorporated⁹.

Practical Example Calculation: If the mechanical pulverization yields exactly 35 grams of powdered tissue, the operator integrates 280 grams of acidic liquid whey⁹. The powder and liquid are continuously processed or ground together until the mixture fully homogenizes into a viscous, thick slurry⁹.

Physical Extraction and Stabilization

The resulting slurry contains a mixture of highly soluble, liberated enzymes and insoluble, inert fibrous connective tissue. The operator transfers the slurry into a fine-mesh cheesecloth or industrial press and applies intense mechanical pressure, squeezing every drop of liquid through the porous matrix⁹. The dense material remaining inside the cloth is a dry wad of fibrous tissue, stripped of its enzymes, which is subsequently discarded⁹.

The substance that passes through the mesh is the final traditional rennet paste (or a highly concentrated liquid, depending on the viscosity of the carrier). To guarantee long-term microbiological shelf stability and prevent the proliferation of spoilage organisms, an additional 4% to 5% by weight of fine sodium chloride is stirred into the final extraction¹³. The finished paste is decanted into sterile glass jars and stored under refrigeration (at approximately 4°C), where it maintains its maximum coagulating efficacy for approximately six months¹⁰. Over time in cold storage, a whitish, dense enzymatic suspension will separate from the saline water; operators must thoroughly agitate the jar prior to use to re-homogenize the matrix and ensure

consistent dosing¹³.

The Biochemical Kinetics of Prochymosin Activation

The technological efficacy of the harvested rennet paste relies entirely on a highly specific sequence of molecular events, beginning with the autocatalytic activation of the precursor protein and culminating in the targeted proteolysis of milk proteins.

Molecular Structure and Steric Hindrance

Within the chief cells of the harvested abomasum, the enzyme is initially synthesized in its inactive zymogen form, preprochymosin, a 381-amino-acid polypeptide³. Upon secretion into the gastric lumen, a 16-residue N-terminal signal peptide is cleaved, yielding the intermediate zymogen, prochymosin⁴.

Prochymosin remains catalytically inactive because its N-terminal prosegment—a sequence of 42 amino acids—physically occludes the active site cleft²⁷. The mature chymosin enzyme

possesses a bilobal tertiary structure consisting primarily of β -sheets, with each lobe contributing one catalytic aspartic acid residue (Asp32 and Asp215) to the active site³. In the inactive state, the third helical segment of the 42-amino-acid prosegment packs tightly against these active site residues²⁸. A highly conserved lysine residue within the prosegment (Lys37p) forms strong electrostatic ion pair interactions directly with the carboxyl groups of Asp32 and Asp215²⁸. Additionally, two tyrosine side chains (Tyr9 and Tyr38p) form symmetric hydrogen bonds with the catalytic aspartates, acting as a physical locking mechanism that absolutely prevents substrate access²⁸.

Acid-Induced Conformational Shifts and Autocatalytic Cleavage

For the rennet paste to function as a milk coagulant, the prochymosin must be activated. This activation is exclusively triggered by exposure to an acidic environment ($\text{pH} < 5.0$), a state naturally achieved when the rennet paste is reconstituted in acidic whey or introduced into the slightly acidic environment of ripened cheese milk³.

As the pH drops, the protonation state of the amino acids shifts, severely disrupting the electrostatic bonds and hydrogen pairs stabilizing the prosegment²⁸. The prosegment undergoes a major conformational reorientation, swinging away from the active site cleft and exposing the catalytic aspartates (Asp32 and Asp215)²⁸.

Once the active site is exposed, an intermolecular reaction occurs: one active chymosin molecule (or exposed zymogen) executes a nucleophilic attack on the prosegment of a neighboring molecule, cleaving it away²⁸. The specific location of this cleavage is highly dependent on the pH of the activation environment:

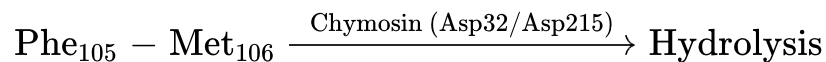
- **Highly Acidic Activation ($\text{pH} < 2.5$):** If the activation occurs rapidly in a highly acidic solution, the cleavage occurs precisely at the 27-28 peptide bond. This yields *pseudochymosin*, a highly active intermediate enzyme that is 15 amino acid residues longer at the N-terminus than mature chymosin³¹.
- **Moderate Acidic Activation ($\text{pH} 4.0 - 5.5$):** When the environment is adjusted to a pH closer to 5.5 (which is highly typical of warm cheese milk), the pseudochymosin undergoes further, spontaneous autocatalytic cleavage. This secondary cleavage

removes the remaining 15 amino acid residues, yielding the fully mature, active chymosin molecule, completely stripped of its 42-amino-acid prosegment³¹.

The Cleavage of κ -Casein and the Mechanics of Flocculation

The primary technological mandate of the mature chymosin in the rennet paste is the destabilization of casein micelles suspended in the milk vat. Bovine milk operates as a highly stable colloidal suspension. The micelles are comprised of hydrophobic α_s - and β -caseins concentrated in the core, surrounded by a hydrophilic outer layer of κ -casein². The κ -casein molecules possess a strong negative electrostatic charge at the native pH of milk (pH 6.7). This negative charge causes the thousands of micelles in the vat to violently repel one another, maintaining the liquid state of the milk².

Chymosin exhibits an extraordinarily narrow and highly specific proteolytic preference for the κ -casein molecule, rendering it the perfect coagulant. The deep, bilobal active site cleft of chymosin flawlessly accommodates the κ -casein chain, allowing the catalytic aspartates (Asp32 and Asp215) to execute a precise nucleophilic attack on a single specific peptide bond: the linkage between Phenylalanine 105 (Phe105) and Methionine 106 (Met106)².



The cleavage of this single Phe105-Met106 bond is catastrophic to the stability of the micelle, splitting the κ -casein into two distinct fragments:

1. **Para- κ -casein:** A highly hydrophobic fraction containing residues 1 to 105, which remains physically attached to the micelle core².
2. **Glycomacropeptide (GMP):** A highly hydrophilic, negatively charged fraction containing residues 106 to 169, which is instantly released and diffuses away into the surrounding aqueous whey¹⁵.

With the negatively charged GMP layer stripped away, the steric hindrance and electrostatic repulsion that kept the micelles apart vanish entirely¹⁵. The hydrophobic para- κ -casein surfaces are now exposed directly to the aqueous environment. Driven by thermodynamic instability, the micelles rapidly seek out one another, aggregating and clustering via massive hydrophobic interactions and the formation of robust calcium-phosphate bridges¹. This system-wide, cascading aggregation forms a solid, three-dimensional protein network that physically traps milk fat, water, and minerals—achieving the fundamental transition of liquid milk into a solid cheese curd¹.

The Lipolytic Profile: The Sensory Signature of Rennet

Paste

While highly purified commercial liquid rennets and modern Fermentation-Produced Chymosin (FPC) contain almost exclusively chymosin and pepsin, traditional animal rennet paste possesses a distinctly richer and vastly more complex enzymatic profile. Because the paste is derived from the extraction of the whole abomasal tissue—and frequently incorporates the coagulated milk clots found within the lumen—it acts as a powerful carrier for specialized lipolytic enzymes⁵.

Pregastric Esterases and Short-Chain Free Fatty Acids

During the physiological act of suckling, the calf secretes pregastric esterase (PGE) from sublingual tissues located at the base of the tongue¹⁸. This enzyme travels down the esophagus suspended in the milk and accumulates in the abomasum alongside resident gastric lipases¹⁸. PGE is highly unique; it exhibits a profound specificity for hydrolyzing triglycerides in milk fat at the primary ester positions, selectively and rapidly liberating short-chain free fatty acids (FFAs), specifically those containing four to ten carbon atoms (C4:0 to C10:0)¹⁷. The enzymatic cleavage of the triglycerides releases immense concentrations of butyric acid (C4), caproic acid (C6), caprylic acid (C8), and capric acid (C10) directly into the cheese matrix during the extended ripening phase¹⁷.

The accumulation of these short-chain FFAs is the primary biochemical driver of the sharp, piquant (*piccante*), spicy, and peppery flavor profiles characteristic of traditional, long-aged Mediterranean cheeses such as *Provolone*, *Pecorino Romano*, and specific artisanal iterations of *Feta*¹⁷. The sensory impact of these lipases is highly species-dependent:

- **Calf Rennet Paste (Dolce):** Typically provides a sweeter, milder, and rounder aroma profile, releasing a highly balanced array of fragrances with a lower intensity of sharpness⁴⁰.
- **Lamb and Kid Rennet Paste (Piccante):** Highly aggressive lipolytic action, rapidly generating goaty, pungent, and intensely spicy notes without introducing the soapy off-flavors associated with the hydrolytic release of long-chain fatty acids (C12-C18)¹⁷.

Modern industrial producers attempting to replicate these complex, heritage sensory profiles using FPC must artificially supplement their vats with exogenous, purified microbial lipases¹⁷. Conversely, the traditional abomasum paste naturally supplies the optimal, historically accurate stoichiometric ratio of proteolytic coagulants to lipolytic flavor-generators in a single application¹⁷.

Quantification of Coagulating Efficacy and Application Standards

Because the enzymatic strength of traditional, home-harvested rennet paste varies wildly based on the age of the calf, the specific diet prior to slaughter, and the efficiency of the brining and pulverization parameters, its absolute coagulating power must be strictly quantified prior to commercial application in the dairy plant.

Measuring Flocculation and the IMCU Standard

The internationally recognized standard metric for quantifying rennet strength is the International Milk-Clotting Unit (IMCU) per gram or milliliter, as defined by IDF Standard 157 (ISO 11815)⁴³. Historically, the industry relied on Soxhlet Units (SU)—defined as the volume of standard milk that can be clotted by one unit volume of rennet in precisely 40 minutes at 35°C—but IMCU provides a vastly more objective, reproducible metric utilizing a standardized reference rennet powder containing exactly 2000 IMCU/g⁴³. Commercial single-strength liquid rennets typically operate at approximately 200 to 250 IMCU/mL⁴⁶.

However, for the artisanal cheesemaker utilizing farm-harvested paste where advanced optical backscatter analysis is unavailable, strength is reliably determined empirically via the Berridge method or a standardized visual flocculation test¹.

1. **Flocculation Testing Protocol:** A highly controlled, small volumetric sample of the reconstituted paste (e.g., 2.5 mL or 0.5 tsp) is diluted in non-chlorinated water and introduced into a standardized volume of milk (e.g., 4 Liters) maintained at a constant 35°C (95°F)¹³.
2. **Visual Tracking Mechanism:** The operator places a lightweight plastic cap top-down on the surface of the milk in the vat. Initially, lightly striking or flicking the cap allows it to glide completely freely over the liquid surface. As the chymosin actively cleaves the κ -casein and the micelles begin their massive aggregation phase, the macroscopic viscosity of the milk increases exponentially¹.
3. **The Flocculation Point:** When the plastic cap ceases to budge upon striking, the milk has reached the exact flocculation point (typically targeted between 10 and 15 minutes post-inoculation)⁹. If the flocculation time is significantly shorter than 10 minutes, the rennet paste is highly concentrated; the volumetric dosage must be immediately reduced for subsequent batches to prevent overly rapid, violent coagulation, which can prematurely trap excessive moisture and whey within the curd matrix, leading to disastrous souring and textural defects during ripening⁹.

A properly executed, highly effective home-harvested calf rennet paste generally functions at an application rate of approximately 60 to 80 IMCU per gram, rendering it capable of rapidly and cleanly setting standard milk volumes with only a single gram of paste⁴⁶.

Conclusion

The harvesting and processing of an unweaned calf's abomasum to produce traditional chymosin rennet paste represents a pinnacle of empirical biotechnology, merging centuries-old artisanal knowledge with precise biochemical mechanics. The process demands absolute, uncompromising adherence to hygienic slaughter protocols, utilizing neurophysiological concussive interventions to ensure humane dispatch and strict two-knife exsanguination techniques to preserve the complete sterility of the internal viscera. By harvesting the abomasum exclusively from neonatal calves, the operator guarantees a dominant, targeted concentration of chymosin over pepsin, effectively neutralizing the risk of non-specific proteolytic bitterness in the final cheese matrix.

The transformation of the raw, highly active gastric tissue into a stable enzymatic paste relies

entirely on a meticulously calibrated 15% sodium chloride brining protocol. This hypertonic environment acts as a severe, multi-functional hurdle: it osmotically extracts the preprochymosin zymogen from the mucosal chief cells, instantly halts the endogenous proteolytic decay of the tissue, and creates an environment completely hostile to the proliferation of enteric pathogens such as *Listeria monocytogenes* and *Salmonella*. Subsequent thermodynamic desiccation below 21°C stabilizes the highly sensitive bilobal structure of the chymosin molecule, allowing for long-term storage and efficient, high-shear mechanical pulverization.

When the desiccated, pulverized powder is reconstituted in an acidic whey carrier, the resulting pH drop triggers the complex autocatalytic cleavage of the zymogen's prosegment, unleashing the fully active chymosin. Unlike highly purified, unidimensional industrial coagulants, this traditional paste delivers not only the targeted, flawless cleavage of the Phe105-Met106 bond

in κ -casein necessary for ideal curd formation but also introduces a rich, historically vital array of pregastric esterases and lipases. These lipolytic agents—liberating massive quantities of short-chain free fatty acids—ultimately define the complex, sharp, and highly sought-after piquant flavor profiles of the world's most distinguished artisanal cheeses. Through the rigorous integration of anatomical knowledge, pathogenic control, and biochemical activation, the traditional extraction of bovine chymosin remains a highly relevant and indispensable technique in the highest echelons of dairy science.

Works cited

1. Determination of Rennet Clotting Time by Texture Analysis Method, <https://www.scirp.org/journal/paperinformation?paperid=37556>
2. (PDF) Plant Milk-Clotting Enzymes for Cheesemaking - ResearchGate, https://www.researchgate.net/publication/359375192_Plant_Milk-Clotting_Enzymes_for_Cheesemaking
3. One-Step Activation, Purification, and Immobilization of Bovine, <https://www.mdpi.com/2311-5637/12/1/66>
4. Article, <http://2.mol.bio.msu.ru/biokhimiya/contents/v85/full/85070916.html>
5. Exploring the Wide World of Rennet | culture: the word on cheese, <https://culturecheesemag.com/stories/research-into-the-wide-world-of-rennet/>
6. Was ist Lab und wofür wird es verwendet? Die Informationsschrift, <https://www.caglifocioclerici.com/de/was-ist-lab-und-wie-wird-es-hergestellt-der-leitfaden-des-caglifocio-clerici/>
7. Production of Sensorily Acceptable Pasta Filata Cheese with Partial, <https://www.mdpi.com/2304-8158/12/9/1766>
8. 46 Bovine Carcass Dressing Guide.pdf
9. How to Make Rennet for Cheesemaking - Homesteaders of America, <https://homesteadersofamerica.com/how-to-make-rennet-cheesemaking/>
10. What is rennet and what is it used for? The Caglifocio Clerici's guide, <https://www.caglifocioclerici.com/en/rennet-what-is-how-is-it-made-caglifocio-clerici-guide/>

11. Gastric chief cell - Wikipedia, https://en.wikipedia.org/wiki/Gastric_chief_cell
12. Chymosin | Request PDF - ResearchGate, https://www.researchgate.net/publication/278718218_Chymosin
13. DIY: Traditional Rennet - Gianacis Caldwell, <https://gianacliscaldwell.com/2016/05/05/diy-traditional-rennet/>
14. How To Use Rennet - Caglificio Clerici, <https://www.caglificioclerici.com/en/our-rennet/how-to-use-tennet/>
15. Rennet - Wikipedia, <https://en.wikipedia.org/wiki/Rennet>
16. Lab - Wikipedia, <https://de.wikipedia.org/wiki/Lab>
17. Altering the Chain Length Specificity of a Lipase from Pleurotus, <https://pmc.ncbi.nlm.nih.gov/articles/PMC9455245/>
18. Influence of lamb rennet paste on the lipolytic and sensory profile of, https://www.researchgate.net/publication/225052141_Influence_of_lamb_rennet_paste_on_the_lipolytic_and_sensory_profile_of_Murcia_al_Vino_Cheese
19. Käse mit Lab selber machen, <https://www.kaese-selber.de/blogs/blog/kaese-mit-lab-selber-machen>
20. Effects of ionic strength on the folding and stability of SAMP1 ... - PMC, <https://pmc.ncbi.nlm.nih.gov/articles/PMC8874027/>
21. Cholinium-Based Ionic Liquids Modulate Protein Stability - MDPI, <https://www.mdpi.com/1420-3049/30/7/1574>
22. Salt of the Earth: All about cheese's most elemental ingredient, <https://culturecheesemag.com/blog/salt-of-the-earth-all-about-cheeses-most-elemental-ingredient/>
23. Microbial Diversity of Traditionally Processed Cheese ... - IntechOpen, <https://www.intechopen.com/chapters/76551>
24. Evaluation of Microbiological Quality and Safety of Milk and Dairy, <http://article.sapub.org/10.5923.j.fph.20180802.03.html>
25. Safety evaluation of the food enzyme rennet paste from ... - PMC - NIH, <https://pmc.ncbi.nlm.nih.gov/articles/PMC8678806/>
26. (PDF) Cloning, Expression, Purification and Refolding of Caprine, https://www.academia.edu/35614233/Cloning_Expression_Purification_and_Refolding_of_Caprine_Prochymosin
27. Analysis of crystal structures of aspartic proteinases: On the role of, <https://pmc.ncbi.nlm.nih.gov/articles/PMC2374050/>
28. (PDF) Structural aspects of activation pathways of aspartic protease, https://www.researchgate.net/publication/12800482_Structural_aspects_of_activation_pathways_of_aspartic_protease_zymogens_and_viral_3C_protease_precursors
29. Mechanism of activation of the gastric aspartic proteinases - PMC, <https://pmc.ncbi.nlm.nih.gov/articles/PMC1219805/>
30. Structural Catalytic Core of the Members of the Superfamily of Acid, <https://pmc.ncbi.nlm.nih.gov/articles/PMC11313796/>
31. Investigations on the activation of bovine prochymosin - PubMed, <https://pubmed.ncbi.nlm.nih.gov/34519/>
32. Chymosin | PDF | Cheese | Protease - Scribd,

- <https://www.scribd.com/doc/81494102/chymosin>
33. Multiple strategies were adopted to optimize the enzymatic ... - PMC,
<https://pmc.ncbi.nlm.nih.gov/articles/PMC12158995/>
 34. US6375947B1 - Recombinant kid pregastric esterase and methods,
<https://patents.google.com/patent/US6375947B1/en>
 35. (PDF) The rennet paste: the effects of the production techniques on,
https://www.researchgate.net/publication/231590079_The_rennet_paste_the_effects_of_the_production_techniques_on_the_enzymatic_activity_and_the_role_of_the_lipolytic_fraction
 36. Pregastric esterase and other oral lipases--a review - PubMed,
<https://pubmed.ncbi.nlm.nih.gov/321489/>
 37. Lamb rennet paste in ovine cheese manufacture. Lipolysis and flavour,
https://www.researchgate.net/publication/233817334_Lamb_rennet_paste_in_ovine_cheese_manufacture_Lipolysis_and_flavour
 38. Effect of artisanal liquid rennet from kids and lambs abomasa on the,
https://www.researchgate.net/publication/248509368_Effect_of_artisanal_liquid_rennet_from_kids_and_lambs_abomasa_on_the_characteristics_of_Feta_cheese
 39. Effect of the use of three different lamb paste rennets on lipolysis of,
https://www.researchgate.net/publication/230806457_Effect_of_the_use_of_three_different_lamb_paste_rennets_on_lipolysis_of_the_PDO_Pecorino_Romano_Cheese
 40. Rennet Paste - Walcoren, <https://www.walcoren.com/rennet-paste/>
 41. The Flavour - Caglifacio Clerici,
<https://www.caglifioclerici.com/en/the-ingredients/the-flavour/>
 42. Does type of rennet (animal vs. vegetable) affect the end flavor of a,
https://www.reddit.com/r/cheesemaking/comments/cknw9q/does_type_of_rennet_animal_vs_vegetable_affect/
 43. Determination of total milk-clotting activity according to the,
<http://www.manuelcastillo.es/pdfs-publications/Comparison%20of%20total%20milk-clotting%20activity%20measurement.pdf>
 44. Milk Clotting Activity - Caglifacio Clerici,
<https://www.caglifioclerici.com/en/the-ingredients/milk-clotting-activity/>
 45. Comparison of total milk-clotting activity measurement precision,
<https://www.cabidigitallibrary.org/doi/full/10.5555/20113375527>
 46. Rennet strength - is it possible to convert from g per kg to IMCU/ml?,
https://www.reddit.com/r/cheesemaking/comments/jifz4/rennet_strength_is_it_possible_to_convert_from_g/
 47. 6. Culture and Rennet Activity Tests - University of Guelph Open Books,
<https://books.lib.uoguelph.ca/cheesemakingtechnologybook/chapter/culture-activity-test-2/>
 48. Coagulation activity - CERTA - UAB,
<https://certa.uab.cat/index.php/en/what-we-offer/coagulation-activity/>
 49. Coagulant Activity Test - Dairy Connection,
<https://dairyconnection.com/coagulant-activity-test/>