

Botanical Coagulation Agents for Artisanal Cheesemaking in the Berlin-Brandenburg Region: Ethnobotany, Biochemistry, and Cultivation Potential

1. Introduction to Plant-Derived Coagulants and Dairy Innovation

The global cheese industry relies fundamentally on the enzymatic coagulation of milk, a biochemical transformation historically mediated by animal rennet—specifically, chymosin extracted from the abomasum of unweaned calves¹. However, an intersection of ethical considerations, religious dietary laws (such as Halal and Kosher restrictions), and the rising prevalence of vegetarianism has fundamentally disrupted the traditional rennet supply chain³. While microbial coagulants and Fermentation-Produced Chymosin (FPC) currently dominate the industrial market to meet the demands of a global cheese production volume exceeding 22 million metric tons annually, there is a renaissance in the application of natural botanical coagulants⁶. This resurgence is largely propelled by the "clean label" movement and a growing consumer demand for artisanal, heritage-rich dairy products featuring unique organoleptic profiles⁸.

The historical precedent for botanical coagulants is extraordinarily robust. The earliest indications of cheesemaking date back to cave paintings from approximately 5000 BC¹. By the time of ancient Greece, figures such as Aristotle and Hippocrates documented the use of fig tree latex to curdle milk⁵. In his first-century AD agricultural treatise *De Re Rustica*, the Roman author Lucius Junius Moderatus Columella provided detailed instructions on cheesemaking, noting that while lamb or kid rennet was standard, milk could also be successfully coagulated with the flowers of the wild thistle, the seeds of the safflower, or the sap of the fig tree⁵. This ancient practice laid the foundation for a Mediterranean cheesemaking tradition that persists in several Protected Designation of Origin (PDO) cheeses today¹¹.

Translating these ancient practices to modern, peri-urban environments such as the Berlin-Brandenburg region of Germany presents a unique matrix of opportunities and challenges. This report exhaustively examines the biochemical mechanisms, ethnobotanical profiles, and technological integration of specific botanical coagulants—namely *Cynara cardunculus* (wild cardoon), *Ficus carica* (common fig), *Galium verum* (Lady's bedstraw), and *Urtica dioica* (stinging nettle). Furthermore, it evaluates the ecological, legal, and toxicological frameworks governing the foraging and cultivation of these species within the specific climatic and geographical context of Berlin, Germany, offering a comprehensive roadmap for artisanal

dairy producers.

2. The Biochemical Architecture of Enzymatic Milk Coagulation

To evaluate the efficacy of botanical coagulants, it is necessary to first understand the structural biology of the milk matrix and the exact kinetics of its destabilization. Bovine milk is a complex emulsion wherein casein proteins—specifically α_{s1} -, α_{s2} -, β -, and κ -casein—assemble into highly hydrated, spherical colloidal structures known as casein micelles⁴. The core of the micelle consists of α - and β -caseins linked by hydrophobic interactions and colloidal calcium phosphate cross-links, while the exterior is stabilized by a hairy layer of κ -casein¹². The C-terminal region of native κ -casein is distinctly hydrophilic and carries a net negative charge at the native pH of milk (approximately pH 6.7), providing the micelle with colloidal stability through both steric hindrance and electrostatic repulsion¹².

2.1 The Two-Phase Coagulation Mechanism

Enzymatic coagulation occurs in two distinct but overlapping phases, a mechanism that remains fundamentally similar regardless of whether calf chymosin or a botanical protease is utilized³.

The primary phase is entirely proteolytic. The coagulating enzyme specifically targets and hydrolyzes the amphiphilic κ -casein molecule. The precise site of this cleavage is the peptide bond between phenylalanine (Phe105) and methionine (Met106)³. The length of the peptide and the sequence surrounding this partitioned bond are critical determinants of enzyme-substrate interaction; studies indicate that while di-, tri-, and tetrapeptides containing the Phe-Met bond are not hydrolyzed, pentapeptides such as Ser-Leu-Phe-Met-Ala-OMe undergo facile enzymatic cleavage³. This highly specific hydrolysis releases the hydrophilic, negatively charged C-terminal region, known as the caseinomacropeptide (CMP), into the soluble whey fraction³. The hydrophobic N-terminal portion, para- κ -casein, remains integrated within the micelle core³.

The secondary phase, which is non-enzymatic, initiates once approximately 85% to 90% of the κ -casein has been hydrolyzed³. The removal of the CMP eliminates the steric barrier and causes a roughly 50% decrease in the zeta potential of the micelle, effectively neutralizing the electrostatic repulsion that previously maintained colloidal dispersion³. The destabilized para-casein micelles rapidly aggregate via a combination of hydrophobic interactions and calcium-mediated ionic cross-links³. This cooperative aggregation forms a three-dimensional fractal gel network that traps fat globules and aqueous phases, eventually expelling the liquid whey during a process known as syneresis to form the solid curd³.

2.2 Milk-Clotting Activity versus Proteolytic Activity (The MCA/PA

Ratio)

The paramount metric for evaluating any alternative botanical coagulant is the ratio of its specific Milk-Clotting Activity (MCA) to its general, non-specific Proteolytic Activity (PA)¹. An ideal coagulant, such as calf chymosin, possesses a highly specific affinity for the

Phe105-Met106 bond of κ -casein with minimal degradation of the internal α_{s1} - and β -casein fractions¹.

Plant proteases frequently exhibit an excessively broad proteolytic specificity, leading to a low MCA/PA ratio¹. Excessive secondary proteolysis is highly detrimental to the cheesemaking process for several interconnected reasons. From an economic standpoint, aggressive general proteolysis breaks down the structural casein matrix into smaller, soluble peptides that are lost into the whey stream, significantly reducing the total dry matter yield of the cheese¹. From a

textural perspective, the over-hydrolysis of the α_{s1} - and β -casein fractions disrupts the structural integrity of the gel network¹⁴. Dynamic rheological monitoring of milk gels reveals

that the storage modulus (G'), which represents the elastic strength of the curd, is generally lower for plant-induced gels compared to chymosin-induced gels, leading to curds that can be overly soft, pasty, or fragile²³.

Furthermore, the non-specific cleavage of β -casein—such as the rapid breakdown of the sequence yielding β -casein f(193-209)—frequently generates low-molecular-weight hydrophobic peptides¹. These peptides accumulate during the ripening phase and bind to human bitter taste receptors, imparting an undesirable, astringent, and distinctly bitter flavor profile to the final product¹². Consequently, the successful application of botanical coagulants requires meticulous species selection, extraction optimization, and processing interventions designed to maximize the MCA/PA ratio and modulate enzyme kinetics¹.

Protease Source	Catalytic Class	Key Enzymes	Specificity & MCA/PA Ratio Characteristics
<i>Cynara cardunculus</i>	Aspartic	Cardosin A, Cardosin B	High MCA; Cardosin A is highly specific (chymosin-like), while Cardosin B exhibits broader action (pepsin-like) ²⁶ .

<i>Ficus carica</i>	Cysteine	Ficin (Isoforms A-D)	Low MCA/PA ratio; aggressive, broad-spectrum proteolysis requiring strict processing control to prevent bitterness ²² .
<i>Galium verum</i>	Aspartic	Progaline A, Progaline B	Moderate MCA; unique α - and β -casein hydrolysis profile distinct from traditional coagulants ²⁹ .
<i>Urtica dioica</i>	Diverse	Unspecified Proteases	High pH dependence (optimal MCA at pH 4.0); moderate clotting strength with high colloidal stability ³¹ .

3. Ethnopharmacology and Biochemistry of Botanical Coagulants

Plant-derived milk-clotting enzymes generally fall into three major catalytic classes based on their active site residues: aspartic proteases, cysteine proteases, and serine proteases³. Aspartic proteases, which utilize two highly conserved aspartate residues to activate a water molecule for nucleophilic attack, are the most abundant and effective class for cheesemaking³. Cysteine proteases, which rely on a catalytic triad including a deprotonated cysteine residue, are less specific but highly potent³. The biochemical properties of these enzymes vary radically across species, dictating their suitability for different cheese varieties.

3.1 *Cynara cardunculus* (Wild Cardoon and Artichoke Thistle)

The *Cynara* genus, belonging to the Asteraceae family, represents the most extensively documented and commercially utilized source of botanical coagulants globally⁹. Aqueous extracts derived from the violet pistils (stigmas and styles) of the *C. cardunculus* flower are mandatory ingredients in the Iberian Peninsula for the manufacture of several Protected Designation of Origin (PDO) ovine and caprine cheeses, including *Queijo Serra da Estrela*, *Queijo de Serpa*, *Torta del Casar*, and *Queso de La Serena*, as well as Italian traditional cheeses

like *Caciofiore di Columella*¹¹.

3.1.1 Structural Biology of Cardosins

The milk-clotting efficacy of *C. cardunculus* is primarily driven by aspartic proteases (APs) designated as cardosins and cyprosins²⁶. At least nine distinct aspartic proteinases have been identified in the pistils of *C. cardunculus*, an unusually high concentration for higher plants, suggesting a specific, localized biological function⁴⁰. The enzymes are synthesized as inactive zymogens (preproenzymes) containing a signal peptide for endoplasmic reticulum translocation, a prosegment necessary for correct folding, and a unique 104-amino acid Plant-Specific Insert (PSI)³³. During maturation in the plant vacuoles or extracellular matrix, the PSI is excised³³. The mature enzymes, such as Cardosin A and Cardosin B, are heterodimeric structures consisting of a heavy chain (approximately 31 to 34 kDa) and a light chain (approximately 14 to 16 kDa) linked by hydrophobic interactions and inter-peptide disulfide bonds¹⁵.

3.1.2 Kinetic Specificity and Cheese Matrix Impact

Cardosin A represents the vast majority of the proteolytic activity within the crude aqueous extract³⁹. Kinetically, Cardosin A mirrors the catalytic specificity of calf chymosin, preferentially cleaving the critical Phe105-Met106 bond of κ -casein with a remarkably high catalytic efficiency (k_{cat}/K_m)²⁶. Conversely, Cardosin B functions more akin to mammalian pepsin, demonstrating broader sequence selectivity and higher non-specific proteolytic activity against the internal α_{s1} - and β -caseins²⁶. The catalytic mechanism relies on two critical aspartate residues, Asp80 and Asp267, located at the base of a deep active site cleft⁴². The dynamic ratio of Cardosin A to Cardosin B dictates the rheological behavior of the resulting cheese²⁶. During the early stages of ripening, cardosin-mediated proteolysis rapidly degrades the protein matrix. In traditional cheeses like *Torta del Casar*, this aggressive, ongoing primary proteolysis results in a distinctive creamy, nearly liquid texture, accompanied by piquant and slightly bitter flavor notes⁴⁴. When extracting the enzyme, macerating the dried flowers in a 0.1 M phosphate-citrate buffer at pH 6.5 for six hours has been shown to yield optimal, highly stable clotting activity¹⁵. The activity is notably enhanced by the addition of Ca^{2+} ions (e.g., $CaCl_2$ concentrations up to 13 mM) and optimal temperatures ranging from $35^\circ C$ to $45^\circ C$ ⁴⁷.

3.2 *Ficus carica* (Common Fig)

The latex exuded from the branches and unripe fruits of the *Ficus carica* tree has been utilized across the Mediterranean basin for millennia to produce specialized artisanal products, such as the Algerian *agugli* (a soft ewe's milk cheese) and the Greek *Teleme* cheese²⁷.

3.2.1 Biochemistry and Isoforms of Ficin

The principal active enzyme in fig latex is ficin (EC 3.4.22.3), a potent cysteine endopeptidase belonging to the papain family (clan CA, subfamily C1A)²⁷. Genomic and proteomic profiling

reveals that ficin exists in multiple structural isoforms, specifically Ficin A, B, C, and D, with distinct molecular weights of 47.20 kDa, 48.56 kDa, 25.12 kDa, and 24.50 kDa, respectively²⁷.

Structurally, the enzyme folds into two main domains—an N-terminal region rich in α -helices and a C-terminal region composed of antiparallel β -strands—separated by a "V" shaped active site cleft³⁴.

The catalytic mechanism is driven by a highly conserved triad of amino acids: Cysteine-25 (Cys25), Histidine-159 (His159), and Asparagine-175 (Asn175)³⁴. During catalysis, the imidazole ring of His159, oriented by Asn175, deprotonates the thiol group of Cys25. The resulting highly reactive thiolate anion performs a nucleophilic attack on the carbonyl carbon of the target peptide bond, forming a transient covalent acyl-enzyme intermediate that is subsequently deacylated by a water molecule⁵⁰.

3.2.2 Technological Application and Thermal Kinetics

Ficin possesses robust proteolytic activity across a broad pH range (5.0 to 8.0) and exhibits a significantly lower MCA/PA ratio compared to chymosin or cardosins²⁸. This aggressive, unspecific proteolysis poses a severe risk of matrix over-hydrolysis, excessive syneresis, and bitterness¹. However, when carefully optimized, ficin is highly effective. For instance, in the production of fresh goat cheese, an optimized concentration of 0.5 mL of fig latex extract per 100 mL of milk, incubated for 120 minutes, yielded an optimal cheese profile featuring 18.56% protein, 2.32% ash, and a robust 803.93 mg/100g of calcium⁵². In *Teleme* cheese production, incorporating purified ficin successfully decreased total syneresis from 95% down to 75%, vastly improving moisture retention²⁷.

A critical consideration for the dairy technologist is ficin's thermal inactivation kinetics. While the enzyme requires temperatures around 45°C to 55°C for optimum catalytic efficiency²⁸, it possesses the lowest inactivation temperature threshold among comparable plant proteases²⁷. Sustained heating to 70°C or 75°C induces rapid and complete thermal denaturation and irreversible inactivation of the enzyme²⁷. This thermal lability allows cheesemakers to precisely halt proteolysis post-coagulation by applying a moderate heat treatment to the curd, preventing runaway bitterness during storage. Furthermore, its potent unspecific activity makes ficin an excellent candidate for generating hypoallergenic milk protein hydrolysates intended for infant nutrition, where the elimination of allergenic epitopes is required²⁷.

3.3 *Galium verum* (Lady's Bedstraw)

Galium verum, a rhizomatous perennial herb of the Rubiaceae family, is natively distributed across Eurasia and is deeply embedded in the historical fabric of European cheesemaking²⁹. Known locally as Lady's Bedstraw or *Echtes Labkraut*, its specific epithet (*verum*, meaning "true") and common names refer directly to its traditional utility as a botanical rennet substitute²⁹. Historically, it was a critical ingredient in traditional English Chester cheese, where its floral compounds imparted both a unique enzymatic action and a distinctive yellow hue to

the curd⁵⁷.

3.3.1 Phytochemistry and Recombinant Progalines

Beyond its coagulant properties, *G. verum* possesses a dense phytochemical profile, rich in bioactive compounds including flavonoids, phenolic acids, iridoids, anthraquinones, phytosterols, and coumarins, which confer significant antioxidant, anti-inflammatory, and antimicrobial properties⁵⁶.

Modern biotechnological research utilizing RACE PCR has successfully isolated two novel aspartic proteases from *G. verum*, designated as preprogaline A and preprogaline B²⁹. The mature form of progaline B is a heterodimeric glycosylated enzyme with an approximate molecular weight of 48 kDa, comprising a 30.7 kDa heavy chain and a 13.5 kDa light chain connected by disulfide bonds²⁹. When recombinantly expressed in the yeast *Pichia pastoris*—achieved by deleting the original plant signal peptide and substituting it with the

yeast α -factor signal peptide from *Saccharomyces cerevisiae*—progaline B demonstrated a

highly favorable MCA²⁹. The recombinant enzyme mirrors the precise κ -casein hydrolysis patterns of commercial industrial coagulants, making it highly suitable for modern dairy

applications, while exhibiting a distinct degradation profile on α - and β -caseins, suggesting a unique potential for novel flavor peptide generation during ripening²⁹.

3.4 *Urtica dioica* (Stinging Nettle)

Urtica dioica is globally recognized for the potent counter-irritant properties of its aerial stinging hairs, which concentrate high levels of histamine, acetylcholine, and formic acid⁵⁹. Furthermore, its subterranean rhizomes (*Urticae radix*) are heavily researched and clinically endorsed by the German Commission E for the treatment of Benign Prostatic Hyperplasia (BPH)⁵⁹. The root extract's efficacy relies on lignans binding to Sex Hormone-Binding Globulin (SHBG) and the inhibition of 5- α -reductase, a critical enzyme that converts testosterone into dihydrotestosterone (DHT)⁵⁹.

However, its utility as a botanical coagulation agent is a subject of intense emerging food biotechnology research³¹. Nettle leaves contain a complex, diverse repertoire of proteases alongside robust defense proteins such as *Urtica dioica* agglutinin (UDA), a heat- and acid-resistant lectin³².

Recent analytical studies leveraging non-destructive methodologies, including Turbiscan stability indexing, FT-MIR, and fluorescence spectroscopy, have demonstrated that the extraction parameters critically dictate the enzymatic yield and stability of the coagulant³¹. Aqueous extracts of *U. dioica* optimized at a highly acidic pH of 4.0 yield the maximum specific milk-clotting activity (0.021 U/mL) and highest overall proteolytic capability³¹. However, at this acidic pH range (3.0–4.0), the resulting extracts exhibit severe colloidal instability, characterized by high Turbiscan Stability Index (TSI) values approaching 20³¹. Conversely, extraction in a nearly neutral pH range (5.0–6.5) yields highly stable enzymatic suspensions (TSI ~1) but with altered catalytic efficiency³¹. While nettle-derived coagulants currently lack the industrial standardization of *Cynara* extracts, they present a highly localized, sustainable

resource for artisanal fresh cheeses where prolonged ripening (and thus, the risk of runaway secondary proteolysis) is not a critical factor⁶¹.

4. The Ecological and Legal Framework of Urban Foraging in Berlin-Brandenburg

For artisanal cheesemakers operating in the Berlin-Brandenburg region, sourcing botanical coagulants through wild foraging requires navigating a stringent legal architecture designed to protect biodiversity. Furthermore, the peri-urban landscape introduces severe toxicological and parasitological risks that demand systematic mitigation⁵⁹.

4.1 Conservation Law and the *Handstraußregelung*

The extraction of wild flora in Germany is governed federally by the *Bundesnaturschutzgesetz* (BNatSchG). The baseline legislative stance, articulated in § 39 Abs. 1 BNatSchG, strictly prohibits the removal, damage, or devastation of wild plants from their natural locations without reasonable cause⁵⁹. However, the law provides a vital exemption for non-commercial, personal foraging known as the "*Handstraußregelung*" (hand bouquet rule)⁵⁹.

Defined under § 39 Abs. 3 BNatSchG, this provision permits individuals to carefully harvest small quantities of wild flowers, leaves, and herbs from areas that are generally accessible to the public and not subject to specific protective designations⁵⁹. A "small quantity" is a qualitative standard interpreted as an amount reasonably held between the thumb and index finger, intended exclusively for the immediate personal consumption (*Eigenbedarf*) of the forager's household⁵⁹.

This legal framework imposes strict limitations on commercial cheesemakers:

1. **Commercial Prohibition:** The *Handstraußregelung* explicitly excludes any commercial harvesting. A cheesemaker intending to forage *Galium verum* or *Urtica dioica* for commercial cheese production, or in volumes exceeding the personal use threshold, must obtain explicit, written consent from the landowner and a formal collection permit (such as a *Holz sammelschein*) from the lower nature conservation authority⁵⁹.
2. **Absolute Prohibition on Subterranean Structures:** The exemption applies solely to the aerial parts of herbaceous plants. The unpermitted excavation of roots, bulbs, or rhizomes—even of ubiquitous species like *U. dioica*—inevitably results in the death of the specimen and disrupts the soil ecosystem. Removing a root without authorization constitutes theft and property damage under the *Strafgesetzbuch* (Criminal Code), carrying severe theoretical fines escalating up to €50,000 for infractions involving protected zones⁵⁹.
3. **Zoning Restrictions:** Foraging is completely nullified on managed agricultural lands and is unequivocally banned within *Naturschutzgebiete* (Nature Reserves), National Parks, and the core zones of Biosphere Reserves, such as the Schorfheide-Chorin or Spreewald in Brandenburg⁵⁹.

4.2 Botanical Literacy and Toxic Lookalikes

Safe urban foraging in Berlin-Brandenburg demands advanced botanical literacy. While gathering common herbs, foragers frequently encounter highly toxic sympatric species sharing

the precise ecological niche and emerging concurrently in the spring understory. For instance, while foraging for aromatic herbs, one must aggressively rule out lethal species. The differentiation of wild garlic (*Allium ursinum*)—which, while endangered and strictly protected in Brandenburg (Red List Category 1), is common in other regions—from its lethal counterparts serves as a primary case study in foraging risk management⁵⁹.

Botanical Feature	<i>Allium ursinum</i> (Edible)	<i>Convallaria majalis</i> (Toxic)	<i>Colchicum autumnale</i> (Lethal)	<i>Arum maculatum</i> (Toxic)
Toxic Principle	Safe/Edible ⁵⁹ .	Cardiac glycosides (convallatoxin); induces severe arrhythmias ⁵⁹ .	Colchicine; a severe mitotic inhibitor, highly lethal ⁵⁹ .	Calcium oxalate raphides; causes severe mucosal burning ⁵⁹ .
Leaf Emergence	Individual leaves, each with its own distinct stalk ⁵⁹ .	Leaves emerge in pairs, tightly clasping a central pseudo-stem ⁵⁹ .	Leaves emerge directly from the soil in a tight rosette, lacking stalks ⁵⁹ .	Leaves are distinctly sagittate (arrow-shaped) ⁵⁹ .
Venation Pattern	Parallel, widely spaced veins ⁵⁹ .	Parallel, very densely packed nervature ⁵⁹ .	Parallel, forming a stiff, rigid structure ⁵⁹ .	Reticulate (net-like) and highly irregular ⁵⁹ .
Texture & Underside	Soft, pliable; distinctly matte on the abaxial (under) side ⁵⁹ .	Firm, leathery; glossy on both adaxial and abaxial surfaces ⁵⁹ .	Fleshy, stiff, rigid; glossy on both surfaces ⁵⁹ .	Firm; frequently exhibits dark maculations (spots) on the adaxial surface ⁵⁹ .

A critical heuristic trap in foraging is olfactory fatigue. After handling heavily aromatic plants, the forager's fingers become coated in essential oils, causing subsequent, potentially toxic plants to smell identical⁵⁹. Visual morphological analysis remains the only secure method of continuous identification. Furthermore, plants within the Apiaceae family—which includes lethal species like *Conium maculatum* (Poison Hemlock) and *Cicuta virosa* (Water Hemlock)—require

extreme caution, as their highly dissected foliage and umbel structures closely resemble edible counterparts⁵⁹.

4.3 Urban Toxicology: Heavy Metal Bioaccumulation

The peri-urban environment of Berlin, characterized by a matrix of dense infrastructure and ruderal wastelands, creates significant sinks for heavy metals (HMs) including lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and chromium (Cr)⁵⁹. Ubiquitous ruderal species frequently targeted by foragers, notably *Urtica dioica* and *Taraxacum officinale*, possess highly adaptable root systems capable of intense phytoextraction and bioaccumulation of these soil contaminants⁵⁹.

Crucially, the physiological distribution of these elements within the plant anatomy is not uniform. Cadmium, copper, and nickel preferentially accumulate in the foliar tissues (leaves), while chromium concentrates heavily in the floral structures⁵⁹. Lead, due to its low mobility within the plant's vascular tissue, tends to remain sequestered in the root system and lower stalks⁵⁹. Because the preparation of botanical coagulants requires the intensive concentration of floral extracts (e.g., *Galium* or *Cynara* flowers) or foliar macerations (e.g., *Urtica* leaves), foraging near high-traffic arteries, former industrial brownfields, or railway corridors introduces a severe risk of heavy metal transfer directly into the cheese matrix⁵⁹. Cultivation in tested, uncontaminated soil substrates or sourcing from verified deep-rural sites is absolutely imperative for commercial safety⁵⁹.

4.4 Zoonotic Parasitology: *Echinococcus multilocularis*

A paramount biological risk associated with foraging low-growing flora in Central Europe is exposure to *Echinococcus multilocularis*, the fox tapeworm⁵⁹. The definitive hosts—primarily the red fox (*Vulpes vulpes*), which maintains extraordinarily high population densities in Berlin's urban spaces, alongside raccoon dogs and felines—excrete highly resilient tapeworm eggs via their feces⁵⁹. These eggs exhibit extreme environmental resilience, surviving in the soil for up to 190 days and remaining viable through severe winter freezes⁵⁹.

Humans act as aberrant intermediate hosts (*Fehlzwischenwirt*). Ingestion of the microscopic eggs leads to alveolar echinococcosis, characterized by a slow, progressive, tumor-like infiltration of the liver²⁸. The disease has a remarkably prolonged incubation period of 10 to 15 years before the onset of clinical symptoms (such as cholestasis, portal hypertension, and hepatomegaly) and is highly lethal if left untreated²⁸.

The Technological Paradox of Zoonotic Mitigation: According to the Robert Koch Institute (RKI), standard washing protocols are insufficient to remove the highly adhesive eggs of *E.*

multilocularis from plant material⁵⁹. Standard domestic freezing (-20°C) is entirely ineffective; the eggs maintain viability until subjected to sustained temperatures of -80°C for several days⁵⁹. The only definitive method to destroy the eggs is sustained thermal processing, specifically cooking or baking the wild edibles at temperatures between 70°C and 80°C for at least five minutes⁵⁹.

However, subjecting botanical coagulants to this thermal threshold induces catastrophic failure of their proteolytic capacity. For instance, the optimal catalytic activity of ficin occurs around 50°C , and the enzyme undergoes rapid thermal denaturation and complete inactivation at temperatures approaching 70°C ²⁸. Similarly, the aspartic proteases in *Cynara* and *Galium* will denature under sustained 80°C heat.

Resolution via Mechanical Exclusion: To resolve the irreconcilable conflict between enzyme preservation and parasitological safety, the cheesemaker must utilize mechanical microfiltration. The taeniid eggs of *E. multilocularis* are physically large on a microscopic scale, measuring approximately 30 to 35 μm in diameter⁶⁶. By preparing the cold aqueous enzyme extract and passing it through a standard commercial polyethersulfone (PES) membrane filter with a pore size of 20 μm or 0.22 μm , the operator guarantees the physical exclusion of the parasite eggs⁶⁵. The soluble, low-molecular-weight protease enzymes (ranging from 14 kDa to 48 kDa) will pass unimpeded into the sterile filtrate, ensuring both maximum coagulant efficacy and absolute biological safety.

5. Climate Change, USDA Hardiness Zones, and Local Cultivation Potential

To bypass the legal ambiguities of the Handstraßregelung, the toxicological risks of urban soils, and the complex zoonotic threats, the localized agricultural cultivation of coagulant species presents the most viable strategy for commercial artisanal production.

Historically, the perennial cultivation of Mediterranean species such as *Cynara cardunculus* and *Ficus carica* in Northern Germany was severely limited by winter frost events⁷⁰. Plant winter hardiness is classified globally by the United States Department of Agriculture (USDA) into geographic zones based on average minimum winter temperatures. For decades, the Berlin-Brandenburg region was firmly situated in Zones 6b to 7a, featuring winter lows of -20.4°C to -15.0°C ⁷⁰. These sustained deep freezes are generally lethal to the overwintering rootstocks of artichoke thistles and cause catastrophic vascular damage to fig trees⁷⁰.

However, anthropogenic climate change and the localized urban heat island effect have fundamentally shifted the agricultural geography of Europe. Recent climatological models incorporating updated measurements from 1991 to 2020 indicate that the interior of Berlin and large swaths of Brandenburg have shifted into Zone 7b (-14.9°C to -12.3°C) and increasingly into Zone 8a (-12.2°C to -9.5°C)⁷⁰. Furthermore, urban centers like Cologne and Duisburg, along with coastal strips, now exhibit Zone 8b microclimates (-9.4°C to -6.7°C)⁷⁰. This upward shift in winter minimums effectively mimics the historical climate

profiles of southern France or northern Italy⁷².

USDA Hardiness Zone	Average Winter Minimum Temperature	Geographical Relevance in Germany (Updated 1991-2020)
Zone 6b	-20.4°C to -17.8°C	Alpine regions in the far south near the Austrian border ⁷⁰ .
Zone 7a	-17.7°C to -15.0°C	South-east Germany, Bavarian Forest, historically Brandenburg ⁷⁰ .
Zone 7b	-14.9°C to -12.3°C	Central Germany, running south-west to north-east, including Berlin ⁷⁰ .
Zone 8a	-12.2°C to -9.5°C	Rhine Valley, Moselle Valley, Bremen, and urban microclimates ⁷⁰ .
Zone 8b	-9.4°C to -6.7°C	Urban areas like the Ruhr region (Cologne) and East Frisian Islands ⁷⁰ .

Consequently, the perennial cultivation of *Cynara cardunculus* is now highly feasible in the Berlin-Brandenburg agricultural periphery. As an added ecological benefit, *C. cardunculus* is a robust, deep-rooted crop highly adapted to low-input agriculture; it exhibits profound drought tolerance, rendering it an excellent candidate for the increasingly arid, low-precipitation summer conditions observed across Brandenburg⁷³. *Ficus carica* can similarly be cultivated in sheltered, south-facing urban microclimates (Zone 8a/8b equivalents), providing a sustainable local source of ficin-rich latex without requiring intensive greenhouse heating infrastructure⁷⁰.

6. Downstream Dairy Technology and Whey Valorization

While cultivating botanicals secures the supply chain, the application of crude plant extracts in cheesemaking inherently introduces biochemical variability. The enzymatic titer of cultivated *Cynara* flowers fluctuates based on soil chemistry, nitrogen fertilization (which has been shown to increase caffeoylquinic acid levels by over 20%), the precise phenological stage of harvest, and the dehydration parameters⁴⁰. Furthermore, managing the aggressive proteolytic activity of plant rennets requires targeted technological interventions to prevent bitterness and yield

loss¹⁴.

6.1 Matrix Manipulation and Yield Optimization

When utilizing high-PA botanicals like *Ficus carica* or *Cynara cardunculus*, dairy technologists can employ several downstream interventions to manipulate the cheese matrix:

1. **Coagulant Blending:** A highly effective strategy involves mixing plant-derived extracts with commercial chymosin (e.g., a 25:75 or 50:50 ratio of cardosin to FPC). The chymosin ensures rapid, specific κ -casein cleavage and a firm, predictable initial gel setup. Simultaneously, the botanical extract operates as a secondary ripening agent, conferring the desired complex, piquant sensory profile without overwhelming the matrix and causing structural collapse¹⁴.
2. **Ultrafiltration (UF) of Milk:** Pre-treating the cheese milk via ultrafiltration increases the concentration of total solids, fat, and native caseins⁷⁵. Utilizing UF milk fundamentally alters the enzyme-to-substrate ratio and increases the chemical buffering capacity of the matrix. Extensive research demonstrates that the use of *C. cardunculus* or *F. carica* extracts in UF milk yields significantly firmer curds, substantially mitigates excessive moisture loss (syneresis), and reduces the incidence of bitterness compared to standard, unconcentrated milk¹⁴.
3. **Salting and Ripening Parameters:** Plant proteases generally exhibit higher temperature optima and lower sensitivity to saline environments than mammalian chymosin²⁸. Increasing the salting time or marginally elevating the NaCl concentration of the cheese brining process can inhibit secondary microbial proteolysis and modulate the plant enzyme's activity. This targeted inhibition decelerates the formation of hydrophobic bitter peptides during prolonged aging¹⁴.

6.2 By-Product Valorization: Sweet vs. Acid Whey

The incorporation of plant coagulants deeply impacts the composition of the resulting whey, presenting unique opportunities for by-product valorization¹⁴. Whey represents 85% to 95% of the total milk volume and retains approximately 55% of the milk's nutrients, including 20% of the original protein⁷⁸.

Whey is broadly classified into two categories:

- **Sweet Whey:** Produced via enzymatic coagulation (using chymosin or botanical rennets like cardosin or ficin) at a pH of approximately 6.0 to 6.7⁷⁸. Sweet whey is rich in lactose (46–52 g/L) and residual whey proteins, making it highly suitable for downstream food processing, sports nutrition supplements (Whey Protein Isolate/Concentrate), and lactose crystallization⁷⁶.
- **Acid Whey:** Produced as a byproduct of acid coagulation (e.g., via lactic acid bacteria fermentation or direct acidification) at a much lower pH of 4.5 to 5.8⁷⁸. Acid whey contains higher levels of lactic acid, calcium, and phosphorus, but its elevated acidity and mineral content make it notoriously difficult to dry into powders or process industrially⁷⁹. High levels of lactose in both whey types contribute to extreme Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), classifying untreated whey

effluent as a severe environmental pollutant⁷⁸.

When utilizing *Cynara cardunculus*, the resulting sweet whey contains high levels of specific caseinomacropptides and is traditionally heated to denature the remaining whey proteins, producing high-value secondary whey cheeses such as Portuguese *Requeijão* or Italian *Ricotta*⁷⁸. The residual liquid from *Requeijão* production, known as Second Cheese Whey (SCW) or *Sorelho*, still contains significant lactose and can be processed via nanofiltration for further carbohydrate recovery⁸². Similarly, the blanching wastewater from cardoon processing can be subjected to ultrafiltration (using 15 kDa membranes) to efficiently recover valuable antioxidant polyphenols⁸³.

Whey Classification	Coagulation Method	Typical pH Range	Characteristics & Industrial Utilization
Sweet Whey	Enzymatic (Calf Rennet, Cardosin, Ficin)	6.0 – 6.7	High lactose and protein; used for Whey Protein Isolate (WPI), <i>Ricotta</i> , <i>Requeijão</i> , and lactose crystallization ⁷⁸ .
Acid Whey	Acidification (Lactic acid, Mineral acid)	4.5 – 5.8	High calcium/phosphorus, highly acidic; difficult to process into powder, utilized in anaerobic biodigesters or specific fermented beverages ⁷⁹ .

7. Industrial Biotechnology, Precision Fermentation, and Market Dynamics

To satisfy the demands of the modern dairy industry for absolute standardization without sacrificing the "vegetarian" label, biotechnology has increasingly looked to precision fermentation to express coagulants, bypassing the physical plant entirely⁸⁴.

7.1 Recombinant Expression of Plant Proteases

Just as the gene for calf chymosin was cloned into microorganisms in the 1990s to create Fermentation-Produced Chymosin (FPC)—which now commands over 60% of the global

market—researchers have successfully mapped and expressed the genes for Cardosin A, Cardosin B, and Progaline B in eukaryotic microbial hosts such as *Kluyveromyces lactis* and *Pichia pastoris*²⁹.

For example, expressing Cardosin B in *K. lactis* involves complex genetic engineering. The gene must be modified to remove the Plant-Specific Insert (PSI)—which otherwise hampers cellular secretion in yeast—and replaced with a small linker sequence⁴². Fascinatingly, the resulting synthetic recombinant Cardosin B actually exhibits a more stringent sequence selectivity and a lower general catalytic efficiency compared to the wild-type plant enzyme⁴⁴. From an industrial perspective, this is highly advantageous: it effectively eliminates the excessive proteolysis associated with crude thistle extracts, allowing for the reliable, continuous production of firm, non-bitter cheeses while retaining the functional properties of a plant enzyme⁴⁴.

7.2 Consumer Perception and the Clean Label Movement

The adoption of botanical coagulants and precision fermentation is heavily influenced by consumer perception and regulatory dynamics⁸. Modern consumers increasingly prioritize "clean label," minimally processed foods⁹⁰. Furthermore, the rise of Alpha-gal syndrome—a

severe, tick-borne allergy to galactose- α -1,3-galactose, a carbohydrate found in mammalian products—has created a critical medical necessity for strictly mammalian-free cheeses, driving the demand for authentic plant and microbial rennets that contain absolutely zero animal-derived components⁹¹.

While FPC and recombinant cardosins offer unparalleled consistency, European consumer markets exhibit high resistance to products derived from Genetically Modified Organisms (GMOs), even when the final enzyme is purified and contains no genetically modified cellular material⁸⁵. Consequently, while precision fermentation represents the apex of industrial scalability, artisanal producers in Berlin seeking to market "locally grown," non-GMO, or "hyper-regional" terroir-driven cheeses will likely continue to favor the agricultural cultivation and controlled aqueous extraction of the physical plants, balancing the inherent biochemical variability with authentic artisanal craft⁸⁵.

8. Strategic Conclusions

The exploration and integration of botanical coagulation agents offers a profound intersection of historical ethnobotany, modern food biotechnology, and climate-adaptive agriculture. For the Berlin-Brandenburg region, the integration of these coagulants into artisanal cheese production is both highly viable and strategically relevant to contemporary consumer trends favoring vegetarian, sustainable, and clean-label foods.

1. **Biochemical Specificity:** The selection of the botanical agent fundamentally dictates the biochemical degradation of the casein matrix. Aspartic proteases from *Cynara cardunculus* (Cardosins) and *Galium verum* (Progalines) offer cleavage mechanisms highly analogous to calf chymosin, yielding stable curds suitable for semi-hard cheeses. Conversely, the potent cysteine protease ficin from *Ficus carica* requires stringent thermal and concentration controls to avoid severe matrix degradation and bitterness, yet holds immense potential for specialized bioactive, hypoallergenic protein

hydrolysates. *Urtica dioica* remains an underexploited resource ideal for rapid-consumption fresh cheeses.

2. **Ecological and Legal Navigation:** Wild foraging for commercial scale is legally prohibited under the *Handstraubregelung* without explicit authorization and is ecologically hazardous in urban environments due to the rapid root bioaccumulation of heavy metals. Furthermore, the ubiquitous presence of *Echinococcus multilocularis* necessitates strict safety protocols. Because the required thermal treatments (70°C - 80°C) will denature the coagulating enzymes, mechanical microfiltration using $20\ \mu\text{m}$ membranes is absolutely required to ensure zoonotic safety without compromising enzymatic integrity.
3. **Climate-Adaptive Cultivation:** The shifting USDA hardiness zones in Germany, moving decisively toward milder 7b and 8a climates, unlock the immediate potential for the local agricultural cultivation of Mediterranean species like *Cynara cardunculus* and *Ficus carica*. Cultivation entirely bypasses the toxicological and legal risks of urban foraging, ensuring a reliable, traceable, and scalable supply of botanical raw materials.
4. **Technological Optimization:** For larger-scale operations, the inherent biochemical variability and aggressive proteolysis of crude plant extracts can be managed through downstream interventions such as milk ultrafiltration, coagulant blending, and careful management of sweet whey streams into secondary products like *Requeijão*. Ultimately, the adoption of recombinant plant enzymes expressed via precision fermentation (e.g., in *Kluyveromyces lactis*) offers industrial standardization, though it must be weighed against regional consumer preferences for non-GMO, authentic artisanal products.

In conclusion, leveraging botanical coagulation agents in the Berlin-Brandenburg region transcends simple ingredient substitution. It represents a multidisciplinary endeavor that requires mastery of protein biochemistry, adaptation to rapidly evolving climatic zones, and rigorous adherence to ecological safety protocols, ultimately yielding distinctive, high-value dairy products.

This is for informational purposes only. For medical advice or diagnosis, consult a professional.

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