THE EDIBLE VESICLE PARADOX

Plant-Derived Extracellular Vesicles, Food Nanostructures and the Boundaries of Nutritional Evidence

From botanical identity and nanoscale isolation to gastrointestinal fate, manufacturing validation, human research and European food law

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Abstract

Plant-derived extracellular vesicles and vesicle-like nanoparticles have become an unusually provocative subject for food biotechnology. Isolated from ginger, grapes and other edible tissues, they combine nanoscale architecture with phospholipids, proteins, metabolites and sometimes detectable nucleic acids. In preclinical systems, selected preparations interact with intestinal epithelial cells, macrophages or microbial communities; engineered variants have been investigated as carriers for exogenous cargo. The opportunity is substantial, but the familiar language of “natural exosomes” hides several separate uncertainties. Particles released from living plant cells are not necessarily equivalent to membrane-rich nanoparticles recovered after grinding, extraction or thermal treatment. A nanoparticle-tracking count is not an identity assay; fluorescent uptake does not establish intact systemic delivery; reduced inflammation in a mouse model does not demonstrate treatment efficacy in humans; and the history of eating ginger or grapes does not automatically establish the lawful status of a concentrated vesicular fraction in the European Union. This critical review combines plant cell biology, membrane chemistry, separation engineering, food processing, gastrointestinal biophysics, experimental design, human evidence, safety assessment, lifecycle analysis and European regulatory science. It examines the 2026 report of thermally reassembled ginger vesicles as a revealing example of how food processing can create a new material identity rather than simply preserve a pre-existing one. A proposed industrial programme uses orthogonal characterisation, mass balance, process-appropriate controls, simulated digestion, human-relevant models and a stepwise legal screen to establish whether a reproducible food ingredient can be developed. The central thesis is that the next generation of bioactive food fractions will be judged not by the novelty of their nanoscale appearance, but by their ability to connect a defined biological source, a reproducible particle population, a justified oral exposure and a consumer-relevant effect within one independently verifiable chain of evidence.

Keywords: *plant extracellular vesicles; food-derived nanovesicles; edible nanoparticles; ginger; grape; gastrointestinal digestion; nanoscale characterisation; MISEV2023; nanoparticle tracking analysis; size-exclusion chromatography; mass balance; oral bioavailability; novel food; engineered nanomaterial; EFSA; European Union; industrial food biotechnology*.

1. The problem begins before the first experiment

A scientifically unusual event occurs when a plant is converted into a food ingredient: a biological structure that may have served a communication or transport function inside its original organism is isolated, concentrated, stored and administered to another organism under entirely different conditions. The resulting material can retain some characteristics of its biological source while acquiring others from mechanical processing, extraction, filtration, aggregation or stabilisation. This distinction is the foundation of any serious discussion of plant-derived extracellular vesicles (PDEVs).

Fresh ginger contains cell membranes, intracellular vesicles, cell-wall structures, proteins, lipids, pigments, nucleic acids and soluble metabolites. A laboratory may homogenise it, separate a particulate fraction and observe nanoscale objects. It is possible that this fraction contains extracellular vesicles naturally released before disruption. It is also possible that it includes membranes fragmented during milling, reassembled lipid assemblies, organelle-derived material, aggregated proteins or particles with entirely different biogenetic histories. An image of a rounded particle cannot determine which possibility is correct. Thus, a discovery statement that “ginger contains exosomes” cannot be deduced from size distribution and morphology alone.

There are legitimate reasons to investigate such materials. Early studies demonstrated that edible plant-derived nanoparticle preparations can interact with mammalian intestinal cells in vivo. Mu and colleagues reported plant nanoparticle interactions with mouse intestinal tissues, while Zhang and colleagues investigated a defined ginger-derived nanoparticle population in experimental models of intestinal inflammation [3,4]. Later work broadened the discussion to possible oral delivery platforms. These experiments provide a scientifically credible starting point. They do not establish that all such particles are naturally secreted vesicles, that the administered fractions remain intact throughout digestion, or that any plant-vesicle supplement improves human health.

The most consequential development in 2026 may be conceptual rather than commercial. A study of ginger material reported that boiling could induce nanoscale reorganisation, creating a thermally reassembled vesicular preparation whose composition and experimental behaviour differed from the original fraction [5]. This challenges

the convenient assumption that every processing operation merely destroys a pre-existing bioactive. Processing may destroy some structures, preserve others, generate new assemblies and alter the relationship between particle count and biological response. In a food factory, these outcomes are not laboratory curiosities: they determine whether the material at the time of consumption resembles the preparation studied in the laboratory.

This article is a critical narrative synthesis, not an original human trial, an exhaustive registered systematic review or a legal determination for an identified commercial material. Its purpose is to develop a defensible framework for research investment, ingredient qualification and regulatory decisions. No industrial results are attributed to INTABIOTECH unless independently documented.

2. A taxonomy that prevents mechanistic confusion

The language of this field is unsettled. Studies use “plant extracellular vesicles”, “exosome-like nanoparticles”, “vesicle-like particles”, “edible nanovesicles” and “plant-derived nanoparticles”, sometimes for materially different preparations. There is a substantive biological reason for this variation. In mammalian cell research, the biogenesis of small extracellular vesicle populations is itself difficult to determine from end-product measurements. Plant cell walls, apoplastic compartments, secretory pathways and tissue-disruption procedures introduce additional challenges.

The International Society for Extracellular Vesicles (ISEV) recommends operational clarity in naming and characterisation under MISEV2023 [1]. The guidance is not a plant-food product authorisation, and plant-specific markers and procedures still require development. Nonetheless, its core principles are valuable: specify the starting material; report isolation and storage conditions; use complementary physical and biochemical characterisation; describe non-vesicular co-isolates; and avoid declaring a particular biogenesis pathway when it has not been established. Plant-focused methodological advice has likewise emphasised multiple independent measurements and contamination controls [2].

For industrial purposes, three working categories are more useful than a single seductive name. **Naturally released plant EVs** are particles for which a credible case can be made that they were secreted by viable plant cells into an extracellular compartment. **Plant-derived vesicle-like particles** are membrane-associated fractions recovered from food tissues without conclusive evidence of the release pathway. **Engineered or reassembled plant-derived nanocarriers** result from deliberate manipulation, loading, reconstruction or processing that materially determines their final structure. These categories may overlap analytically but should not be merged in a technical dossier.

Working category	What the name may responsibly convey	Evidence needed beyond particle size	Main industrial uncertainty
Naturally released plant EVs	Extracellular origin supported by collection from a relevant plant compartment	Biological source, isolation without substantial cell rupture, compatible membrane and cargo markers, negative controls	Scale-up from viable biological secretion to a food-grade output
Tissue-derived vesicle-like nanoparticles	Nanoscale membrane-associated fraction recovered from processed edible tissue	Orthogonal particle identity, exclusion of debris and aggregates, source and process definition	Variation caused by cutting, grinding, homogenising and extraction
Thermally reassembled vesicular structures	Nanoscale assemblies arising or changing following a specified thermal treatment	Before/after structural data, composition, mass balance and process causality	Whether production conditions and resulting material can be reproduced
Loaded or surface-engineered nanocarriers	Deliberate incorporation of external cargo or modification of a vesicular fraction	Encapsulation efficiency, free-cargo removal, release kinetics, toxicity and manufacture	Potential transition away from a conventional-food regulatory route

Table 1. *Operational nomenclature for plant-associated nanoscale preparations. None of these terms independently establishes a food authorisation, a therapeutic indication or clinical efficacy.*

The problem of identity extends to the source material. A supplier should specify the botanical Latin name, cultivar or variety when relevant, plant part, growing system, season, post-harvest conditions, processing delays and intermediate storage. Juice from a ripe fruit, freeze-dried peel, cultured plant cells and a mechanically comminuted rhizome are not interchangeable feedstocks. Each contains a different set of membranes, metabolites, enzymes and potential contaminants. Even a genetically stable plant can produce a chemically variable vesicle-rich fraction when exposed to drought, temperature shifts, pest stress or changes in maturation.

3. What is inside a plant vesicle-like fraction?

A nanoscale membrane compartment is not a single active substance. Its outer boundary may contain phospholipids, glycolipids, sterols and associated proteins; its internal or co-isolated phase may contain small metabolites, proteins, carbohydrates, oligonucleotides or free molecules retained during purification. Lipid composition governs membrane packing, curvature, permeability, phase behaviour and interaction with bile salts. Surface charge can influence colloidal stability and contact with mucus or epithelial interfaces, although a zeta-potential measurement alone does not establish behaviour in a complex gastrointestinal environment.

The biochemical argument for plant nanovesicles has two components that should be kept separate. One is **intrinsic activity**: a defined fraction may influence a cell because of its own membrane composition or associated constituents. The other is **carrier activity**: the particle may protect, concentrate or deliver an endogenous or deliberately loaded constituent. A material can perform one role without the other. Moreover, a cell response attributed to the vesicle may arise from gingerols, shogaols, flavonoids, free fatty acids or other small molecules that co-purified with the fraction.

This distinction can be addressed experimentally. When feasible, the researcher should compare the purified fraction, the relevant particle-depleted supernatant, a preparation in which membrane organisation has been disrupted, a free-cargo control and a reconstituted carrier with matched exposure. Each control answers a different question. A particle-depleted fraction tests whether soluble non-vesicular material can reproduce the result. Membrane disruption tests whether an intact assembly is necessary, but the disruption procedure itself may chemically alter constituents. A synthetic liposome control helps distinguish structural membrane delivery from uniquely plant-derived effects. None is a perfect comparator on its own.

Small RNAs deserve especially careful treatment. Detection of RNA within a plant-derived fraction establishes analytical presence under specified assay conditions, not necessarily stability during digestion, delivery into human cells or functionally significant regulation of human gene expression. Sequencing libraries can contain contamination, degraded fragments and ambiguous mapping. Evidence for protected intravesicular RNA requires appropriate nuclease treatment, membrane-permeabilization controls, validated extraction, sequence annotation and exclusion of free RNA. Claims of cross-kingdom gene regulation additionally require delivery, intracellular target engagement, an exposure-compatible dose and a reproducible biological consequence. A single statistically altered messenger-RNA abundance in cultured cells is a very early observation in that chain.

Protein signatures require similar scrutiny. Many plant fractions contain proteins from plasma membranes, intracellular compartments and extracellular spaces; there is no universally validated plant equivalent of a single mammalian EV identity marker. The practical answer is not to abandon protein analysis but to use it comparatively: report candidate enriched markers, contaminants, sample preparation, detection limits and batch variability. Lipidomics and proteomics should be interpreted together with particle-level measurements rather than used as substitutes for them.

4. The industrial act of isolation changes the material being studied

Extraction technologies deserve as much attention as molecular biology because they determine the composition of the commercial candidate. Differential centrifugation can enrich particles according to sedimentation behaviour, yet it also sediments aggregates, organelle fragments and other dense material. Ultracentrifugation can expose fractions to high forces and create aggregation or loss. Ultrafiltration can concentrate the target while retaining large proteins and polysaccharides. Size-exclusion chromatography may separate some soluble contaminants but generally has limited ability to discriminate particles with overlapping hydrodynamic dimensions.

Density gradients improve certain separations at the cost of time, scale and recovery. Tangential-flow filtration is attractive for industrial throughput but demands careful membrane, pressure and shear validation. No single operation establishes a pure EV product [1,2,6].

A process should consequently be specified as a sequence of unit operations rather than described by one headline technology. Source washing, size reduction, pressing, filtration, clarification, concentration, polishing, buffer exchange, formulation, packaging and cold-chain management can each alter the fraction. For every operation, three questions should be asked: What target material is retained? What non-target material is co-retained? What structural or chemical alteration is introduced?

Mass balance is central. Researchers often report particles per millilitre of final preparation, but a concentrated fraction can show an impressive numerical count while representing a poor yield from the original biological input. Particles per gram of fresh plant tissue, per kilogram dry mass, per litre of process water and per kilogram of saleable product communicate different properties. The industrial mass balance should quantify starting biomass, dry solids, lipid mass, particle-number distribution, recovered protein and RNA where relevant, product lost to filters or columns, water and energy use, rejected batches and final output meeting specification.

An instructive set of process metrics is:

Saleable vesicular-fraction yield = mass of released specification-compliant fraction ÷ mass of incoming botanical feedstock.

Particle recovery = post-process particle count within a defined, validated measurement window ÷ corresponding pre-process count, with the explicit warning that processing can change particle aggregation, fragmentation or generation; numerical recovery is not necessarily material recovery.

Effective exposure at consumption = measured fraction present in the final serving × fraction retaining specified critical attributes after storage and digestion, where the second factor must be experimentally defined rather than assumed to equal one.

These expressions are not universal formulas for biological potency. Their purpose is to expose the missing denominators in supplier claims. A claim of “ten billion exosomes per capsule” is scientifically incomplete unless particle identity, measuring instrument, calibration, size window, background subtraction, batch variation and the relation between counted particles and biological activity are all described.

5. Orthogonal analytics: how to avoid counting impurities as innovation

Nanoparticle tracking analysis (NTA) estimates the hydrodynamic size and number of light-scattering particles within an instrument-dependent detection range. Dynamic light scattering (DLS) describes intensity-weighted diffusion behaviour and is disproportionately influenced by larger scattering objects. Electron microscopy can visualise morphology and membrane-like structures but introduces preparation artefacts, especially during dehydration, staining or freezing. Cryogenic electron microscopy can reduce some artefacts while imposing substantial cost and methodological requirements. Atomic-force microscopy, tunable resistive pulse sensing and asymmetric-flow field-flow fractionation provide additional orthogonal information in suitable workflows. None should be treated as a definitive standalone assay of plant EV purity.

This is not technical pedantry. A preparation containing free pectin, lipid droplets and cell-wall fragments can display a plausible “exosome-sized” peak. A lipid dye can label non-vesicular hydrophobic components or form its own particles. A fluorescent spot inside a cell may represent free dye, dye-bearing fragments or true vesicle-associated cargo. A meaningful uptake study must remove unbound label, include dye-only and processed-vehicle controls, quantify labelling-induced changes in particle properties and distinguish surface attachment from internalisation. Where possible, independent tracking of membrane and cargo should be used.

An industrial reference panel should address identity of source; fractionation history; particle size distribution and concentration; morphology; lipid and protein fingerprint; cargo relevant to the hypothesised function; co-isolated soluble and non-vesicular fractions; microbiology; relevant food contaminants; process residues; and batch

stability. A certificate reporting only particle diameter, “exosome count” and an antioxidant assay cannot establish a research-grade identity, let alone a saleable food ingredient.

Measurement	What it can establish	What it does not establish	Essential complementary control
NTA or particle tracking	Instrument-specific count and hydrodynamic size profile	EV biogenesis, purity, intact membrane or biological dose	Dilution linearity, blank, calibration, orthogonal microscopy
DLS	Ensemble diffusion behaviour, aggregation trend	Number-weighted abundance or unique vesicle identity	NTA or fractionation and microscopy
Cryo-TEM/TEM	Morphology and some membrane architecture	That every particle in bulk has this structure	Representative sampling, compositional and count analyses
SEC / density gradient / AF4	Operational separation of populations	Absolute purity or function	Recovery balance and pre/post fraction fingerprint
Lipidomics / proteomics	Chemical composition and comparative signatures	Intracellular origin or causal activity	Contaminant controls and functional perturbation
Small-RNA sequencing	Detected RNA sequences under specified conditions	Protected intravesicular RNA or human gene regulation	Nuclease/permeabilisation, blanks, validation and functional assay
Fluorescent cellular uptake	Cell-associated optical signal	Intact particle transport to target organ	Free dye, membrane/cargo dual labels, localisation and mass balance
In-vitro functional assay	A response under particular model conditions	Human efficacy or authorised health claim	Particle-depleted, free-cargo, dose-matched and toxicity controls

Table 2. *Analytical measurements and the inferential boundaries relevant to plant-derived vesicular fractions*.

MISEV2023 remains a valuable scientific reference for reporting the preparation and measurement chain [1]. It should not be cited as evidence that a food has been legally authorised or that a plant-derived fraction satisfies a medical manufacturing standard. Plant-specific performance qualification remains necessary.

6. Food processing does not have one predictable effect on nanoscale structure

An edible plant ingredient is rarely consumed in the state in which its constituent particles were first characterised. Washing, homogenisation, pasteurisation, fermentation, acidification, freezing, freeze-drying, reconstitution, extrusion and storage transform the surrounding food matrix. Some changes are mechanical; others are chemical or biological. Membrane oxidation, protein denaturation, phospholipid hydrolysis, aggregation, coalescence and surface adsorption may occur simultaneously. Food-derived EV processing has therefore become a separate subject of investigation in 2026 [7].

It would be misleading to assume that a vesicle-rich fraction is necessarily destroyed by modest thermal treatment, or that apparent particle survival demonstrates retention of function. A heat-treated sample may retain an NTA peak while losing specific surface proteins. It may show fewer large particles because aggregates have broken apart. Conversely, heating may promote reorganisation of endogenous lipids and proteins into new assemblies detectable in the nanoscale range. The direction of change depends on the botanical matrix, lipid composition, pH, salts, oxygen, heat transfer and shear.

The 2026 original investigation entitled *Thermally Induced Reassembly of Ginger Extracellular Vesicles for Oral Therapy of Intestinal Inflammation* is important precisely because it treats boiling as a structure-changing operation rather than a neutral preservation step [5]. Its authors reported thermally reassembled ginger-derived preparations with altered surface architecture and investigated cell interactions and therapeutic cargo delivery in laboratory and mouse models. The study included mouse experiments involving dextran-sulphate-sodium-induced colitis and comparisons of freshly obtained and thermally reassembled fractions, including siRNA-loaded variants. Those data support a mechanistic hypothesis about processing-induced assembly and oral delivery in the models used.

The appropriate interpretation has several boundaries. First, the work did not demonstrate clinical efficacy in patients with inflammatory bowel disease. Second, a loaded siRNA preparation is not equivalent to a conventional

food containing ginger extract. Third, boiling a biological fraction may create a new product identity, an issue with direct implications for safety and legal classification. Fourth, a favourable result for thermally reassembled material does not establish that any kitchen-boiled ginger beverage produces the same exposure, particle distribution or biological effect. Translating that result to food manufacturing would require a new process specification and an independently reproduced material fingerprint.

The observation also creates an opportunity. Industrial food technology can investigate deliberately controlled assembly and stabilisation, rather than merely trying to preserve laboratory-isolated structures unchanged. Yet this opportunity should be approached through compositional and functional studies. Thermal reassembly cannot be used as a generic synonym for improved bioavailability, and its legal status cannot be deduced from a history of consuming the source plant.

One 2026 study of vesicle-like particles recovered from differently processed *Gastrodia elata* material likewise illustrates why processing history matters [8]. Its interest in ingredient science lies in the comparative physicochemical and model-based data rather than a universally established neurological benefit. An edible or traditionally used plant may produce different nanoparticle-rich fractions depending on drying and other processing operations. Different fraction compositions can alter an in-vitro assay result, which is why a future commercial dossier should define the processing method as part of the ingredient, not as a minor manufacturing footnote.

For an industrial trial, heat and pressure should be expressed as distributions and process histories, not just nominal setpoints. “Pasteurised at 85°C” does not reveal holding time, heating and cooling profiles, oxygen availability or local shear. A valid experiment would record these factors, then assess particle count, size, membrane signatures, cargo retention and application-specific function before treatment, immediately after treatment and after storage. It would also measure free non-vesicular material generated by processing. A process producing a higher count of smaller objects might be less successful if it has fragmented the relevant assemblies rather than increased their functional output.

7. The gastrointestinal tract is not simply a transport corridor

Oral exposure begins a second manufacturing-like sequence inside the consumer. Saliva changes ionic strength and exposes structures to enzymes and mucins. Gastric acidity and pepsin act on protein-rich components. Bile salts, pancreatic lipases, phospholipases and other digestive factors can restructure lipid interfaces. Intestinal mucus, digestive products, resident microbiota and epithelial barriers determine which constituents remain locally available. A vesicle counted in the finished beverage may therefore become an intact particle, a partially disrupted membrane complex, a micellarised lipid fraction, a soluble metabolite or a collection of digestive fragments before any interaction with host cells.

The meaningful question is not whether the original particle remains visible at the end of a simulated digestion tube. It is what exposure reaches a biologically relevant site and in which chemical form. Survival of a subset of nanoscale structures through digestion would be an interesting intermediate result. It would not prove that these structures cross the intestinal epithelium, distribute systematically, reach an intended tissue or release active cargo. Conversely, local interaction with the gut epithelium or immune cells could be biologically relevant without intact systemic transport. These are competing pathways that require different experiments.

A rigorous digestion study should begin with a standardised protocol appropriate to the food product and consumer population, such as an established static in-vitro digestion framework adapted with scientifically justified parameters. It should report the actual matrix, gastric and intestinal conditions, enzyme and bile activity, pH trajectory, dilution, timing and sampling. At each stage, it should test particle identity, soluble co-extractives and any nominated functional cargo. Comparators should include the untreated fraction, a disrupted-particle control, and the same marker or compound added in free form where appropriate. The laboratory must explicitly recognise that simulated digestion cannot reproduce all aspects of intestinal transit, microbial ecology and host uptake.

Bio-accessibility, uptake, bioavailability and efficacy are distinct. **Bio-accessibility** concerns the fraction accessible in a form potentially available for absorption or local action after digestion. **Cellular uptake** describes entry or association in an experimental cell model. **Bioavailability** generally concerns the fraction and rate

reaching systemic circulation or a defined biological site, depending on the endpoint. **Efficacy** demands a specified functional outcome. A preparation can show high uptake in Caco-2 cells while failing to deliver the same amount in a living human intestine. Fluorescence alone cannot quantify intact cargo transport if the label is released or redistributed.

An especially important problem is dose realism. Cell studies may expose monolayers to particle numbers or extract concentrations many times greater than are achievable from plausible food servings. Scaling up from a cell-culture well to an adult's gut volume, mucus, residence time and digestion chemistry is not a linear calculation. An in-vitro effect should be assessed against the maximum credible dietary exposure from a characterized finished food, not against an arbitrarily concentrated laboratory fraction.

The associated microbiome may complicate the interpretation. Bacteria can act on plant metabolites and liberated lipids; particles can change the distribution of food components between soluble and particulate phases; and some reported microbial effects may result from carbohydrates or polyphenols co-isolated with the vesicular fraction. An apparent shift in a bacterial taxon does not establish that the particle population itself acted on that organism, nor that the change improves host health. Functional assays, substrate accounting and controls for the accompanying matrix are necessary to distinguish direct vesicle effects from ordinary food fermentation chemistry.

8. Preclinical results deserve neither dismissal nor clinical inflation

The earliest influential studies of edible plant-derived nanoparticles demonstrated experimental interactions that were not trivial. Mu and colleagues described uptake and signalling effects of plant-derived nanoparticle preparations in mice [3]. Zhang and colleagues characterised multiple nanoparticle populations isolated from ginger and examined one of them in models of colitis and colitis-associated cancer [4]. These studies offered a conceptual alternative to the assumption that oral bioactive molecules must act only after being molecularly dissolved. They remain valuable because they generated testable hypotheses concerning membrane-associated food fractions.

Their limitations are equally informative. Mouse colitis models induce inflammatory injury under experimental conditions and are not equivalent to the heterogeneous biology of human ulcerative colitis or Crohn's disease. A treatment that affects disease severity in an animal model may act through mechanisms not dominant in human disease. Dosing schedules, administered particle amounts, batch identity, microbiota, intestinal transit and immune responses vary across species. Some studies use deliberately purified or enriched fractions at exposures that cannot readily be achieved by eating a normal portion of the source food. The difference is not one of scientific quality alone; it is a difference of intervention.

The 2026 thermally reassembled ginger-vesicle study extended this research in an especially instructive direction: a food-processing operation was used to modify the platform, and an engineered siRNA cargo was compared in preclinical systems [5]. This is stronger than a simple observational report of particles, because manipulation and comparison can help test a causal mechanism. Yet the experiment also crosses toward pharmaceutical delivery research. Engineered nucleic-acid cargo, therapeutic targets and delivery to inflamed tissue cannot be translated into claims for an ordinary ginger-based drink without a wholly different evidence and regulatory programme.

A balanced research review should distinguish **mechanistic credibility**, **biological reproducibility**, **exposure relevance**, **human efficacy** and **market eligibility**. Evidence can be strong at one level and weak at another. A clear cell uptake mechanism may coexist with unknown gastrointestinal stability. A consistent mouse response may coexist with unknown human safety at repeated doses. An edible source may coexist with an unestablished legal position for an isolated, concentrated and technologically manipulated fraction. These are not contradictions; they represent the different questions being tested.

Evidence level	Typical observation	Legitimate conclusion	Missing step before a stronger claim
Particle characterisation	Reproducible membrane-associated nanostructures	A defined material can be measured	Source of particles, purity and biological identity
Cellular model	Uptake or altered inflammatory marker	Interaction under specified laboratory exposure	Dose realism, free-cargo controls, tissue relevance
Simulated digestion	Retention of specified physical/cargo attributes	Partial stability under model conditions	In-vivo exposure and molecular-form tracking
Animal model	Altered disease-associated endpoint	Preclinical activity in a particular system	Replication, toxicology, human dose and clinical validation
Registered human study	Protocol and planned outcomes exist	A study was initiated or registered	Actual outcome data and critical appraisal
Human controlled trial with results	Prespecified endpoint and effect estimate	Preparation-, population- and outcome-specific inference	Replication, generalisability, lawful claims
EU authorisation and claims review	Exact permitted food status and allowed language	Defined market access and communication	Continued compliance, quality and surveillance

Table 3. *A translational evidence ladder. The table describes types of evidence, not a claim that every stage has been completed for edible plant vesicles.*

9. What the human clinical record supports nowadays

ClinicalTrials.gov contains investigations involving plant-derived exosome or nanoparticle preparations. Their existence is frequently quoted as proof that the platform is “clinically validated”. That inference is incorrect. Registration can establish a protocol, sponsor, planned intervention, population and outcome; completion status does not independently establish a positive result; and a trial without published or posted results cannot be used to assert efficacy.

Among the better-known examples is **NCT01668849**, concerning grape-derived material investigated in a setting of chemoradiotherapy-associated oral mucositis in patients with head and neck cancer. The available register and 2026 reviews describe the study as completed, but do not provide a corresponding publicly posted result set sufficient to establish efficacy [9,10]. A second investigation, **NCT04879810**, concerned ginger-derived preparations and curcumin in inflammatory bowel disease. Available summaries describe recruitment substantially below the original plan and no posted efficacy results [10,11]. Another registered study, **NCT01294072**, was designed to investigate delivery of curcumin to colon tissue using plant-derived vesicular material; its available status information does not justify a claim of proven clinical benefit [10]. Registry details can change and must be consulted directly at the date of any scientific or commercial communication.

The research questions are not identical. A study of mucositis during cancer treatment, an exploratory comparison in inflammatory bowel disease and an investigation of curcumin distribution in colon tissue measure different mechanisms and clinical endpoints. They should not be aggregated rhetorically into an imaginary body of positive human trials for “plant exosomes”. Nor should the presence of registered studies be dismissed. They demonstrate that some research groups have moved beyond animal models toward human feasibility questions. The appropriate next step is transparent outcome reporting, including adverse events, analytical identity of the actual administered preparation and dose verification.

The central conclusion at the November 2026 evidence cut-off is therefore narrow and important: **there is not a sufficiently developed, openly reported body of controlled human evidence to support general therapeutic or specific disease-prevention claims for edible plant-derived EV fractions as a class.** This does not establish that all proposed products lack activity. It means that the claimed activity has to be tested for the exact preparation in an adequately designed study, and that available registrations should not be substituted for published outcomes.

For a future human study, adequate reporting should include botanical and process identity, particle composition, batch-to-batch reproducibility, dose expression, contaminant and microbial testing, the administered matrix, stability and intended biochemical exposure. The placebo should be carefully designed. A “vesicle-free” plant extract may differ in lipids, polyphenols, sugars and sensory properties; any differences can confound the trial. A useful, controlled design could compare the intact fraction, an appropriately matched disrupted fraction and a process-matched food matrix, but feasibility and ethical considerations must guide final design. Investigators should predefine a clinically meaningful endpoint and resist the practice of presenting an exploratory microbial or cytokine signal as proof of the intervention’s stated health objective.

10. Safety is a property of an exposure, not of the plant’s reputation

Food history matters. Ginger and grapes have substantial histories of human consumption in conventional forms. This does not automatically confer a complete safety profile on fractions isolated at much higher particle concentration, altered structurally, loaded with additional cargo or administered daily over prolonged periods. The relevant exposure is to the exact manufactured preparation, not to an idealised botanical name.

Safety evaluation starts with agricultural and manufacturing inputs. Plants can carry pesticide residues, environmental contaminants and microorganisms. Extraction may enrich or deplete these components unpredictably. A vesicle-associated lipid fraction may partition certain hydrophobic substances differently from the starting juice; filtration may remove microorganisms but leave endotoxin-like or other microbial constituents; concentration can amplify minor compounds that were insignificant in the original food. These possibilities are material-specific hypotheses, not allegations that any specific supply contains contaminants. They define what must be measured.

The particle fraction itself raises questions of chronic exposure, gastrointestinal tolerance, local immune responses and interactions with the intestinal barrier. Some plant proteins may present allergen or sensitisation considerations in specific botanical sources. Residual extraction aids, processing membranes or formulation excipients introduce additional exposures. An engineered or loaded vesicle system adds the safety profile of the cargo and of the loading method; free cargo, membrane-bound cargo and intra-vesicular cargo can have different fate and risk profiles. A formulation that protects a molecule from degradation may also alter its distribution in undesirable ways.

The toxicological plan should be proportionate to the claimed use and expected exposure. For a naturally occurring trace fraction within an ordinary conventional food, a novel high-dose toxicology programme may not be justified. For an isolated, concentrated, intentionally reassembled and repeatedly consumed nanofraction, more detailed characterisation and route-specific assessment may be necessary. The legal and scientific classification of the material should determine which guidance applies; the word “nano” is not a complete toxicological conclusion.

In nanospecific assessment, the European Food Safety Authority has published guidance on the risk assessment of nanomaterials in the food and feed chain and technical guidance for identifying small particles within regulated products [12,13]. These documents require attention to physical-chemical characteristics, particle size and number distribution, aggregation, dissolution/degradation, exposure and toxicology under appropriate conditions. They do not declare that all naturally occurring vesicles are harmful; nor do they exempt an intentionally engineered material because its original feedstock was edible. The applicable analysis depends on the product’s real characteristics and the legal route being considered.

A standard genotoxicity or cell-viability assay is not a complete oral safety evaluation. Experimental concentrations must be relevant, assay interference from particles and plant pigments must be controlled, and adverse outcomes should be assessed over time appropriate to the intended use. If particles appear to cross a biological barrier, the evidence needed to understand their distribution and elimination becomes more demanding. Claims that plant nanoparticles are inherently “non-immunogenic” or “non-toxic” should be replaced by measured statements about a specific preparation and test system.

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11. The European legal question has three different starting points

A common misconception in the emerging plant-vesicle sector is that a preparation must be either "a food because it comes from food" or "a medicine because it is nanoscale". European law demands a more precise analysis. The source plant, the fraction that is placed on the market, the manufacturing process, the intended function, the composition, the degree of human consumption before the historical cut-off, and the proposed communication each have legal significance. The evidence needed to classify a conventional juice is not identical to the evidence needed for a concentrated and deliberately engineered nanovesicle platform.

First: conventional food status and the novel-food boundary. Regulation (EU) 2015/2283 governs foods and food ingredients that fall within its definition of novel food. The important historical reference is significant human consumption within the Union before 15 May 1997, together with the relevant categories in Article 3(2)(a) [14]. A traditional history of consuming the whole botanical source can be important but does not automatically prove significant historical consumption of a purified, concentrated, structurally modified or newly manufactured fraction. An operator must establish the identity of the marketed substance and the relevant consumption history. The Commission's procedures include a consultation mechanism, implemented by Regulation (EU) 2018/456, for operators who are uncertain of novel-food status [15].

A central problem is the risk of replacing an ingredient-level history with a source-level history. Consumers may have eaten grapes for centuries. That fact does not prove that they consumed a separate concentrated vesicle fraction at the same dose, in the same chemical state or with the same biodistribution potential. Conversely, evidence that a preparation is enriched in nanoscale structures does not automatically prove novelty. A careful Article 3 assessment requires facts concerning the exact fraction, processing route and relevant consumption practices; the conclusion cannot be inferred from fashion in terminology.

Second: processing and engineering. Article 3(2)(a) of Regulation 2015/2283 includes categories relevant to food obtained from production processes not used before the cut-off where these result in significant changes affecting nutritional value, metabolism or undesirable substances, and to food consisting of engineered nanomaterials [14]. The definition of an engineered nanomaterial, in Article 3(2)(f), is concerned with intentionally produced materials with nanoscale dimensions or functional parts and the relevant characteristics. It is not scientifically or legally sound to classify every naturally occurring membrane particle as an engineered nanomaterial merely because it is 50–200 nm across. Equally, intentionally engineered, reassembled or loaded particles may require more detailed examination than a conventional unmanipulated food fraction.

The food-technology pathway therefore needs an explicit legal decision record. Was the material naturally present as a minor part of a historically consumed food? Was it concentrated by a conventional process? Was it isolated into a composition not previously consumed to a significant degree? Was a new structure deliberately assembled? Were synthetic lipids, foreign nucleic acids or modified surfaces introduced? What chemical and physical features survive in the final product? A meaningful decision cannot be made until those questions are answered.

Third: legal authorisation, labelling and communication. Where a material is a novel food, the Union list established by Commission Implementing Regulation (EU) 2017/2470 and its amendments determines the permitted entries, specifications and conditions of use [16]. A source plant or a different novel ingredient cannot be used as a proxy for authorisation of a newly isolated vesicle fraction. The publicly reviewed Union list does not establish a general, family-wide authorisation for "plant exosomes". This is a deliberately limited statement: it is not a definitive declaration that no possible specific commercial material has a lawful route; each proposed ingredient must be checked against the current consolidated list, status consultations and any applicable national facts immediately before market entry.

Regulation (EU) 1169/2011 governs the information communicated about foods and ingredients. Article 18(3) requires the name of an ingredient present in the form of an engineered nanomaterial to be followed by "nano" in brackets in the ingredient list [17]. Whether that requirement applies to an actual plant-derived preparation depends on its legal classification. It should neither be ignored when clearly relevant nor automatically imposed on every natural nanoscale food constituent. The general prohibition on misleading food information is important even before a product makes an explicit health claim. Words such as "cell-programming", "DNA-repairing", "colon-

targeted therapy" or "clinically proven exosomes" can create scientifically ungrounded impressions if they are derived only from preclinical work.

Regulation (EC) No 1924/2006 establishes the framework for nutrition and health claims on foods [18]. A favourable experiment in a cell line or mouse is not a substitute for a relevant authorised consumer health claim. Even a later positive human intervention does not automatically create a new permitted statement; the claims system has its own scientific and legal requirements. Statements that a product treats colitis, heals mucositis, prevents cancer or modifies a disease may additionally raise medicinal-product classification concerns under Directive 2001/83/EC, depending on product presentation and intended use [19]. It would be a serious category error to present engineered siRNA delivery research as a food-health benefit.

Finally, general food law and hygiene remain applicable. Regulation (EC) No 178/2002 prohibits placing unsafe food on the market and provides the basic framework for responsibility and traceability [20]. Regulation (EC) No 852/2004 requires hygienic production and procedures based on HACCP principles where appropriate [21]. An absence of a specific, category-wide numerical limit for plant vesicles is not an exemption from assessing hazards associated with the source, process and product.

12. EFSA nanospecific guidance should be integrated without legal shortcuts

The EFSA Scientific Committee's 2021 guidance for nanomaterials in the food and feed chain and its companion technical guidance for establishing the presence of small particles are particularly relevant when the proposed material contains a nanoscale fraction [12,13]. Their scientific value lies in separating ordinary compositional assessment from questions that arise because the material is particulate: dimensions, size distribution, number concentration, surface area when appropriate, aggregation, agglomeration, solubility, dissolution, degradation, in-vitro and in-vivo behaviour, exposure and potentially nanospecific toxicity.

These guidances do not decide the legal definition of an engineered nanomaterial for every applicant. The classification question and the scientific question interact but should not be conflated. A material may contain a small-particle fraction requiring further nanospecific characterisation even if the operator disputes or does not meet the legal engineered-nanomaterial definition. Conversely, a particle formed during ordinary food processing cannot be presumed biologically hazardous merely because a measurement detects nanoscale dimensions. The correct approach is a decision tree grounded in the complete physical-chemical evidence.

A useful pre-submission exercise would compare the measured particle population with the anticipated exposure after realistic storage and digestion. If a fraction is destroyed or dissolved before systemic exposure, the assessment may focus on its transformation products and any local gastrointestinal effects. If intact particles persist, an investigator may need more detailed information on uptake and tissue distribution. Where information is insufficient, the answer is not to assume an adverse effect but to define the missing measurement needed to make a defensible risk assessment.

The European framework also requires careful attention to use as an additive or carrier. A material deliberately included to perform a technological function in the finished food may engage the food-additive framework under Regulation (EC) No 1333/2008 [22], depending on its precise identity and circumstances. Describing a nanovesicle fraction as a "natural delivery system" is not a legal category that overrides additive rules. Similarly, if a formulation is designed to deliver a pharmacologically active molecule for treatment or prevention of disease, a food route cannot be secured by choosing an edible botanical carrier. The border between foods, supplements, medical foods and medicinal products depends on composition, intended use, claims and applicable legislation; it must be resolved before commercial investment.

Commercial proposition	Central legal question	Necessary evidence	Unsafe shortcut to avoid
Conventional ginger or grape food	Is the product a conventional food made by established methods?	Exact composition, manufacturing history, general food compliance	"It contains nano-sized particles, therefore it is an approved nanomedicine"
Concentrated plant	Does the exact fraction require	Source and process, pre-1997 history,	"The botanical source is

Commercial proposition	Central legal question	Necessary evidence	Unsafe shortcut to avoid
vesicle fraction	novel-food authorisation?	composition, intended exposure, Article 4 consultation where needed	traditional, therefore the isolate is traditional"
Intentionally reassembled or loaded particles	Is a novel food, engineered nanomaterial or other category implicated?	Process engineering, particle identity, carrier and cargo, safety and exposure	"Its ingredients are natural, therefore engineering is irrelevant"
Nanomaterial-containing ingredient label	Does Article 18(3) of 1169/2011 apply?	Legal nanomaterial classification and exact ingredient form	"Everything under 100 nm must always have the same label"
Food with proposed anti-inflammatory claim	Is the claim authorised and is medicinal classification implicated?	Actual wording, proposed function, authorised claims and trial evidence	"Mouse colitis improvement permits an IBD claim"
Engineered cargo delivery for treatment	Does the intended use place the product outside ordinary food law?	Mode of action, target disease, dose, formulation, regulatory classification	"An edible plant carrier makes a drug-delivery claim a food claim"
Ongoing manufacture and market supply	Are food safety, traceability and controls demonstrated?	HACCP, supplier controls, validated assays, release and surveillance	"Novel-food clearance removes ordinary safety duties"

Table 4. *European regulatory decision points. This is a reasoning framework rather than an authorisation opinion for any specific material.*

13. A possible industrial platform for INTABIOTECH: begin with a constrained question

A research programme should not begin with "make the world's most bioactive plant exosomes". Such language fails to identify a consumer, food category, measurable function, manufacturing target or lawful route. A more useful initial brief might be: *Determine whether a food-grade ginger-derived membrane-rich fraction can be produced reproducibly and remain chemically and colloidally stable in a defined acidic beverage and establish whether its legal status permits the intended use*. This is a legitimate first-stage industrial research question even before any claim of human health benefit is contemplated.

The proposed platform would have four interdependent workstreams: botanical source qualification; chemical and particulate characterisation; finished-matrix processing and stability; and European legal feasibility. An optional fifth workstream, biological mechanism research, should commence only after there is sufficient confidence that the tested particle population can be produced reproducibly and is not dominated by uncharacterised co-isolates. Human clinical development would be a later, separately budgeted and ethically reviewed endeavour if the intended commercial proposition genuinely requires it.

An initial source screen could compare authenticated ginger rhizomes from two or three documented suppliers and at least two independent harvest or production lots per source. The panel would quantify fresh and dry mass, moisture, soluble solids, characteristic botanical metabolites, baseline microbiology and risk-based contaminants. Each lot would enter a standardised small-scale extraction with recorded mechanical input, temperature, pH, holding times and clarification conditions. Replication should involve independent plant lots and extraction batches, not multiple instrument injections from a single pooled sample.

The analytical workstream would use a tiered characterisation strategy. A screening tier could include NTA with method qualification, light scattering for aggregation trends, basic lipid/protein measurements, microscopy on representative batches and HPLC or LC-MS methods for characteristic free plant constituents. A reference tier could add cryo-electron microscopy, detailed lipidomics, targeted proteomics, orthogonal fractionation and, only where relevant to a defined hypothesis, small-RNA characterisation. Routine release methods should be selected from measurements that can be validated against the deeper reference profile. A high-cost assay that cannot be deployed at industrial batch scale may be useful for development but unsuitable as the sole release criterion.

A critical step is to define *negative controls before collecting favourable results*. The project should retain the liquid supernatant after particle enrichment and analyse it for free gingerols and other metabolites. It should compare a

process-matched fraction depleted in the target particle population. It should introduce a membrane disruption control with documentation of collateral chemical changes. If the goal is technological stability, biological claims need not appear in the first phase. If the goal is an epithelial response, the experiment must ensure that the response is not attributable to unrelated free constituents, acidity, osmotic pressure or assay interference.

Manufacturing scale-up should proceed through a mass-balance model: kilograms of rhizome received, kilograms dry matter, volume of pressed or extracted material, membrane area and throughput, product losses, quality failures, cleaning demand, energy, water and packaging. A promising laboratory particle count per millilitre can be rendered economically irrelevant by low mass recovery, sterile filtration bottlenecks or instability. The mass balance should reflect the grade that can be released under an agreed specification, not a theoretical product measured before processing losses.

14. A worked formulation case: a plant beverage with a characterised nanofraction

Consider a hypothetical company developing a 250 mL chilled, mildly acidic botanical beverage. A supplier proposes adding a membrane-rich ginger fraction and argues that it can improve intestinal function. The correct industrial response is to separate three possible projects. The first is an ordinary beverage containing ginger juice or extract for flavour. The second is a beverage with a standardised purified particle-rich fraction, subject to exact food-status review. The third is an intervention designed to deliver an engineered bioactive or therapeutic cargo. The scientific and legal needs of these projects differ substantially.

For the second case, the development team would begin with a written legal and compositional screening of the proposed fraction. If its lawful status for the intended category and use level cannot be established, a commercial launch cannot be assumed. Research may continue under appropriate non-market experimental conditions, but marketing, packaging commitments and broad health claims should be deferred. This gate is essential to protect clients against investing in process development that cannot legally reach the intended market.

Assuming that a lawful research or development path is identified, a fixed beverage matrix should be established for pH, titratable acidity, soluble solids, carbohydrate profile, colour, ginger flavour, microbial preservation and packaging. The vesicular-fraction candidate should be added at several deliberately selected concentrations justified by measurable physicochemical objectives rather than unsupported human dose assumptions. At this stage, the primary endpoints should be recoverable particle-associated markers, fraction stability, sensory acceptance and microbiological safety. Any exploratory epithelial assay would be secondary.

A factorial pilot could compare selected conditions: before versus after a defined heat treatment; two pH levels within the intended product range; and two packaging oxygen-barrier properties. Factorial designs become unwieldy quickly, so the team should use preliminary screening to identify the dominant sources of variation and reserve confirmatory experiments for the most informative combinations. All comparisons should involve independent manufactured lots, with process records sufficient to reproduce them. If a heat treatment produces a new particle population, that population should receive a separate material identity rather than being described as the unchanged original fraction.

The storage protocol should include immediate post-processing measurement, intermediate time points, declared end of shelf life and reasonably foreseeable temperature excursions. Analytical release should include food microbiology, fraction identity, particle aggregation, relevant plant metabolites, pH, colour, sedimentation and sensory changes. Digestive simulation, if undertaken, should be performed on the beverage at the end of realistic storage as well as at manufacture; otherwise, a successful digestion result for a freshly prepared laboratory fraction may not represent the product consumed by a customer.

A go/no-go decision should be based on the combined technical and legal evidence. A formulation that delivers a stable particle-associated marker but cannot establish legal food status is not ready for sale. A legally permissible beverage with poor sensory acceptability is commercially weak. A pleasant beverage with a strong in-vitro anti-inflammatory signature but no human evidence cannot be marketed as a treatment. And a fraction requiring an expensive refrigerated supply chain may be inferior to an ordinary ginger extract for the same consumer proposition. These are rational findings, not failures of scientific ambition.

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Illustrative pilot endpoints, to be defined experimentally rather than treated as existing INTABIOTECH results: (i) lot-to-lot reproducibility of specified fraction attributes; (ii) acceptable microbiological quality and stability; (iii) recovery of the defined particle-associated marker after processing; (iv) validated analytical method performance; (v) sensory and packaging acceptability; (vi) complete source and process traceability; (vii) demonstrated legal feasibility for the exact ingredient and category. Quantitative acceptance ranges should be established from pilot variability, safety assessment and customer requirements, not invented in advance as generic industry standards.

15. Seven evidence gates for the development programme

An advanced development project can be organised as seven decision gates, each of which asks a different question and has a defensible stopping rule.

Gate I - Biological identity and source provenance. Establish the species, plant part, supply chain, harvest and storage conditions. Verify that the source is suitable for the intended category and that the project does not depend on an unsubstantiated claim of traditional consumption of the isolated fraction. Reject sources for which critical traceability or agricultural safety evidence is unavailable.

Gate II - Fraction identity and orthogonal measurement. Demonstrate a reproducible particle-associated population using more than one independent measurement. Define non-vesicular co-isolates, cargo relevant to the hypothesis, reference standards and instrument qualifications. Reject preparations whose apparent potency depends on an unexplained batch-specific analytical artefact.

Gate III - Process causality and mass balance. Show how each extraction and formulation operation affects yield, composition, structure and losses. Distinguish preservation from reassembly and track any new structures created by the process. Reject manufacturing routes that cannot reproduce the critical attributes at the intended scale or that introduce unacceptable process residues.

Gate IV - Finished-product compatibility. Measure the exact formulation after the relevant heating, homogenisation, filling and storage. Determine whether the desired fraction survives in a useful form and whether sensory and microbiological performance remain acceptable. Reject projects that extrapolate stability from purified laboratory buffer to an unrelated food matrix.

Gate V - Exposure and safety. Estimate likely consumption, foreseeable cumulative exposure, particle state after digestion and the significance of contaminants and process impurities. Where nanospecific safety questions arise, define the studies using relevant EFSA guidance. Reject unsupported assumptions that edible source or cell viability guarantees repeated-dose safety.

Gate VI - Biological and clinical substantiation. State a precise functional hypothesis and select a validated endpoint. Use particle-depleted and free-cargo controls, independent replication and exposure-relevant concentrations. If a consumer-health benefit is proposed, use an ethically approved and appropriately powered human intervention with prespecified outcomes. Reject any decision to replace a failed primary endpoint with favourable exploratory biomarkers in the final scientific interpretation.

Gate VII - Legal and commercial release. Verify the exact ingredient status, any applicable novel-food authorisation, nanomaterial determination, food category, labelling, claims and supply documentation. Incorporate costs per compliant serving, real-time stability, change control and post-market surveillance. Reject marketing language that exceeds the scientific outcome or the legal permission.

Gate	Required documentation	Acceptance question	Example of a reason to stop
I. Source	Botanical authentication, lot and origin, harvest and pre-processing chain	Is the feedstock defined and traceable?	Critical source variation cannot be controlled
II. Identity	Orthogonal particle, lipid, protein and contamination profile	Is the actual fraction reproducible?	Counts measure mixed debris with no defined target
III. Process	Mass balance, unit operations, losses, process change control	Can the fraction be produced at scale?	High laboratory count but negligible compliant yield

Gate	Required documentation	Acceptance question	Example of a reason to stop
IV. Matrix	Product-specific processing, storage, safety and sensory studies	Does the finished food preserve the specified attributes?	Rapid degradation, off-flavours or microbial instability
V. Safety	Exposure scenarios, contaminants, digestion and nanospecific assessment as needed	Is intended exposure responsibly assessed?	Material lacks critical toxicological or purity data
VI. Efficacy	Appropriate controls, defined primary endpoint, independent trials	What function or benefit is genuinely demonstrated?	Only high-dose cell assay or unreported clinical registration
VII. Market	Exact status and conditions, claims, labels, cost and traceability	Can this product lawfully and profitably launch?	Novel-food status unresolved or proposed claims impermissible

Table 5. *Proposed evidence-gated development framework. The table specifies research decisions and does not imply any of these tests has been conducted by INTABIOTECH.*

16. Computational discovery: why a nanovesicle model is only as valid as its batches

An industrial platform will generate multivariate data: botanical source, tissue maturity, dry matter, process pH, shear, filtration pressure, particle distribution, lipid composition, protein fingerprints, free metabolites, storage and assay responses. Statistical design of experiments can identify combinations that influence recovery and stability. Machine learning may help identify high-risk input lots or predict the most efficient purification strategy. Such approaches can be useful, but a central methodological risk is data leakage.

If particle spectra, NTA replicates and chromatography measurements from one production lot are divided randomly between training and test sets, the model may recognise the identity of that lot rather than learn a transferable relationship between process variables and product attributes. The correct validation split may need to be based on independent harvests, different suppliers, manufacturing batches and, ultimately, facilities. Repeated microscopy fields from one specimen or ten injections of one fraction are not ten independent biological replicates.

For structure–function models, the target variable must also be defensible. Training on a broad “anti-inflammatory potency score” constructed from different assays and different dose scales can create attractive predictions that have no reproducible biological meaning. Better endpoints include physically defined recovery, validated chemical retention, prespecified marker concentration, stability under realistic conditions and clearly documented cellular assays at exposure-relevant doses. If the proposed application concerns consumer health, predictive models should inform experiment selection rather than replace controlled human evidence.

Artificial intelligence may assist in interpreting complex lipidomic and proteomic profiles, but unsupervised grouping of samples does not prove the existence of a discrete biological vesicle subtype. Apparent clusters may reflect extraction batches, analytical instrument settings or the abundance of co-isolated plant compounds. Model interpretability, uncertainty, independent reference measurements and traceable raw data should be incorporated into the technical dossier. The most valuable digital asset will be a system that reduces failed experiments and manufacturing variability, not one that generates increasingly precise-looking graphs from poorly identified material.

17. Sustainability and economic realism: the nanoparticle is not the unit of value

The appealing narrative that plant-derived vesicles are sustainable because plants are renewable is incomplete. A concentrated fraction may require many kilograms of biomass, chilled transport, extensive washing, energy-intensive centrifugation, membrane consumption, buffer exchange, lyophilisation and refrigerated storage. An environmental comparison with a conventional ingredient must use a consistent functional unit and include the upstream and downstream processes. Counting particles does not solve the economic problem if the biologically meaningful fraction is uncertain or the finished product cannot lawfully enter the intended market.

A proper cost model would identify the price and variability of incoming plant material; dry-mass yield; water use; extraction energy; membrane lifespan and fouling; labour and sanitation; analytical testing; fraction losses; reject

rates; packaging; warehousing and stability. The economically relevant denominator is the number of compliant, specification-controlled servings that can be supplied, not the nominal particle count obtained in a laboratory tube. A process that delivers a spectacular enrichment factor, but poor overall recovery may be less competitive than a simpler process giving reproducible, moderate enrichment.

Co-product recovery is a legitimate option. After particle-rich fractionation, the remaining botanical streams may contain soluble carbohydrates, dietary fibres, flavours or other compounds. Their valorisation must be justified by composition, lawful food or non-food uses, contamination control and actual demand. A biorefinery does not become circular merely because a diagram assigns a hypothetical use to every residual stream. The life-cycle assessment should account for the avoided burden only where there is a credible displacement and an evidence-based methodology.

The strategic value for an ingredient company may initially lie in *analytical and regulatory competence* rather than exclusive production. Source qualification, independent fraction characterisation, matrix validation and credible EU status assessment can already help clients avoid expensive development errors. Partnerships with specialised plant cell-biology groups, particle-characterisation laboratories, food technologists and clinical investigators may be more efficient than immediately investing in industrial vesicle manufacturing. In a technically immature sector, disciplined negative findings can be valuable assets: knowing that a given process generates only cell debris, that an unstable fraction fails a beverage specification, or that a proposed use lacks a clear legal route prevents sunk investment.

18. The research agenda: five questions that would genuinely change the field

The first question is biological origin. Can plant-derived EV populations be isolated from food-relevant material using methods that minimise mechanical generation of vesicle-like debris, and can they be distinguished from other membrane fragments using reproducible markers? More accurate origin attribution would improve the comparability of studies and reduce the risk that experimental findings concern different materials sharing one name. Plant-specific guidance being developed through ISEV's specialist activities may assist the field, but the value of consensus depends on experimental validation [23].

The second question is dose. What fraction of the orally consumed candidate survives food processing and digestion in a form capable of producing the proposed local or systemic effect? This question requires realistic, finished foods, independent batches, quantitative recovery and careful tracking of chemical form. It should be answered before selecting a human trial dose from a particle count in a concentrated laboratory preparation.

The third question is causality. When a fraction changes a cell or animal endpoint, which component is responsible? Is membrane structure necessary, or do co-extracted gingerols, polyphenols or other compounds explain the result? Appropriate fraction-depleted controls and intervention studies are needed. It is not necessary to identify every molecule before all research can proceed, but the causal hypothesis should become progressively more precise rather than remain a generic claim that "the exosome mixture is bioactive".

The fourth question is human relevance. Can a characterised, quality-controlled preparation produce a meaningful benefit in a prespecified human endpoint at an exposure compatible with the intended food category? Published results should include negative trials, adverse events, protocol changes and statistical uncertainty. The field would benefit more from a few transparent, adequately designed studies than from a long list of enthusiastic secondary interpretations of unpublished registrations.

The fifth question is market identity. Can an operator obtain a clear determination of the exact preparation's status under EU law and, where relevant, assemble the technical and safety data required for authorisation? The answer is linked to the first four questions. A commercial proposal cannot remain legally generic while its scientific argument is highly dependent on nanoparticle structure, source and processing. The requirements for a conventional botanical drink, concentrated nanofraction and engineered drug carrier must remain separate.

These questions create a demanding but productive interdisciplinary agenda. Cell biologists can improve particle provenance; analytical chemists can establish compositional fingerprints; engineers can design reproducible separation; toxicologists can assess exposure; clinicians can select meaningful outcomes; and regulatory

specialists can determine the lawful route. The field needs less enthusiasm about the mere presence of nanoscale structures and more evidence that remains intelligible when transferred between these disciplines.

19. Discussion: a useful technology at an early evidentiary boundary

The intellectual appeal of edible plant vesicles is genuine. Living cells package material into membrane-bounded structures, and food processing can recover membrane-rich fractions with distinct physical properties. Nanoscale assemblies offer plausible ways to modify solubility, protect labile constituents and change interactions with biological interfaces. The 2014 and 2016 investigations opened a productive experimental direction; studies published during 2026 have improved attention to processing, characterisation and intentional reassembly [3–8]. These advances should not be dismissed simply because they have not yet produced a mature consumer-food market.

The opposite error is to treat the emerging field as though it has already established a new therapeutic class of everyday foods. Names borrowed from mammalian extracellular vesicle biology can obscure uncertain plant biogenesis. Particle number can substitute rhetorically for purity. Photographs of cellular uptake can substitute rhetorically for bioavailability. A mouse model can substitute rhetorically for human benefit. A registered clinical study can substitute rhetorically for positive results. A history of consuming a whole plant can substitute rhetorically for authorisation of a concentrated manufactured fraction. Each substitution makes the story simpler, but less accurate.

The 2026 thermally reassembled ginger-vesicle work supplies a constructive way out of this dilemma. It suggests that a process variable can be used to investigate the properties of a defined nanoscale fraction, rather than treating botanical origin as a sufficient explanation of function. It also makes the regulatory question harder. If heating generates a substantially different material, the processed fraction must be characterised as such. A scientist should welcome that distinction because it permits better causal experiments. A food manufacturer should welcome it because it improves process control. A regulatory assessor must insist on it because a different material can present different exposures.

There is no necessary conflict between innovation and caution. A well-characterised vesicular fraction may eventually become a valuable ingredient or delivery platform. At present, the most responsible applications may be research tools, ingredient-development programmes and defined industrial collaborations, with commercial food use considered only after exact legal status is established. Some therapeutic proposals may belong under medicinal-product regulation rather than food law. A technology does not lose scientific value when the appropriate regulatory pathway is different from the one initially hoped for.

A further implication concerns ownership of evidence. Publications from research groups can inform candidate selection, but they cannot replace batch qualification for material purchased from an unrelated supplier. Two preparations described as “ginger-derived exosomes” may differ in lipid composition, soluble co-extractives, purity and processing-induced structure. A company wishing to rely on an external study should perform an explicit evidence-bridging assessment: identity of source, extraction, analytical methods, biological dose and finished-food form. Where correspondence cannot be shown, the proper description is that the proposed commercial material belongs to a related research category, not that its clinical effectiveness has been demonstrated.

20. Conclusions

Edible plant-derived extracellular vesicles and vesicle-like nanoparticles represent a credible research frontier, but their scientific status is more heterogeneous than their commercial names suggest. The material placed into a food may be a naturally released extracellular vesicle; a particle recovered from disrupted plant tissue, a process-generated lipid assembly or an intentionally engineered carrier. Those possibilities differ in provenance, composition, expected gastrointestinal fate, safety evidence and European regulatory analysis. Size alone cannot resolve them.

The existing literature demonstrates important preclinical biological interactions and provides examples of process-induced changes to particle architecture. The 2026 ginger reassembly study is a significant experimental contribution, not a completed human efficacy programme. Publicly discussed human registrations have not, at the

evidence cut-off used here, supplied a sufficiently robust and openly reported evidence base for general therapeutic claims. Food developers should therefore distinguish analytical detection, mechanistic activity, oral exposure, human benefit and lawful commercial communication throughout their programmes.

European food law offers neither a universal prohibition on nanoscale plant constituents nor a general authorisation for concentrated “plant exosomes”. Novel-food status depends on exact material identity, processing and relevant historical use. Engineered nanomaterial questions require their own legal and scientific assessment. Labelling, claims, food safety and, where applicable, medicinal classification create separate obligations. A favourable laboratory experiment does not waive any of them.

For INTABIOTECH and its research partners, the most defensible strategy is a staged ingredient-development platform with authenticated botanical sources, orthogonal characterisation, process mass balance, realistic finished-food stability, exposure-appropriate biological models, independent clinical evidence where justified and documented EU regulatory screening. The achievement worth pursuing is not the largest quoted number of particles. It is a reproducible, safe and legally admissible ingredient whose identity, function and commercial value can survive independent scrutiny.

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