Review
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Citrus IntegroPectin: A Family of Bioconjugates With Large Therapeutic Potential

Rosaria Ciriminna,*[a] Valentina Di Liberto,[b] Lorenzo Albanese,[c] Giovanna Li Petri,[a] Chiara Valenza,[b] Giuseppe Angellotti,[a] Francesco Meneguzzo,*[c] and Mario Pagliaro*[a]

Citrus IntegroPectin is a family of bioconjugates sourced from different citrus fruits first isolated in 2019 via hydrodynamic cavitation of industrial citrus processing biowaste conducted in water only. Having a low methoxyl homogalacturonan backbone, these new pectins are enriched in citrus flavonoids, terpenes, and RG-I rhamnogalacturonan regions. Investigating lemon, grapefruit, orange, red orange, and mandarin IntegroPectin in numerous in vitro and in vivo studies unveiled their multitarget biological activity, including antioxidant, anti-inflammatory, cardioprotective, anti-apoptotic, immunomodulatory, neuroprotective, mitoprotective, antimicrobial, and anticancer properties. This review summarizes research achievements in the first five years following the discovery of IntegroPectin. A critical perspective toward production and practical uptake of this new family of bioconjugates for the treatment and prevention of numerous ailments concludes this account.

1. Introduction

Pectin is a bioactive polysaccharide widely investigated for its therapeutic properties as well as in tissue engineering and regenerative medicine for its gelling behavior, wound healing ability, and high biocompatibility.[1] Besides apple pomace, lemon or orange citrus processing waste (CPW), dried, or fresh, if the pectin plant is close to the citrus juice production plant) is the main raw material for commercial pectin production based on prolonged acid hydrolysis using mineral acid in hot water followed by vacuum evaporation and alcohol precipitation.[2] Though vastly degraded during the hydrolytic extraction with mineral acid,[3] pectin has metal chelating properties and multitarget bioactivity, including immunomodulating, anticarcinogenic, antimetastatic, anti-inflammatory, and gastroprotective properties.[1,4] Furthermore, having wound-healing properties and readily forming gels in water, highly biocompatible pectin is extensively researched for regenerative medicine and biomedical engineering applications.[5]

Widely employed in numerous food and nutraceutical products, citrus flavonoids[6] and citrus essential oils[7] in their turn, have significant bioactivity and therapeutic properties.

IntegroPectin is the name given in 2020 to a new pectin-based phytocomplex obtained in Italy via hydrodynamic cavitation (HC) in water of the fresh residue of industrial manufacturing of lemon juice sourced from organically grown lemon fruits.[8] Generally extracted from dried lemon and orange peel or dried apple pomace using mineral acid in hot water followed by precipitation with isopropyl alcohol, pectin is the most valued food hydrocolloid,[9] and nature's structurally most complex polysaccharide.[10]

In brief, pectin is a heteropolysaccharide consisting of a linear homogalacturonan (HG) polymer of α-1,4-D-galacturonic acid (GalA) monomers, many of which are methyl-esterified at *O*-6 position (some also acetyl-esterified at *O*-2 or *O*-3). The HG linear polymer is interrupted by branched regions composed of (1→2)-α-l-rhamnose units (rhamnogalacturonan-I, RG-I, regions) further binding neutral sugars including galactose, arabinose, xylose, and fructose, as well as by rhamnogalacturonan-II (RG-II) regions consisting of highly branched HG, with side chains at C-2 and C-3 including arabinose, apiose, fucose, galactose, rhamnose, aceric acid, glucuronic acid, galacturonic acid, xylose, and fucose.

Between 2013 and 2018, close to 500 new food and beverage products employed pectin as stabilizer, whereas the related number for the second most used hydrocolloid (carboxymethylcellulose) slightly exceeded 100.[9] Commercial pectin, however, is very different from the native heteropolysaccharide abundant in the pericarp of citrus fruits. Prolonged hydrolysis with hot dilute mineral acid at pH∼2 in the industrial process, indeed, results in loss of most RG-I and RG-II "hairy" chains.[3]

A new HC-based extraction process from CPW carried out in water only, followed by freeze-drying of the aqueous extract to isolate citrus IntegroPectin, was first reported in 2019.[11] The structural analysis of the newly obtained orange pectin

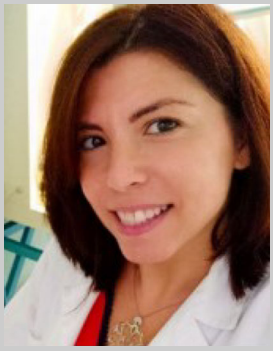
[a] *Dr. R. Ciriminna, Dr. G. Li Petri, Dr. G. Angellotti, Dr. M. Pagliaro*
Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, Palermo 90146, Italy
E-mail: rosaria.ciriminna@cnr.it
mario.pagliaro@cnr.it

[b] *Prof. V. Di Liberto, Dr. C. Valenza*
Dipartimento di Biomedicina, Neuroscienze e Diagnostica Avanzata, Università di Palermo, Corso Tukory 129, Palermo 90134, Italy

[c] *Dr. L. Albanese, Dr. F. Meneguzzo*
Istituto per la Bioeconomia, CNR, via Madonna del Piano 10, Sesto Fiorentino FI 50019, Italy
E-mail: francesco.meneguzzo@cnr.it

unveiled a low (17.05%) degree of methylation (DM). Furthermore, we ascribed the high stability of this new pectin during prolonged storage at room temperature in direct contact with air to "powerful antioxidant orange biophenols."[11] The subsequent year, high antioxidant activity and lack of cytotoxic activity of lemon IntegroPectin were reported.[8] The study opened the route to a vast number of in vitro and in vivo studies that in the following five years unveiled the multitarget bioactivity and large therapeutic potential of this new family of bioconjugates derived from industrial biowaste of organically grown citrus fruits. Significant antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antimicrobial, and anticancer proper-

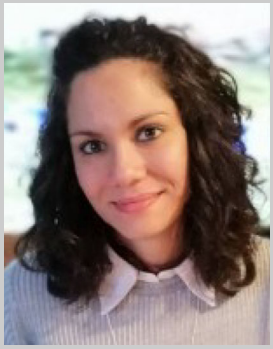
Rosaria Ciriminna. Research Director at Italy's Research Council Institute for the Study of Nanostructured Materials based in Palermo. Developed in collaboration with researchers from over 20 countries, her research focuses on the development of advanced materials for energy and environmental applications, green chemistry and the bioeconomy. Co-author of over 300 frequently cited scientific publications, Rosaria is renowned also for excellence in student mentoring. She coordinates work of numerous young researchers within national and international research projects.



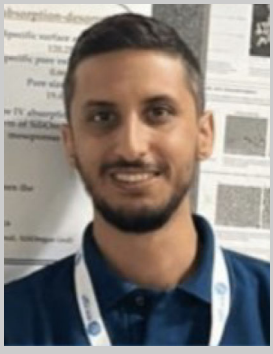
Valentina Di Liberto. Associate Professor of Physiology at the University of Palermo, Valentina Di Liberto graduated in Medical Biotechnology and Molecular Medicine in 2006. After completing her PhD, she worked abroad first in the laboratory of Prof. Cavalli at Washington University in St. Louis School of Medicine, and subsequently at the University of Barcelona in the neuropharmacology laboratories of Prof. Ciruela, and then at Ludwig Maximilian University of Munich. In 2019 she joined the University of Palermo as research fellow where she has also taught Molecular Neurobiology. Her research is chiefly focused on the study of new therapeutic strategies to combat Alzheimer's and Parkinson's diseases.

Lorenzo Albanese. Senior researcher at the Institute of Bioeconomy of Italy's Research Council (CNR) in Sesto Fiorentino, Dr Albanese started his career at the Institute of Biometeorology of the CNR in 2008. He has jointly developed and advanced numerous technologies based on controlled hydrodynamic cavitation phenomena, from new beermaking to extraction of natural products. Research interests expanded to include the analysis and valorization of forest ecosystem services, focusing on human health benefits from forest exposure. Actively involved in regional and national projects with various scientific organizations, he has co-authored over 50 research articles and book chapters.

Giovanna Li Petri is researcher at the Institute for the Study of Nanostructured Materials (ISMN) of Italy's Research Council in Palermo. Her research focuses on green chemistry applied to chemical synthesis, natural product extraction and the development of application of novel bionanomaterials. She holds a doctorate in Molecular and Biomolecular Sciences jointly awarded by the University of Palermo and Vrije Universiteit Amsterdam. After working first at the University of Sunderland, and for nearly three years at the Ri.MED Foundation, in 2024 she joined the ISMN Labs.



Chiara Valenza. Chiara recently (June 2025) got a PhD in Biomedicine, neuroscience and advanced diagnostics from the University of Palermo after defending a Thesis on the biological properties of citrus IntegroPectin, including its antiproliferative and antioxidant action. In 2023, she spent 9 months at the Department of Biochemistry and Developmental Biology of the University of Helsinki. She holds a Master's degree in Medical Biotechnologies and Molecular Medicine.



Giuseppe Angellotti is a researcher based at Palermo's Institute for the Study of Nanostructured Materials (ISMN) of Italy's Research Council. His research focuses on green and sustainable chemistry applied to a variety of chemical processes and novel functional materials including solid biopesticides. After being granted a PhD defending a Thesis on the development of multifunctional biobased sponges for the promotion of healing processes for use in oral surgery, in 2023 he joined the ISMN Labs.

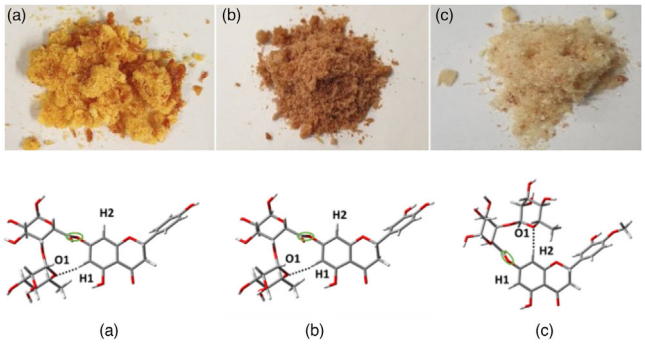


Figure 1. Top: Sweet orange a), red orange b), and lemon c) IntegroPectin obtained by acoustic cavitation. Bottom: Optimized structures of IntegroPectin conjugates with naringenin a), eriocitrin b), and hesperidin c) [Top Image reused from Ref. [50], created by Authors, available at https://doi.org/10.1101/2025.01.15.633201, under the CC-BY-NC 4.0 license; bottom image reused from Ref. 16, created by Authors, available at http://doi.org/10.1007/s44345-025-00013-z, under the CC-BY-NC-ND 4.0 license].

ties of different IntegroPectin bioconjugates employing lemon, orange, red orange, grapefruit, and mandarin IntegroPectin were demonstrated in a series of in vitro and in vivo studies.

In this review we thus summarize results obtained studying citrus IntegroPectin bioconjugates sourced from different citrus fruits. A critical perspective toward production of this new family of phytocomplexes using the "CytroCav" circular economy process, subsequently demonstrated also using acoustic cavitation,[12] for the treatment and prevention of numerous ailments concludes the study.

2. Structure and Bioactivity of IntegroPectin

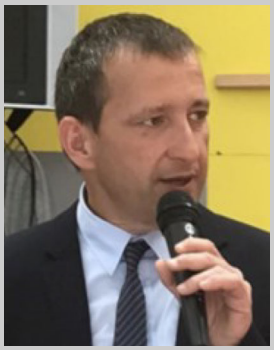
Comprised of citrus pectin rich in RG-I regions, flavonoids[13,14] and terpenes[15] citrus IntegroPectin obtained either via HC or AC of industrial CPW from organically grown citrus fruits has vastly enhanced bioactivity when compared to commercial citrus pectin. Imparting to IntegroPectin its distinctive vivid colors (Figure 1, top), flavonoids in these flavonoid-pectin conjugates are molecularly bound (Figure 1, bottom).[16]

A list of the first studies identifying the multitarget biological activity of lemon, grapefruit, orange, red orange, and mandarin IntegroPectin bioconjugates is shown in Table 1.

Showing evidence of decrystallization of the HG regions, cavitation of CPW destroys the "fringed-micellar" structure of the crystalline regions in semicrystalline pectin.[17] The XRD spectra of the citrus IntegroPectin bioconjugates shown in Figure 1 with a broad peak centered around 18.5° show evidence that all citrus IntegroPectin phytocomplexes obtained by cavitation (AC, in this case) of CPW consist of amorphous pectin. For comparison, the XRD spectrum of commercial citrus pectin sourced via acid hydrolysis of citrus peel shows many diffraction peaks between 12.4° and 40.2° due to partly crystalline arrangement of the HG chains.[18]

All citrus IntegroPectin bioconjugates are low methoxyl (LM) pectin, namely pectin with DM $<$ 50%, with large and negative zeta-potential values that are reflected also in the high (and quick) solubility of citrus IntegroPectin bioconjugates in water at room temperature.

LM pectins are more bioactive than HM pectins. The unique physiological properties of LM pectin, for example, include its



Francesco Meneguzzo. Senior research physicist at the Institute for Bioeconomy, National Research Council, Italy. His career started in 1992 as a researcher and meteorologist, first as an Officer in the Italian Air Force, next at Tuscany's Laboratory for Meteorology and Environmental Modelling (LaMMA) regional weather service he helped to create along with the late Giampiero Maracchi. He has developed and advanced numerous technologies based on controlled hydrodynamic and acoustic cavitation, including new beermaking replacing conventional processes from dry milling to wort boiling, to the green extraction of natural products. His research interests today include the analysis and valorization of forest ecosystem services, focusing on human health benefits from forest exposure. Since 2000, he jointly worked at the organization of numerous international conferences and symposia. He has co-authored over 100 highly cited research papers and book chapters.

Mario Pagliaro. Research Director at Italy's Research Council based in Palermo, Italy, where he leads a research Group focusing on nanochemistry, solar energy, green chemistry and the bioeconomy. In 2021 he was elected ordinary member of the Academia Europaea. Developed in co-operation with leading researchers based in more than 20 countries, his Group's research is reported in nearly 400 research papers. IntegroPectin, CuproGraf, NiGraf, CytroCell, GrafeoPlad, CytroCav, AquaSun, SiliOrange, AnchoisOil, LimoFish, AnchoisFert, SiliaSun, and HyTan are some of the names created by Dr Pagliaro to identify new functional materials and new enabling technologies jointly developed by his Lab. Some of the 22 books he co-authored have become important references in their field.

Table 1. List of studies dedicated to the bioactivity of IntegroPectin between 2020 and early 2025.

Property	Study	Reference
Antioxidant	Exceptional antioxidant, noncytotoxic activity of integral lemon pectin from hydrodynamic cavitation (HC)	[8]
Antimicrobial	Superior antibacterial activity of integral lemon pectin extracted *via* HC	[24]
	A new water-soluble bactericidal agent for the treatment of infections caused by Gram-positive and Gram-negative bacterial strains	[23]
	Cross-linked natural IntegroPectin films from citrus biowaste with intrinsic antimicrobial activity	[28]
	Antibacterial activity of lemon IntegroPectin against *Escherichia coli*	[25]
Cardioprotective	Cardioprotective effects of grapefruit IntegroPectin extracted *via* HC from by-products of Citrus fruits industry: role of mitochondrial potassium channels	[33]
Mito- and neuroprotective	New neuroprotective effect of lemon IntegroPectin on neuronal cellular model	[36]
	Protective, antioxidant, and antiproliferative activity of grapefruit IntegroPectin on SH-SY5Y cells	[37]
	The new phytocomplex AL0042 extracted from red orange by-products inhibits the minimal hepatic encephalopathy in mice induced by thioacetamide	[45]
Anti-inflammatory, anti-apoptotic, and immunomodulatory	Anti-apoptotic and anti-inflammatory properties of grapefruit IntegroPectin on human microglial HMC3 cell line	[41]
	Acute toxicity evaluation and immunomodulatory potential of HC extract of citrus peels.	[44]
Anticancer	Protective, antioxidant, and antiproliferative activity of grapefruit IntegroPectin on SH-SY5Y cells	[37]
	In vitro activity of citrus IntegroPectin against lung cancer cells	[50]
	In vitro activity of citrus IntegroPectin against breast cancer and colon cancer	[51]

ability to bind galactin-3 binding protein abundant in cancer cells through the galactose and arabinose sugars present in RG-I and RG-II regions, preventing the aggregation of cancer cells.[19] Due to the low DM and the consequent presence of anionic carboxylate groups, the zeta-potential values of IntegroPectin bioconjugates are large and negative, ranging from −14.6 mV for lemon IntegroPectin to −22.7 mV for red orange IntegroPectin. [12] The lower zeta-potential observed in lemon integroPectin is likely due to higher esterification degree of galacturonic acid groups with flavonoids. Computational insight[16] and recent experimental outcomes[20] suggest that flavonoids in IntegroPectin are chemically bound to the pectin GalA units.

2.1. Antioxidant Activity

The first remarkable biological properties were noted for lemon IntegroPectin in 2020, after about two years of storage at room temperature of the aqueous extract.[8] The substance had a total phenolic content (TPC) measured in gallic acid equivalents (GAE) per dry gram of pectin, of 0.88 mg GAE/g), namely two orders of magnitudes higher than the amount of polyphenols contained in the lemon peel. The latter, indeed varies, depending on the cultivar, between 5.12×10^{-3} and 8.30×10^{-3} mg GAE/g.[21] The antioxidant activity of lemon IntegroPectin was assessed by Oxygen Radical Absorbance Capacity (ORAC) and Folin–Ciocalteu assays of IntegroPectin in phosphate-buffered saline (PBS) solution. Results of the dichloro-dihydro-fluorescein diacetate (DCFH-DA) quantitative assay for oxidative stress assessment of A549 human epithelial cells unveiled strong bioactivity of lemon IntegroPectin in preventing oxidative stress caused by *tert*-butyl hydroperoxide (TBH). After 24 hours of incubating the cells with IntegroPectin, che citrus bioconjugate inhibited the TBH-induced stress, driving also a significant recovery of the TBH-induced altered cell morphology and size.[7]

Subsequent study of the TPC and DPHH radical scavenging power of fresh citrus IntegroPectin bioconjugates sourced via AC showed exceptionally high values. For example, the TPC lemon and red orange IntegroPectin range from 24.1 mg GAE/g in the case of sweet orange IntegroPectin to 33.6 mg GAE/m in the case of lemon IntegroPectin (Table 2)

The kinetic curves of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay displayed in Figure 2 show that the antioxidant power of all the IntegroPectin bioconjugates, showing steep curves rather than a curve reaching a *plateau* as it happens for single flavonoids, indicates into an antioxidant power that is *growing* with time. This outcome is in full agreement with what previously observed for lemon and grapefruit IntegroPectin sourced from CPW by HC.[22] In detail, lemon IntegroPectin sourced via AC exhibits the highest and sweet orange IntegroPectin the lowest radical scavenging activity.

Red orange IntegroPectin sourced via AC, in its turn, showed a kinetic profile similar to that of lemon IntegroPectin.

Table 2. DPPH residual and TPC of IntegroPectin bioconjugates.

IntegroPectin	DPPH [%] [mg GAE/g] after 30 minutes	DPPH [%] [mg GAE/g] after 60 minutes	TPC [mg GAE /g]
Lemon	3.85±0.19	4.24±0.20	33.61±0.71
Sweet orange	1.24±0.19	1.56±0.17	24.16±0.64
Red orange	3.44±0.21	3.90±0.28	30.91±0.84

[Table reused from Ref. [50], created by Authors, available at https://doi.org/10.1101/2025.01.15.633201, under the CC-BY-SA 4.0 license].

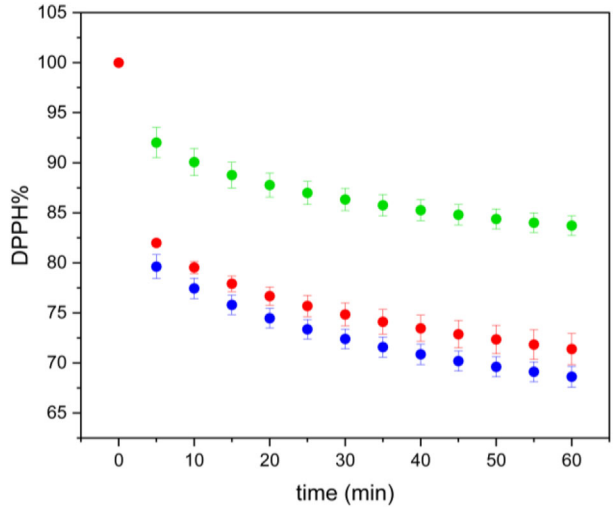


Figure 2. DPPH residual kinetic curves of lemon (blue), sweet orange (green), and red orange (red) IntegroPectin. [Image reused from Ref. [50], created by Authors, available at https://doi.org/10.1101/2025.01.15.633201, under the CC-BY-SA 4.0 license].

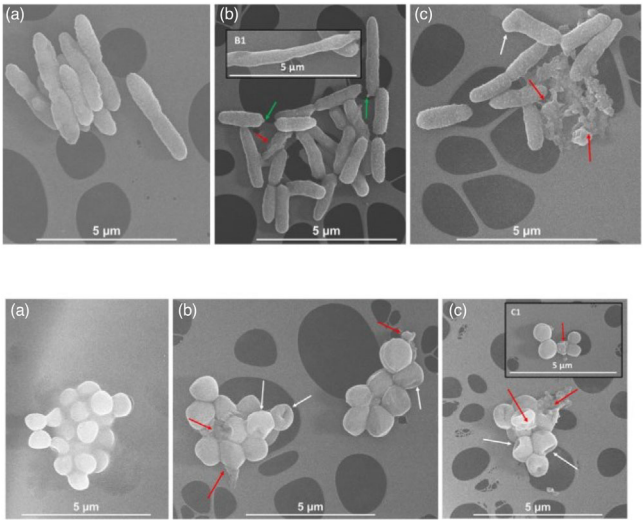


Figure 3. Top: SEM images of *P. aeruginosa* cells unchallenged a) or challenged with lemon b) or grapefruit c) IntegroPectin. The inlet B1 shows an elongated and undivided cell. Non-orthodox cell morphology is indicated by white arrow; cell death events by red arrows; green arrows highlight the presence of blebs of the cell envelope; Bottom: SEM images of *S. aureus* cells unchallenged a) or challenged with lemon b) and grapefruit c) IntegroPectins. Non-orthodox cell morphologies are indicated by white arrows; cell death events by red arrows. [Image reused from Ref. [23], created by Authors, available at https://doi.org/10.3390/antibiotics9090586, under the CC-BY-SA 4.0 license].

2.2. Antimicrobial Activity

Early indirect evidence of antimicrobial activity of lemon IntegroPectin was noted by incubating for 2 weeks samples of both fresh and heat-stressed lemon IntegroPectin in a PBS buffered solution. No mold formation was observed.[8] In May 2020, the discovery of the antimicrobial activity in vitro of lemon IntegroPectin against *Staphylococcus aureus* was reported.[23] Challenging a culture of *S. aureus* cells with 3 and 6 mg mL^{-1} of lemon IntegroPectin or commercial pectin, the newly extracted lemon IntegroPectin was able to significantly reduce the number of viable Colony Forming Units (CFU) per mL of culture (Log_{10} CFU viable bacterial count decreased from 8.3 to 6.2 with at 6 mg mL^{-1} IntegroPectin concentration but remained nearly unvaried from 7.2 to 7.1 employing commercial citrus pectin).

Shortly afterwards the same team published the outcomes of an extensive investigation of the antibacterial properties of both grapefruit and lemon IntegroPectin against Gram-negative and Gram-positive bacteria.[24] Both showed a powerful antimicrobial activity that completely killed the initial amount of inoculated *P. aeruginosa* cells at a concentration of 15 mg mL^{-1}. The commercial citrus pectin exerted the same effect at a higher microbial bactericidal concentration (MBC) of 40 mg mL^{-1}. In the case of *S. aureus*, grapefruit IntegroPectin was the only pectin able to completely kill the initial microbial load. In 2 hours of contact, both lemon or grapefruit IntegroPectin in solution altered the morphology of *P. aeruginosa* and *S. aureus* cells (Figure 3).

The presence of longer bacilli or collapsed cocci suggests that both lemon and grapefruit IntegroPectin may affect the cell viability by hampering a proper cellular division. Additionally, *P. aeruginosa* cells exposed to lemon IntegroPectin showed the occurrence of blebs at the cell envelope level, which is the route exploited by Gram-negative strains to secrete both insoluble and soluble molecules in response to stress. The first mode of action explaining the antimicrobial activity of these new citrus pectins was thus ascribed to the induction of defects during the cell division process.

Another mode of action was identified in the large amount of reactive oxygen species (ROS) released by these new pectins when contacted with bacteria, discovered by studying inhibitory activity of lemon IntegroPectin against *Escherichia coli*.[25] Dissolved in 1.0 mg/mL concentration lemon IntegroPectin was readily adsorbed at the surface of the microbes rapidly driving the formation of ROS in higher amount than that generated by 0.5 M H_2O_2, to eventually almost double the oxidative stress

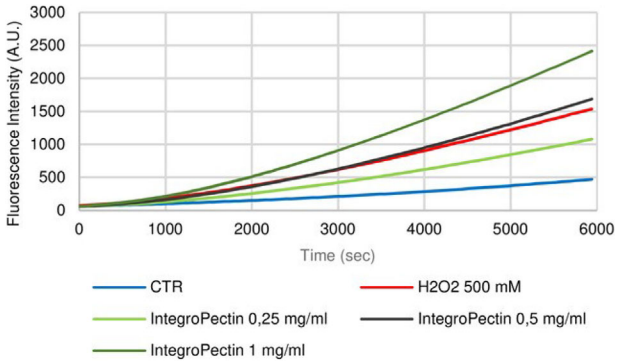


Figure 4. *E. coli* oxidation kinetics driven by aqueous H_2O_2 0.5 M and by increasing concentrations of lemon IntegroPectin. CTR stands for control sample. [Image reused from Ref. [23], created by Authors, available at https://doi.org/10.3390/antibiotics9090586, under the CC-BY-SA 4.0 license].

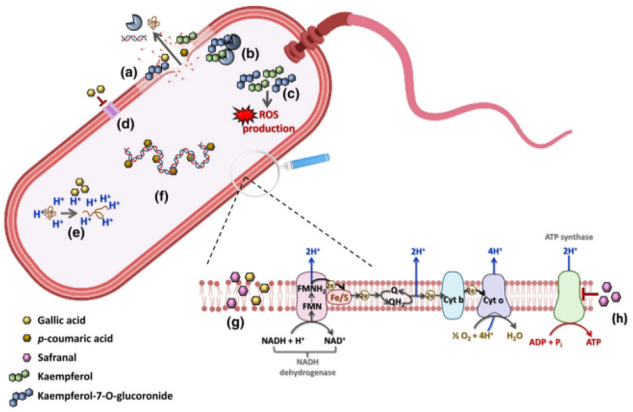


Figure 5. Schematic representation of putative mechanisms of action of lemon IntegroPectin against Gram-negative bacterial strains. [Image reused from Ref. [28], created by Authors, available at https://doi.org/10.3390/antibiotics9090586, under the CC-BY-SA 4.0 license].

exerted by the latter strong oxidant 1.7 hours after the addition of the new pectic substance (Figure 4).

Pectin is an antimicrobial agent whose antibacterial activity, first identified in 1937 but virtually ignored until the late 1990s, is higher against Gram-negative bacteria.[26] Associated to the number of free galacturonic acid residues, and thus to a low DM of pectin, said activity is highest at low pH, preferably at pH not higher than 5.0. Citrus flavonoids, on their turn, are antimicrobials that do not function by means of traditional bactericidal or bacteriostatic mechanisms, but rather through multiple mechanisms including alteration of bacteria quorum sensing and disruption of bacterial cell membrane.[27]

Indeed, studying the antibacterial activity of cross-linked lemon and grapefruit IntegroPectin cross-linked films against *P. aeruginosa* and multidrug-resistant isolate *Klebsiella pneumoniae* unveiled a slow and controlled-release of bioactive flavonoids and terpenes up to 72 hours via a quasi-Fickian diffusion processes.[28] The films acted as biocides against *P. aeruginosa* ATCC 10145, while the other two strains tolerated the challenge represented by these film formulations better. Nevertheless, the number of *K. pneumoniae* colonies decreased by at least three orders of magnitude (> 99.9% of killing), in terms of logarithmic units, when challenged with lemon or grapefruit IntegroPectin films.

The antimicrobial activity of the films was ascribed to the release over time of diverse citrus flavonoids and tepenes, that can synergistically act along with the IntegroPectin structure rich in RG-I regions and carboxylate groups to inhibit bacterial growth and eventually kill the bacteria through a multitarget mechanism displayed in Figure 5 for lemon IntegroPectin film against Gram-negative bacterial strains.

In brief, low-methoxyl IntegroPectin rich in RG-I regions acts as vehicle of both hydrophilic and lipophilic flavonoids and terpenes, resulting in: a) disruption of membranes with consequent leakage of macromolecules in the extracellular environment, b) the targeting of enzymes crucial for cell functioning and vitality, c) ROS production, d) inhibition of efflux pumps, e) acidification of the cytoplasm and protein denaturation, f) intercalation with the DNA double helix, g) permeabilization of the bacterial membrane, and h) inhibition of ATP synthase.

In detail, lemon IntegroPectin film chiefly released flavonols such as kaempferol (a flavonol able to generate ROS through the interaction of its phenoxyl radical with oxygen),[29] phenolic acids (*p*-coumaric acid and gallic acid) having a higher affinity for Gram-negative bacteria, and monoterpenoid safranal. The grapefruit IntegroPectin film chiefly released flavanones such as naringin, naringenin, and hesperidin that more efficaciously inhibit Gram-positive growth. Remarkably, the activity of films made with commercial citrus pectin was null.

This multitarget mode of action and the noncytotoxic nature of citrus IntegroPectin combined with the ease of its reproducible production in large amount and at low cost from citrus juice industry's waste, support further investigation for the clinical uptake of a new antimicrobial treatment against polymicrobial infections leading to biofilm formation. Strains of Gram-positive *S. aureus*, indeed, are often present in conjunction with *P. aeruginosa* forming hazardous polymicrobial complex communities particularly resistant to antibiotics.[30] The use of IntegroPectin, furthermore, may not drive multidrug resistance since bacteria cannot change their membranes without losing functions,[31] and also thanks to the multifactorial antimicrobial activity acting against different molecular targets in the pathogen instead of having one specific action site.[32]

2.3. Cardioprotective and Mitoprotective Activity

In 2022, the first in vivo study concerning a citrus IntegroPectin was reported describing powerful cardioprotection in mice of grapefruit IntegroPectin.[33] Evaluated by an ex vivo ischemia/reperfusion protocol as well as pharmacologically characterized to evaluate the involvement of mitochondrial potassium channels, grapefruit IntegroPectin showed significantly higher anti-ischemic cardioprotective activity when compared to pure naringenin (the bioactive aglycone of naringin). In detail, rats were treated with intraperitoneal injection (i.p.) of naringenin (100 mg/kg), vehicle (dimethyl sulfoxide, DMSO, 1 mL/Kg), or grapefruit IntegroPectin (45 mg/kg; 135 mg/kg; 450 mg/kg), 2 hours before heart removal. The selected doses

Table 3. Main parameters (RPP, dp/dt, and Ai/Avs) at the end of the ischemy/reperfusion protocol on rats administered with grapefruit IntegroPectin at 45 mg/kg, 135 mg/kg e 450 mg/kg dosage.

	Dose (Grapefruit IntegroPectin)		
	45 mg/kg	135 mg/kg	450 mg/kg
RPP (%)	14±9	34±10	34±10
dp/dt (%)	22±6	42±9	41±7
Ai/Avs (%)	33±5	24±3	20±2

Abbreviations: *RPP = myocardial contractile function; dP/dt = myocardial performance; Ai/Avs = ischemic area calculated as % of left ventricle total area.
[Table reused from Ref. [33], created by Authors, available at https://doi.org/10.3390/foods11182799, under the CC-BY-SA 4.0 license].

of IntegroPectin, based on the amount of naringin in the phytocomplex (74 mg/g),[13] are equivalent to pure naringin doses of 3 mg/kg, 10 mg/kg, and 30 mg/kg, respectively.

Table 3 summarizes the functional and histological results at the end of the ischemy/reperfusion protocol.

Cardioprotective effect observed already at the 135 mg/kg dosage, corresponding to a 10 mg/kg dosage of naringin, is particularly relevant, as it is nearly identical to that observed in animals administered with naringenin at 100 mg/kg dosage. Hence, this outcome confirms that the grapefruit IntegroPectin has an improved pharmacokinetics that ensures anti-ischemic protection at a dosage 10-fold lower than that of the pure flavonoid.

Furthermore, the study unveiled that the activation of mitoK channels, cardioprotective against ischemia–reperfusion injury,[34] might be responsible for the protective activity of grapefruit IntegroPectin. Indeed, addition of the bioconjugate to isolated cardiac mitochondria in concentration range 0.01, 0.03, 0.1, 0.3 mg/mL corresponding to about 1, 3, 10, and 30 μM concentration of naringin, resulted in a concentration-dependent depolarization, eventually reaching a maximum membrane depolarization value of 25±2 mV at 0.3 mg/mL, identical to that of naringenin 30 μM. Depolarization of mitochondrial membrane potential reduces the accumulation of Ca^{2+} ions in the matrix, reducing apoptosis and preserving cell viability.[35] Accordingly, the addition of grapefruit IntegroPectin reduced the uptake of Ca^{2+} ions into the mitochondrial matrix in a concentration-dependent manner.

First evidence of the mitoprotective activity of citrus IntegroPectin had been reported in 2021 describing the activity of lemon IntegroPectin in reversing mitochondrial damage in neuronal SH-SY5Y human cells due to addition of 0.2 M H_2O_2.[36] Following cell exposure to concentrated H_2O_2, the JC-1 red/green fluorescent signal significantly diminished. Pretreatment and cotreatment with lemon IntegroPectin fully counteracted the significant H_2O_2-induced mitochondrial shape remodeling. On the contrary, citrus pectin failed in producing a similar effect. The morphology of mitochondria, that during normal culture conditions is characterized by a well-interconnected mitochondrial network, was changed from tubular networks to fragmented puncta (circular) upon treatment of the cells with aqueous H_2O_2 0.2 M. When lemon IntegroPectin was administered immediately before or directly along with the strong oxidizer H_2O_2, the morphology and mitochondrial parameters were partially recovered.

A similarly powerful mitoprotective action was reported shortly afterwards also for grapefruit IntegroPectin.[37] Remarkably, changes in mitochondrial morphology occur in many neurological diseases including Alzheimer's,[38] Parkinson's,[39] and amyotrophic lateral sclerosis[40] diseases.

2.4. Anti-Inflammatory and Anti-Apoptotic Activity

In 2023, grapefruit IntegroPectin was found to exert a multispectral beneficial activity on microglia HMC3 cells exposed to oxidative stress conditions by inhibiting the intracellular pathways typically associated with apoptotic, oxidative, and neuroinflammatory responses.[41] Again, treatment of cells with grapefruit IntegroPectin allowed to prevent loss in cell viability due to a strong oxidizer agent such TBH. HMC3 exposure for 24 hours to TBH led to a concentration-dependent decrease in cell viability. On the other hand, HMC3 cells treated with IntegroPectin (1 mg/mL) immediately before TBH exposure fully prevented the cell death induced by the TBH treatment. In detail, the protective effects of the newly extracted citrus IntegroPectin on microglia HMC3 cells, including neuroinflammatory response inhibition and basal microglia activation, is due to:

- decrease in the amount of ROS
- activation of the phosphoinositide 3-kinase (PI3K)/Akt cascade
- down-regulation of the PI3K-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)-inducible nitric oxide synthase (iNOS) cascade.

The anti-inflammatory power of grapefruit IntegroPectin was further investigated by the mRNA expression of three major genes involved in the modulation of the inflammatory response in microglia cells: the main pro-inflammatory cytokines IL-6 and IL-1β, and the iNOS enzyme, a major mediator of inflammation.[42] Through a combination of several experiments, including nuclei morphology, Annexin V binding-PI uptake, and quantification of Caspase-3 (an enzyme catalyzing the specific cleavage of many key cellular proteins in apoptosis),[43] the study explored the time-related activation of three important intracellular modulators of the neuroinflammatory response: ERK1/2, Akt, and NF-kB. A short-time (4 hours) treatment with IntegroPectin led to a significant increase in p-ERK1/2 levels and to a parallel decrease in p-Akt and p-NF-kB levels, compared to Ctrl cells. Plots in Figure 6 show evidence that grapefruit IntegroPectin was able to counteract TBH-induced apoptosis in the human microglial HMC3 cell line.

Cells in the untreated (Ctrl) sample retained high viability (Annexin V − PI−, 95.5%) with only a small percentage of Annexin V + PI − cells (4.3%) undergoing intermediate apoptosis. In a co-treatment with TBH, grapefruit IntegroPectin exerted protective action preventing oxidative stress-induced apoptosis: the treated sample yielded a high percentage of Annexin V − PI − cells (95%) and a small percentage (4.5%) of Annexin V + PI − cells.

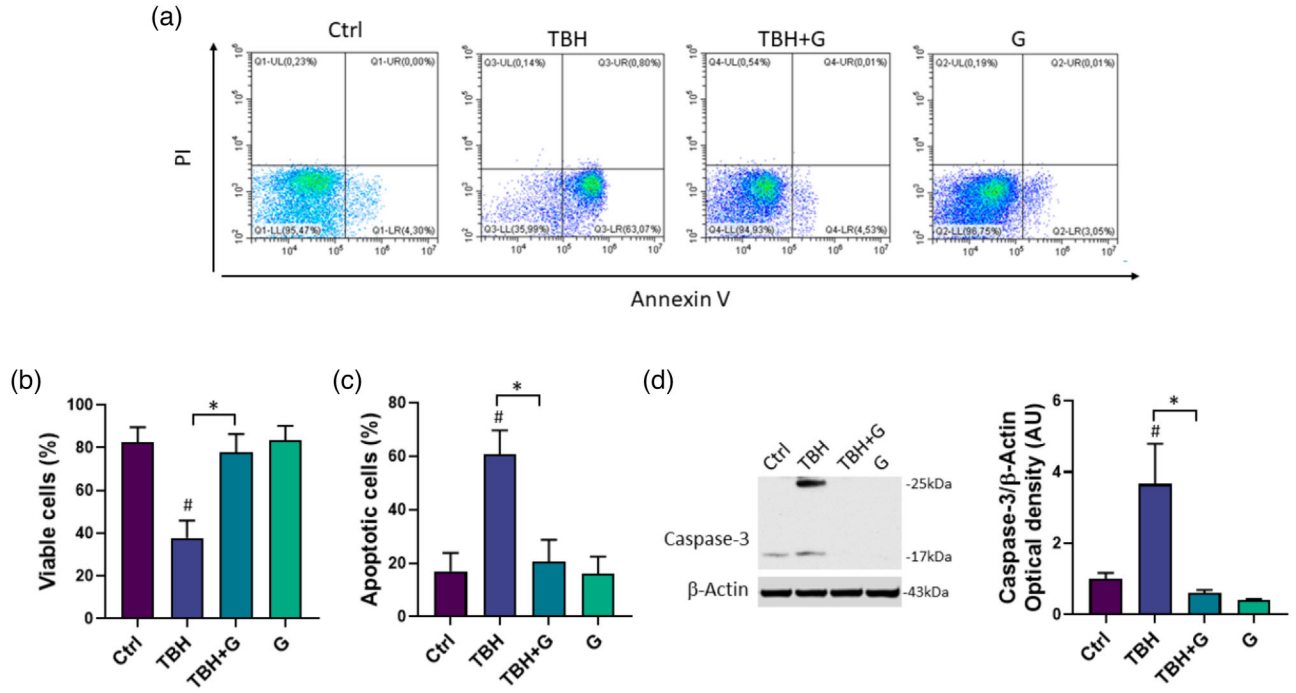


Figure 6. Anti-apoptotic effects of grapefruit IntegroPectin: a) representative plot indicating the percentage of Annexin V- PI- cells (on the lower left quadrant), Annexin V + PI- cells (on the lower right quadrant), PI + Annexin V- cells (on the upper left quadrant), and PI + Annexin V + cells (on the upper right quadrant) of different conditions: untreated sample (Ctrl), sample treated with TBH (200 μM, 24 hours), sample co-treated with TBH (200 μM, 24 hours), and IntegroPectin (G) (1 mg/mL, 24 hours), and sample treated with IntegroPectin (G) alone (1 mg/mL, 24 hours); b) Histogram showing the cumulative Annexin V- PI- cell (viable cells) percentages from all the experiments; c) histogram showing the cumulative Annexin V + PI- cell percentages (apoptotic cells) from all the experiments; d) representative images of Caspase-3 and β-actin western blotting bands and histogram of Caspase-3 normalized to β-actin Optical density in Ctrl cells, cells treated with TBH (200 μM, 24 hours), TBH (200 μM, 24 hours) + G (1 mg/mL, 24 hours), and G alone (1 mg/mL, 24 hours). Tukey test: # $p < 0.05$ as compared to Ctrl group; * $p < 0.05$. AU (Arbitrary Units). [Image reused from Ref. [41], created by Authors, available at https://doi.org/10.3390/cells13040355, under the CC-BY-SA 4.0 license].

Furthermore, whereas treatment with TBH alone induced a significant increase in the cleaved forms of Caspase-3 (25 kDa and 17 kDa), grapefruit IntegroPectin was able to substantially counteract this effect. Caspase-3 is a thiol protease involved in the execution phase of apoptosis.[43] In brief, the radical scavenger activity of grapefruit IntegroPectin blocks the cross-linked extrinsic and intrinsic apoptotic pathways, reestablishing the mitochondrial homeostasis, as it happens in SH-SY5Y neuronal cells using both grapefruit[37] and lemon[36] IntegroPectin.

Interestingly, while the grapefruit IntegroPectin treatment in SH-SY5Y cells produced a decrease in the proliferation rate and a cell cycle arrest at the G2/M phase,[37] the same treatment did not affect cell viability and cell proliferation in HMC3 cells, suggesting a cell type-specific activation by grapefruit IntegroPectin.

As put it by Silverman and coworkers, there are no iNOS inhibitors approved for human use because promising results found in iNOS inhibitor development in animal studies have not translated to man.[42] The fact that new phytocomplex grapefruit IntegroPectin modulates the inflammatory response and basal microglia activation by inhibiting the cascade of PI3K-NF-kB-iNOS is therefore promising toward the development of a safe and effective iNOS inhibitor.

Supporting the findings related to the anti-inflammatory activity of grapefruit IntegroPectin, an in vivo study from Ikawati and coworkers in Indonesia reported in 2022 the immunomodulatory activity and a reduction in LPS-induced lymphocytes in rats administered with a similar citrus pectin derived by HC from the dried peels of *Citrus reticulata* (mandarin).[44] In immunomodulatory evaluation, the new pectin was given orally daily for 11 weeks, inflammation induced with lipopolysaccharide (LPS), and the blood sampled 5 hours after LPS induction.

Another important outcome of the latter study was that oral administration of mandarin pectin sourced via HC in the acute toxicity test prolonged until day 15 did not cause lethality in rats, even at the highest tested dose of 5,000 mg/kg body weight (BW). The flavonoid analysis was not reported, but the team ascribed the anti-inflammatory activity to hesperidin and hesperetin with bioinformatics analysis suggesting that the two flavonoids target 14 proteins in lymphocytes inflammed with LPS.[44]

2.5. Neuroprotective Activity

The effects of lemon IntegroPectin and commercial citrus pectin on cell viability, cell morphology, ROS production, and mitochondria perturbation driven by treatment of neuronal SH-SY5Y human cells with H_2O_2, the neuroprotective in vitro activity of lemon IntegroPectin was reported in 2021.[36]

In detail, a dose-effect investigation on SH-SY5Y cells viability in response to treatment with commercial citrus pectin

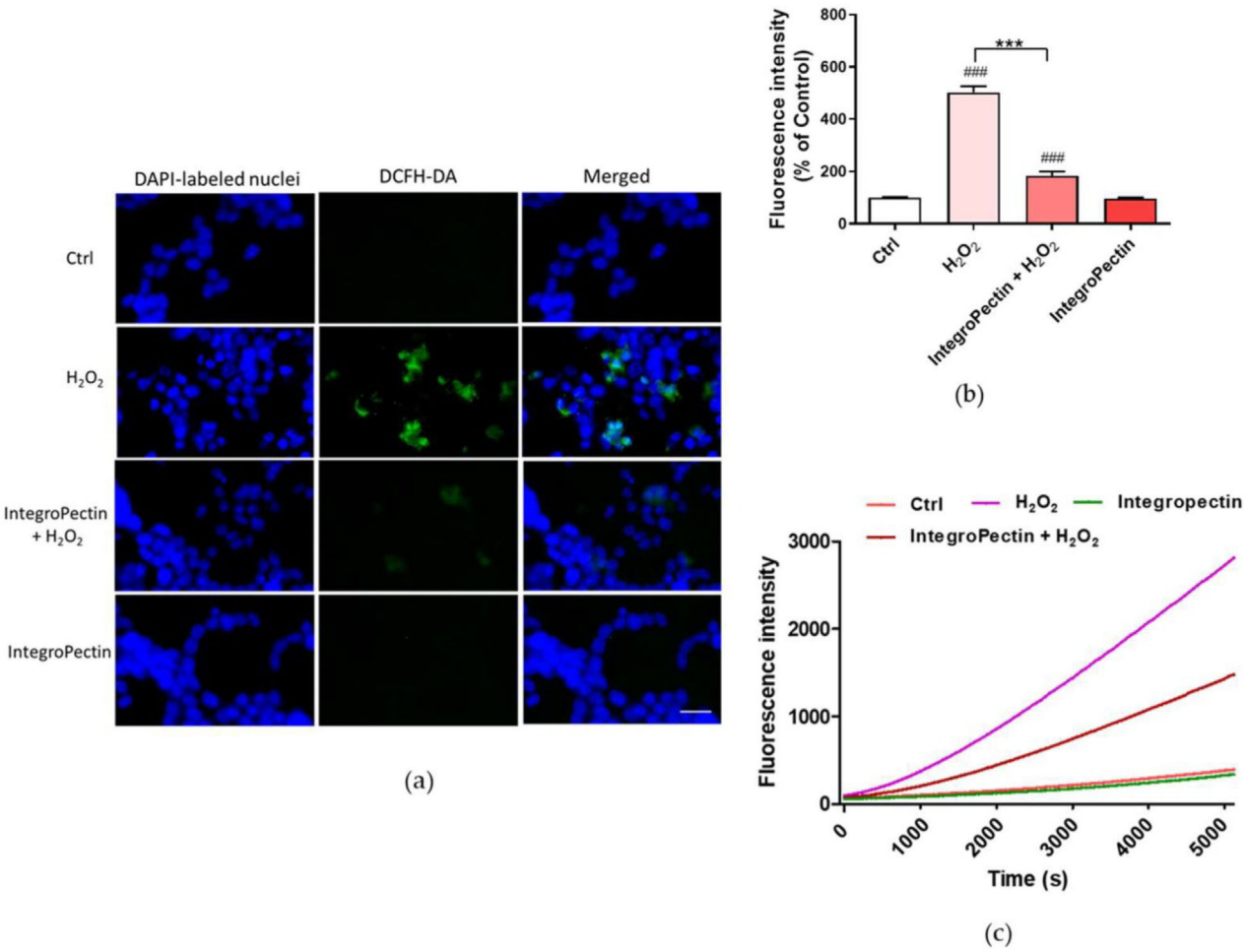


Figure 7. Effects of grapefruit IntegroPectin on ROS production driven by exposure to concentrated (200 μM) aqueous H_2O_2: a) DCFH-DA, DAPI, and merged fluorescence microscopy images of untreated cells (Ctrl), treated with grapefruit IntegroPectin, with H_2O_2 alone or in combination with IntegroPectin; b) histogram of fluorescence intensity of untreated cells (Ctrl) treated with IntegroPectin, with H_2O_2 alone or in combination with IntegroPectin measured using DCFH-DA fluorescence assay (n = 26); c) oxidation kinetics of untreated cells (Ctrl), treated with IntegroPectin, with H_2O_2 alone or in combination with IntegroPectin. Scale bar: 50 μm. Tukey test: ### $p < 0.001$ as compared to control (Ctrl) group, *** $p < 0.001$. [Image reused from Ref. [37], created by Authors, available at https://doi.org/10.3390/ijms22179368, under the CC-BY-SA 4.0 license].

and lemon IntegroPectin was conducted via the MTT cell sensitivity assay testing 0.01, 0.1, and 1 mg/mL doses for 24 hours of contact. Results showed evidence that both pectins did not induce any significant change in cell viability. Treatment of neuronal SH-SY5Y human cells with grapefruit IntegroPectin fully counteracted the significant H_2O_2-driven oxidative damage due to concentrated H_2O_2.[37] Fluorescence microscope inspection (Figure 7a) and fluorescence intensity measurement (Figure 7b) showed that the treatment of neuronal cells with grapefruit IntegroPectin almost completely counteracted the ROS formation driven by exposure to concentrated H_2O_2. Analysis of the kinetics of ROS production after exposure of SH-SY5Y cells to H_2O_2 further showed that treatment with IntegroPectin is highly effective in substantially lowering and delaying ROS production (Figure 7c). Mediated by ROS, including H_2O_2 and its derivatives, oxidative stress alters numerous cellular processes, such as mitochondrial regulation and cell signaling, propagating cellular injury that leads to neurodegenerative disease.

Finally, evidence of powerful neuroprotective activity for red orange IntegroPectin was lately reported studying the protective in vitro activity of IntegroPectin on the epithelial integrity of Caco-2 cells challenged by a pro-inflammatory cocktail, and its therapeutic in vivo effect on minimal hepatic encephalopathy in mice induced by thioacetamide (TAA).[45] Results unveiled powerful protective activity of red orange IntegroPectin on the epithelial integrity of Caco-2 enterocytes (comparable to that of the reference compound butyrate); whereas the in vivo murine tests revealed a significant therapeutic effect on the minimal hepatic encephalopathy induced by TAA, dramatically decreasing oxidative stress and peripheral and central inflammation.

2.6. Anticancer Activity

First evidence of anticancer activity of IntegroPectin was obtained in 2021 reporting the in vitro antiproliferative activity

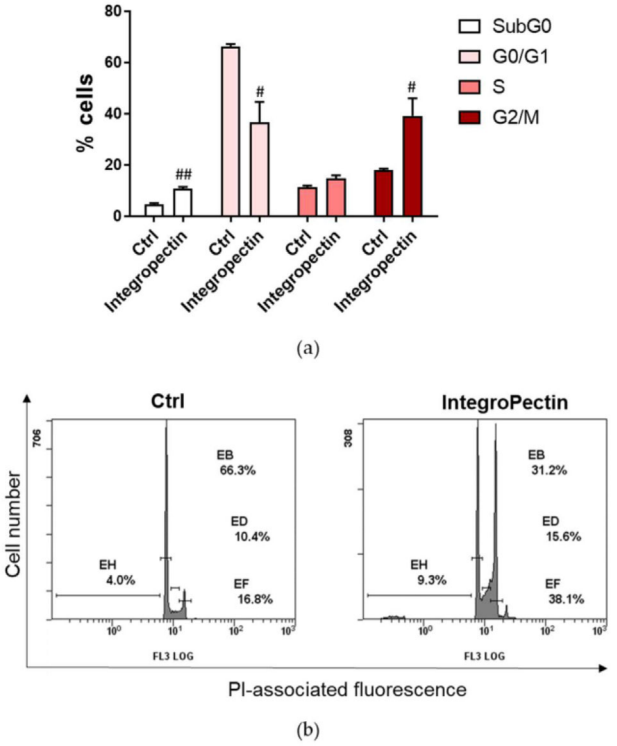


Figure 8. a) Effect of grapefruit IntegroPectin on the cell cycle distribution of SH-SY-5Y cells. Percentage (%) of cell distribution of untreated (Ctrl) cells and cells treated for 24 hours with IntegroPectin (1 mg/mL) in different phases of the cell cycle, assessed by flow cytometry analysis after propidium iodide (PI) staining (n = 12); b) Representative images. t-test: # $p < 0.05$, ## $p < 0.01$ as compared to control (Ctrl) group. [Image reused from Ref. [37], created by Authors, available at https://doi.org/10.3390/ijms22179368, under the CC-BY-SA 4.0 license].

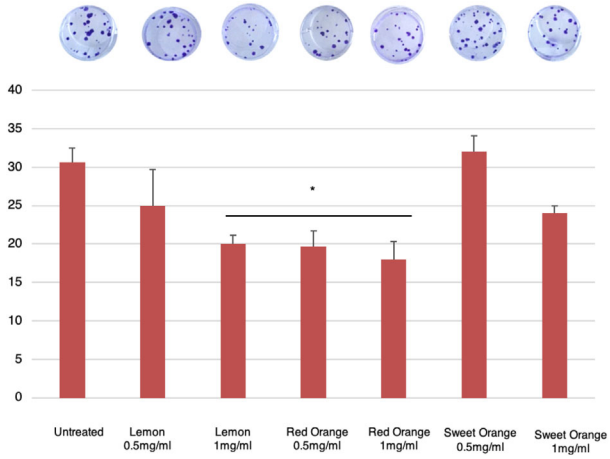


Figure 9. Effect of different citrus fruit IntegroPectin bioconjugates on colony formation ability in A549 cell line. [Image reused from Ref. [50], created by Authors, available at https://doi.org/10.1101/2025.01.15.633201, under the CC-BY-SA 4.0 license].

of grapefruit IntegroPectin on SH-SY5Y neuroblastoma cells.[37] The distribution of cells in the different phases of the cell cycle, analyzed by the flow cytometry analysis of cellular DNA content following cell staining with propidium iodide (PI), shed insight on the cytostatic effect of the new grapefruit pectin on neuronal model cells.

As shown in Figure 8, treatment of SH-SY5Y cells with grapefruit IntegroPectin produces a cell cycle arrest exactly at the G2/M phase. In the growth two phase (G2 phase) of the cell cycle preceding mitosis, the cell replenishes its energy and synthesizes the proteins needed for chromosome manipulation. Cell cycle arrest at the G2/M phase indicates that the damage of intracellular DNA is difficult to repair.[46] The G2/M-phase checkpoint usually prevents cells with damaged DNA from undergoing mitosis by the inhibition of the mitotic complex CDK1-cyclin B and activation of the apoptosis cascade.[47] However, no increase in cell death was observed when treatment was prolonged up to 5 days. Pretreatment of the SH-SY5Y cells with grapefruit IntegroPectin at a dosage of 1 mg/mL, per se cytostatic, was able to significantly counteract the cell death induced by the cell treatment with 0.2 M H_2O_2.

The same treatment with grapefruit IntegroPectin was also able to recover cell morphology and cell body area, impaired by H_2O_2 treatment. Furthermore, pretreatment with the newly sourced citrus pectin was able to reduce the amount of cell debris, indicative of cell protection.

The high antiproliferative activity of the grapefruit IntegroPectin was partly ascribed to its low DM of 22%[37] LM pectin suppresses the expression of cancer cells by binding to galactin-3, a lectin present on multiple cellular locations which plays a vital role in metastasis of cancers and in tumorigenesis, preventing their adhesions to normal cells or spreading to other parts of the body.[48] Thanks to its numerous carboxylate groups, LM citrus pectin binds and inhibits Toll- like receptor-2 involved in inflammation, whereas galactose and arabinose sugars present in RG-I (and RG-II) regions interact with galactin-3 binding protein expressed on cancer cells, preventing their aggregation.[49]

Subsequent evidence of citrus IntegroPectin anticancer activity was reported in 2025 describing substantial in vitro activity against human nonsmall cell lung cancer cells of IntegroPectin bioconjugates obtained through acoustic cavitation of CPW from lemon, red orange, and sweet orange organically grown fruits.[50] Dissolved in PBS, pH 7.4 at different concentrations, all IntegroPectin bioconjugates tested (Figure 9) affected long-term proliferation and cell migration of adenocarcinoma cells of line A549.

Compared to sweet orange, IntegroPectin from lemon and red orange were particularly effective in reducing proliferation (colony-forming ability). Histograms in Figure 9 show the significant reduction in A549 cell colony number after treatment with lemon IntegroPectin at 1 mg/mL concentration, and with red orange IntegroPectin at 0.5 and 1.0 mg/mL concentration.

Increased cancer cell motility is a feature of aggressive tumors.[19] It is therefore promising that all three IntegroPectin bioconjugates were able to decrease both and cell migration.

Further tested against Caco-2 colon cancer and MCF-7 breast cancer cells, the same citrus IntegroPectin bioconjugates dissolved in PBS substantially reduced cell migration of both tumor cell lines already at 0.5 and 1.0 mg/mL concentration.[51] Furthermore, red orange and lemon IntegroPectin phytocomplexes at

Table 4. Main technical and economic advantages of the CytroCav circular economy process for the production of IntegroPectin.

Zero emissions	Using only electricity, water, and fresh citrus processing waste (CPW), the process affords no waste
High material and energy efficiency	No drying of CPW required. High energy efficiency of HC and AC processes ensure low electricity consumption
High productivity	Using the HC extraction, tens of kg of IntegroPectin from 500 kg of CPW in 2 hours
Reproducibility	Digitally controlled cavitation process consistently affords IntegroPectin bioconjugates with the same characteristics from the same raw material

5 and 10 mg/mL load reduced cell viability for both cell lines. Adding to promising results against lung cancer, these outcomes support further investigation of this family of bioconjugates as broad-scope therapeutic agents for the treatment of cancer.

3. The CytroCav Process

Looking to forthcoming industrial uptake of citrus IntegroPectin for the prevention and treatment of numerous ailments, it is instructive to investigate the technical and economic feasibility of IntegroPectin industrial production via the CytroCav process based on cavitation (hydrodynamic or acoustic) of fresh CPW from organically grown citrus fruits.[12] Table 4 summarizes the main technical and economic advantages of the cavitation-based extraction of IntegroPectin from CPW conducted in water only.

First, the process does not use any acid, base, or organic solvent degrading or potentially contaminating the extracted bioproducts. No extensive water purification of the spent acidic aqueous phase prior to discharge water in the environment is required. The need for expensive treatment of said aqueous effluents, for example, was responsible in the early 1990s for the relocation to Latin America of all pectin plants formerly based in the USA.[52]

Second, the CytroCav process has high material and energy efficiency. This allows to valorize the uniquely high organic matter content (95% of total solids) of CPW. No energy-intensive drying of CPW containing 80% water by burning natural gas followed by pelletization of dried CPW is required.[53] Similarly, no valued components of the CPW are wasted. The cellulosic insoluble fraction obtained is a valued nanocellulose, dubbed "CytroCell", suitable for example to vastly enhance the mechanical and chemical stability of polymers for advanced applications such as in anion exchange membranes.[54]

From the energy viewpoint, using a HC-based complete extraction plant including a closed- hydraulic loop (total volume capacity around 230 L), a centrifugal pump (7.5 kW nominal mechanical power, rotation speed 2900 rpm), and a Venturi-shaped cavitation reactor, employed in the treatment of 34 kg of grapefruit processing waste in 120 L water requires just 1 hour, with overall consumed energy at the level of 0.2 kWh per kg of CPW.[11]

Third, the modular cavitation-based CytroCav process has high productivity. Figure 10 shows the main components of an

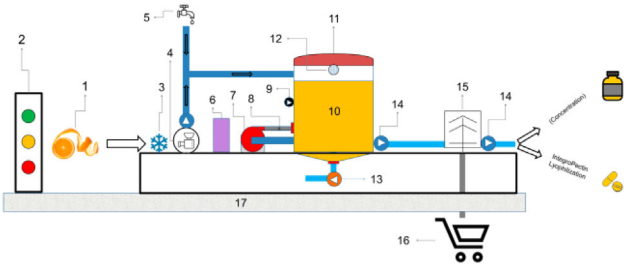


Figure 10. Main technological components of the CytroCav HC-based extractor to produce IntegroPectin capsules. 1—citrus waste peel; 2—electronic control panel; 3—ice machine; 4—grinder; 5—water supply; 6—inverter; 7—centrifugal pump; 8—HC reactor, such as a Venturi tube; 9—dosing pump; 10—working vessel; 11—hatchway; 12—washing sphere; 13—lobe pump; 14—multistage pump; 15—filter/separator; 16—discharge of residues; 17—skid. [Image reused from Ref. [57], created by Authors, available at https://doi.org/10.3390/pr8050549, under the CC-BY-SA 4.0 license].

industrial HC-based natural product extractor applied to CPW developed by the teams of Meneguzzo and Pagliaro during the COVID-19 public health crisis.[55]

The complete plant includes electronic control panel, grinder, water supply, centrifugal pump, the HC reactor, and filter/separator. One such plant with a nominal capacity of about 2000 L, undertaking the processing of 500 kg CPW in 1500 L water, would be able to extract tens of kg of IntegroPectin per cycle, hence several hundreds of kg of pectin per day (in 12 cycles).[55]

Fourth, the digitally controlled cavitation-based extraction process consistently affords IntegroPectin with the same structure and composition, and thus bioactivity, independent of the harvesting season. This was lately shown testing the cell protective and therapeutic activity of red orange IntegroPectin sourced from CPW obtained from oranges harvested in early 2022 and 2023, within 24 hours of receiving the respective frozen batches of red orange by-products resulting from fruits harvested and processed within a week.[45] What is necessary is that CPW originates from organically grown citrus fruits. Otherwise, the extraction of industrial biowaste originating from fruits containing pesticides or fungicides on the peel will result in the extraction and concentration of said contaminants. Conventionally grown citrus fruits, indeed, are heavily treated with pesticides and even with fungicides post-harvest.[56]

In general, the low cost of manufacturing, low capital expense, lack of noxious emissions, ease of scale-up, and highly controllable conditions affording lot-to-lot product

consistency make HC applied to natural product extraction from biological resources the enabling technology of the bioeconomy.[57]

Similarly, the extraction of natural products using AC is an economically viable alternative to conventional natural product extraction techniques allowing to lower extraction time, the amount of energy and unit operations.[58]

4. Conclusions

In conclusion, summarizing the main biological properties of citrus IntegroPectin identified in the first five years following the discovery of its first significant bioactivity in 2020,[8] this account provides an overview on the multispectral bioactivity of this new family of citrus bioconjugates sourced from industrial processing waste of different citrus fruits using cavitation in water only.

Explaining the vastly enhanced biological activity of citrus IntegroPectin when compared to conventional citrus pectin, numerous in vitro and in vivo studies suggest a synergistic mode of action justifying the multitarget activity of this new bioconjugate. The enhanced bioactivity would be due to: i) the low DM of the pectin backbone; ii) the relative abundance of highly bioactive RG-I regions; iii) the abundance and prolonged release of citrus flavonoids (and terpenes) concentrated at the surface of the polysaccharide. Computational insight[16] and recent experimental outcomes[20] suggest that flavonoids in IntegroPectin are chemically bound to the pectin GalA units,[59] eventually making them both soluble and orally bioavailable. Flavonoid lack of solubility indeed has been the main limitation to practically useful flavonoid employment in biomedicine as pharmaceutical and nutraceutical products.[60]

Given the global health relevance of the IntegroPectin bioactivity properties listed in Table 1, the study will hopefully assist in guiding both new fundamental and applied (in vivo and preclinical) studies across the world aimed at the uptake of IntegroPectin in new pharmaceutical and nutraceutical products for the treatment and prevention of several serious illnesses. *Citrus* are cultivated in more than world's 140 countries (in tropical, subtropical, and Mediterranean climates) in the "citrus belt" between approximately 40° N and 40° S latitude.[61] It is also instructive, in conclusion, to learn that a significant and rapidly increasing fraction of *Citrus* is organically farmed (share of organic *Citrus* tree fruit crops was already 16% in 2013).[62] In conclusion, considering that with citrus IntegroPectin we are dealing with highly health-beneficial substances (citrus pectin, flavonoids, and terpenes) widely approved for human consumption in food, nutraceutical, and pharmaceutical (diosmin, for instance)[63] products, start of preclinical and clinical trials using IntegroPectin will be eased by the IntegroPectin bioconjugate composition as well as by its extraction and isolation route requiring only water. Hopefully, said studies will confirm the activity identified in the first in vitro and in vivo studies, and citrus IntegroPectin will be shortly used as active ingredient in numerous new pharmaceutical and nutraceutical products for the treatment and prevention of several ailments worldwide.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: citrus pectin · flavonoids · IntegroPectin · pectin bioactivity · RG-I pectin

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