Food Hydrocolloids for Health 8 (2025) 100246

Contents lists available at ScienceDirect

Food Hydrocolloids for Health

journal homepage: www.elsevier.com/locate/fhfh

# Citrus flavonoid-pectin conjugates: Towards broad scope therapeutic agents

Caterina Di Sano [a], Claudia D'Anna [a,1], Giovanna Li Petri [b], Giuseppe Angellotti [b], Francesco Meneguzzo [c,*], Rosaria Ciriminna [b,*], Mario Pagliaro [b,*]

[a] Istituto per la Farmacologia Traslazionale, CNR, via U. La Malfa 153, 90146 Palermo, Italy
[b] Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy
[c] Istituto per la Bioeconomia, CNR, via Madonna del Piano 10, 50019 Sesto Fiorentino FI, Italy

ARTICLE INFO

*Keywords:*
Flavonoid-pectin conjugates
Pectin
Bioconjugates
Citrus flavonoids

ABSTRACT

Obtained via synthetic organic chemistry, enzymatic, free radical or cavitation-based routes, citrus flavonoid-pectin conjugates are bioconjugates showing substantial bioactivity. Reviewing research achievements concerning the biological activity of citrus flavonoid-pectin conjugates since their recent introduction, this study suggests that the use of water-soluble and biocompatible citrus pectin to chemically bind and deliver citrus flavonoids offers a synergistic solution to the poor bioavailability of flavonoids that so far limited their uptake. Merging the powerful and broad scope bioactivity of citrus flavonoids with that of pectin, the approach is promising towards the development of new pharmaceutical and nutraceutical products for the treatment and prevention of numerous diseases.

## 1. Introduction

Ubiquitous in the cell wall of plants and fruits where it acts as glue enabling cell adhesion (Bou Daher & Braybrook Siobhan, 2015) promoting morphogenesis of individual cells and contributing to the development and structural strength of organs (Saffer, 2018), pectin is a galacturonic acid polymer approximately consisting of a linear homogalacturonan (HG) made of (1→4)-α-d-GalA (galactopyranosyluronic acid) units partly methyl-esterified at O-6 position. 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 (Ropartz & Ralet, 2020).

Soluble in water, pectin is an anionic polyelectrolyte in which the linear charge density depending on the number and protonation state of the nonesterified carboxyl groups along the chain, and degree of branching by neutral sugars play a key role in the conformation of the molecule in solution (Zdunek et al., 2021). Manufactured since the early 1900s first by simple extraction from apple pomace in hot water, and subsequently (since 1941) via acid hydrolysis in hot water of citrus fruit peel or apple pomace followed by precipitation with alcohol and solvent removal under vacuum (Muhidinov et al., 2021), pectin is the most valued hydrocolloid used by the food industry (Seisun & Zalesny, 2021). New research in the first two decades of the 2000s has opened the route to new and unexpected applications of this uniquely complex heteropolysaccharide well beyond its traditional use as food hydrocolloid (Ciriminna et al., 2022). Reviewing said advances often based on pectin sourced via new green extraction routes, some of us wrote in 2022:

> "In this rapidly evolving context, the pectin industry, including many new players using the aforementioned new green extraction technologies, will progressively start supplying the valued biomaterial and its derivatives, such as pectin-derived oligosaccharides no longer to customers in the food and beverage industries only, but also to companies using pectin to produce advanced biobased materials, nutraceutical, medical and pharmaceutical products" (Ciriminna et al., 2022).

Perhaps not surprisingly, three years later researchers from one of the world's largest pectin manufacturers jointly published along with academic researchers the first systematic scoping review of human intervention studies on nutrition and health effects of pectin conducted between 1961 and 2022 (Weber et al., 2025). In detail, the team identified 134 human intervention studies. Said clinical studies show

---

* Corresponding authors.
*E-mail addresses:* francesco.meneguzzo@cnr.it (F. Meneguzzo), rosaria.ciriminna@cnr.it (R. Ciriminna), mario.pagliaro@cnr.it (M. Pagliaro).

[1] Equally contributed as first author.

https://doi.org/10.1016/j.fhfh.2025.100246
Received 20 June 2025; Received in revised form 22 September 2025; Accepted 7 October 2025

evidence that pectin administration improves gut health, glycaemic response and appetite (reduction in post-prandial blood glucose and insulin peaks, increased satiety and delayed gastric emptying); fat metabolism (lowering fat absorption), immune response (enhanced immune response and reduced disease symptoms); and body detoxifying ability (detoxification from radioactivity and heavy metals) (Weber et al., 2025).

Though being extensively degraded (hydrolysis and degradation of RG-I and RG-II “hairy” regions) (Wang et al., 2016) during the industrial extraction with mineral acid, commercial citrus pectin is highly biocompatible and has also wound-healing properties for which it is researched for regenerative medicine and biomedical engineering applications (David, 2023).

Increasingly employed in new nutraceutical formulations, and chiefly consisting of flavanones, flavones, flavonols (and anthocyanins, in red oranges), citrus flavonoids exert significant health beneficial properties (Alam et al., 2022). Clinical studies support their use to enhance endothelial function (Jalili et al., 2024), as well as lipid-lowering, antihypertensive and anti-inflammatory agents (Assini et al., 2013). Selected clinical studies suggest their use also for treatment of autoimmune disorders (Musumeci et al., 2023) and atherosclerosis (Ebrahimi et al., 2023). Many citrus flavonoids, furthermore, exert powerful antimicrobial activity (Ciriminna et al., 2025), and antimicrobials based on a mix of different citrus flavonoids have been commercialized in the early 2010s. Finally numerous studies support the use of citrus flavonoids as anti-cancer and neuroprotective agents (Qiu et al., 2023), as well as for the treatment of metabolic diseases such as obesity and diabetes (Gupta et al., 2023).

Favonoids are polyphenolic molecules having a fundamental structure phenylpropanoid chain (C6–C3–C6), consisting of two aromatic rings (A and B) linked by a heterocyclic pyran ring (C). As such, they have extremely low solubility in water leading to poor oral bioavailability that so far limited biomedical use of these biomolecules. Researches carried out worldwide to enhance their bioavailability generally focus on the development of effective delivery systems (Elmeligy et al., 2021). First reported by Lee and co-workers who synthesized flavonoid-pectin conjugates from quercetin, rutin, and hesperidin via epichlorohydrin conjugation reaction (Ahn et al., 2017), the approach to use pectin as a carrier for poorly soluble citrus flavonoids by synthesizing flavonoid-pectin conjugates in principle merges the powerful and broad scope bioactivity of citrus flavonoids with that of pectin. The approach goes beyond the use of pectin as a carrier for partly soluble biophenols such as hydroxytyrosol that can be dissolved in water and adsorbed at the outer surface of pectin (Rubio-Senent et al., 2025). Reviewing research achievements concerning the bioactivity of citrus flavonoid-pectin conjugates since their recent introduction, this study suggests that the use of water soluble and highly biocompatible citrus pectin to chemically bind and deliver citrus flavonoids offers a promising synergistic solution to the poor bioavailability of citrus flavonoids that so far limited the uptake of these biomolecules in the development of new pharmaceutical and nutraceutical products for the treatment and prevention of disease.

## 2. Citrus flavonoid-pectin conjugates

To identify research conducted on citrus flavonoid-pectin conjugates we carried out an online search on a comprehensive research database (Google Scholar, 2025). Search returned 245 results that were inspected to identify research studies relevant to this study.

### 2.1. Epichlorohydrin route

As mentioned above, in 2017 Lee and co-workers in South Korea synthesized flavonoid-pectin conjugates from quercetin, rutin, hesperidin via epichlorohydrin conjugation reaction (Ahn et al., 2017), demonstrating the approach to use water-soluble pectin as a carrier for poorly soluble citrus flavonoids imparting pectin with bioactivity of citrus flavonoids. The two-step reaction affording the bioconjugate involves formrmation of intermediate by reaction of the flavonoid with epichlorohydrin, followed by reaction of the intermediate with pectin to form the flavonoid-pectin conjugates.

Evaluating the antioxidant activity of pure flavonoids and pectin conjugates in the concentration range of 0.5–3 mg/mL, the team showed that while no meaningful antioxidant activity was detected for unmodified commercial citrus pectin, the pectin conjugates showed significant, dose-dependent antioxidant activity, comparable to that of pure flavonoids. Finally, they reported how the new flavonoid-pectin conjugates were readily soluble in water, whereas neat pectin was “difficult to dissolve” with the hydrogels formed by the conjugates in the presence of small amount of $Ca^{2+}$ having higher shear moduli than pure pectin due to ion binding ability of flavonoids promoting further crosslinking of the conjugates.

> “These results”, the researchers concluded, “reveal that the conjugation creates an antioxidant property in the pectin macromolecule, which can also create new application areas for pectin” (Ahn et al., 2017).

### 2,2. Pectin hydroxyls activation using DCC

In 2019 a joint team based in India and South Africa reported the conjugation of pectin to naringenin with the aim “to enhance its therapeutic efficacy” (Mundlia et al., 2019). In brief, naringenin was conjugated to an expensive high methoxyl (HM) commercial citrus pectin specifically commercialized for wound healing bandages, ostomy bandages, pharmaceutical emulsions, and pharmaceutical suspensions. The activated –COOH functionality of pectin interacted with –OH group of naringenin to form the Pec-Nrg conjugate. In detail, pectin (500 mg) was added to DMSO (50 mL) to form a dispersion kept under stirring at room temoerature. The carboxylic groups of polymer were activated using *N, N′*-dicyclohexylcarbodiimide (DCC, 80 mg) while dimethylaminopyridine (DMAP, 30 mg) was added as a coupling agent to the pectin dispersion, and stirred for another 2 h. A 10 mL solution of naringenin in DMSO (25 mg/mL) was slowly added under $N_2$ atmosphere to the activated polymer dispersion and stirred for 6 h at 60–65 °C. The solution obtained was subjected to dialysis against DMSO for 12 h to remove the unreacted molecules followed by dialysis against distilled water for 72 h to remove the solvent. The dialyzed polymer-drug conjugate mixture was frozen at − 75 °C for 4 h prior to lyophilization. Chromatographic analysis returned a 37.069 mg/g load of naringenin in the conjugate obtained after lyophilization.

Molecular mechanistic simulations performed for pectin and naringenin revealed the energetic stability of the conjugate. In detail, computing the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, the team found that the conjugation of naringenin to pectin is accompanied by $\Delta E_{\text{form}}$ of $\approx -18$ kcal/mol confirming favorable and energetically stable conjugation. The bioconjugate is stabilized primarily by the non-bonding energy terms: electrostatic interactions ( − 18.642 kcal/mol) and van der Waals forces (− 9.680 kcal/mol).

The team also showed that release of naringenin from the conjugate obeys Higuchi’s square root kinetics, with diffusion being the primary mechanism of release. At pH 1.2, nearly all (99.4 %) narigengenin was released over a period of 30 h, whereas at pH 7.4 <<60 % (57.62 %) flavonoid was released in 48 h. The synthesized conjugate showed anticancer activity against NIH: OVCAR-5 cells. In case of control and pectin, the cells showed spindle formation and good adherence on the culture plate. For Pec-Nrg conjugate, the cells showed early detachment, spherical cells, and cell death was evident confirming the anti-cancer potential of the Pec-Nrg conjugate.

Comparative evaluation of the antioxidant activity revealed a significantly higher radical scavenging activity of conjugate over unmodified naringenin. Furthermore, the conjugate exhibited significantly higher antimicrobial action against *Staphylococcus aureus, Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* while a comparable antimicrobial activity was observed against *Escherichia coli* and *Bacillus subtilis*.

In detail, the Pec-Nrg conjugate against *Staphylococcus aureus* (MTCC 7443), *Staphylococcus epidermis* (MTCC 435), *Bacillus subtilis* (MTCC 441), *E. coli* (MTCC 1652), *Pseudomonas aeruginosa* (MTCC 424), exhibited minimum inhibitory concentration (MIC) values of 2.5, 12, 11.5, 12.5, 12.5 μg/mL in comparison to 6.5, 24.5, 12.5, 12.5, 23.5 μg/mL of naringenin, respectively. In the case of commercial pectin no inhibition of microbial growth was observed. Both enhanced antibacterial and anti-cancer activities of amphiphilic Pec-Nrg conjugate over the lipophilic naringenin could be attributed to the greater aqueous solubility and penetration of the colloidal Pec-Nrg conjugate over the hydrophobic naringenin.

### *2.3 Free radical route using $H_2O_2$ and ascorbic acid*

In 2022, Aiello and co-workers in Italy reported remarkable biological performance for quercetin-pectin conjugates successfully synthesized (Fig. 1) by chemical modification of the flavonoid **1** protected with α,α-dichlorodiphenylmethane, followed by reaction of quercetin protected as 3′,4′-diphenylmethylketal derivative **2** with docosahexaenoic acid (Carullo et al., 2022). Pectin conjugation with the quercetin derivatives **2** and **3** by radical-based grafting using ascorbic acid and hydrogen peroxide afforded flavonoid-pectin conjugates P_2 and P_3.

Photographs and plots in Fig. 2 show that P_2 and P_3 conjugates reduced the migration of Caco-2 and HepG2 intestinal cancer cell lines, while the blank polymer (PB) synthesized with pectin undergoing the same reaction but without the quercetin derivatives, had no effect. Similarly, the outcomes of treatment with increasing concentrations of PB, P_2, and P_3 clearly indicate a dose-dependent reduction in cell viability after treatment with P_2 and P_3 (particularly for the P_3 conjugate) whereas treatment with PB did not produce any effect.

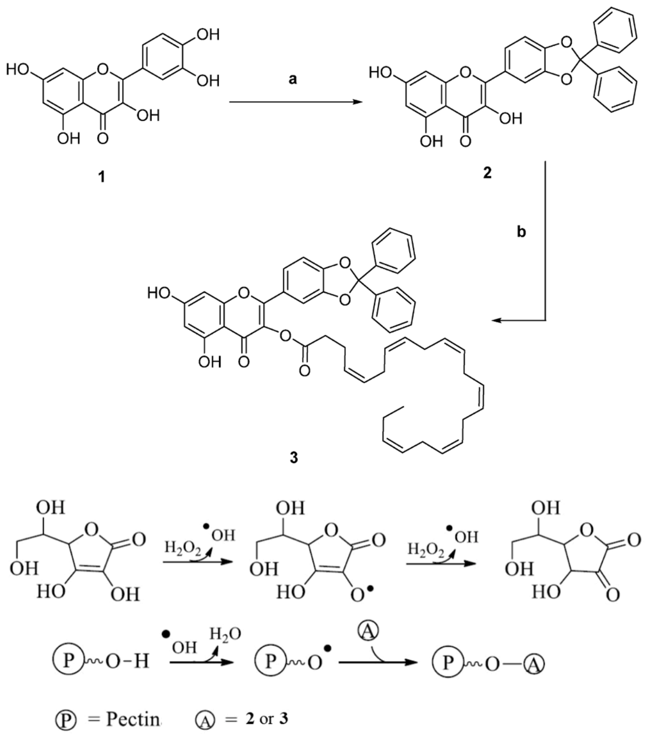

**Fig. 1.** . Top: reagents and conditions, (a) α,α-dichlorodiphenylmethane, 180 °C, 10 min; (b) cervonic acid, *N,N*'-dicyclohexylcarbodiimmide, dry dichloromethane, 0 °C to RT, 24 h; bottom: Schematic representation of the synthesis of the macromolecular conjugate. [Copyright: The Authors. Reproduced from Carullo et al., 2022, Creative Commons CC BY 4.0 Attribution License].

The team concluded that the covalent binding of the quercetin derivative to the pectin chain offers a suitable alternative strategy to improve the bioavailability of the quercetin thanks to a versatile and highly biocompatible polysaccharide, improving also protection of the conjugated flavonoid molecule.

In 2023 Tian and co-workers in China reported grafting of hesperetin (HT) on citrus pectin sourced from citrus canning processing water, namely a pectin rich in RG-I branched regions (Shen et al., 2021), via free radical-induced reaction employing green reactants only, including $H_2O_2$ and ascorbic acid to generate ascrobate and pectin radical species, and EtOH to precipitate the modified pectin (Ren et al., 2023). The latter route enables binding of HT to the hydroxyl functions of the GalA monomers of the pectin molecule, until most hydroxyl groups were derivatized. The conjugate showing the highest HT content had a flavonoid content exceeding 103 mg/g.

Evidence of successful grafting was obtained from the UV and FTIR spectra. Pectin showed no absorption peak in the UV–vis wavelenght range of 200–400 nm while hesperetin showed two characteristic peaks at 225 nm and 280 nm. Accordingly, the PB-HT conjugates showed two characteristic absorption peaks at 230 and 288 nm, with the UV absorption peak of the PB-HT conjugates showing a slight red shift, due to the covalent bond between hesperetin and pectin. In agreement with higher grafting ratio, the intensities of the two bands (~230 and 288 nm) of PB-HT-0.5 was higher than those in the other two PB-HT conjugates. The FTIR spectra of PB-HT conjugates clearly showed the two absorption peaks at 1307 $cm^{-1}$ and 1504 $cm^{-1}$ typical of aromatic ring *C* = *C* stretching, with the broad absorption peak at 3424 $cm^{-1}$ due to -OH vibration modes narrowed, due to less extensive hydrogen bonding.

Each bioconjugate, including PB-HT-0.3 (57.3 mg/g) and PB-HT-0.8 (39.98 mg/g) showed excellent cytocompatibility when tested against L929 fibroblast cells, and good hemocompatibility with hemolysis rate being almost zero when placed in contact with blood. The team evaluated the antibacterial activity of the conjugates against *Staphylococcus aureus*. B: *E. coli* and found that whereas pectin did not inhibit the activity of *E. coli* and *S. aureus*, hesperidin and the flavonoid-pectin bioconjugates did. In the case of e bioconjugate with the highest contents of hesperetin (PB-HT-0.5, 103.18 mg/g) the antibacterial activity of the conjugate was comparable to that of HT.

### *2.4 Enzyme-mediated conjugation*

In late 2024, Zheng and co-workers in China reported the lipase-mediated enzymatic synthesis of two citrus flavonoid-pectin conjugates of high flavonoid load (hesperidin, HD: 20.21 %; naringin dihydrochalcone, ND: 16.11 %) (Liu et al., 2025). In detail, commercial citrus pectin dissolved in water (3.3 %, w/v) was mixed with a solution of each flavonoid (HD or ND; 7.5 or 30 mmol/L) in tetrahydrofuran. A small amount (0.2 g) of lipase added was enough to promote the esterification reaction at 50 °C for 24 h. Each solution was then stirred, evaporated to dryness, and the flavonoid-pectin conjugate solution freeze-dried. The HD-pectin and ND-pectin conjugates in powder form obtained (HDCP and NDCP) with medium and high grafting rates were tagged with -M and -H, respectively. Successful conjugation was confirmed by proton NMR spectroscopy (Fig. 3).

Comparison of the area of the 3.73 ppm peak, corresponding to $OCH_3$ groups of GalA in pectin (Hu et al., 2021), with the total areas of all hydrogen peaks (including peaks at 5.01, 3.64, 3.93, 4.10, and 4.38 ppm corresponding to H-1, H-2, H-3, H-4, H-5) unveiled ester bond formation between pectin GalA moieties and flavonoids because the peak areas of the flavonoid-pectin conjugates (HDCP-H: 33.8 %, NDCP-H: 36.7 %) were lower than the peak area of citrus pectin (CP, 40.7 %) due to lower methyl content.

Conjugation with hydrophobic flavonoids makes the resulting flavonoid-pectin conjugates amphiphilic, thereby improving the

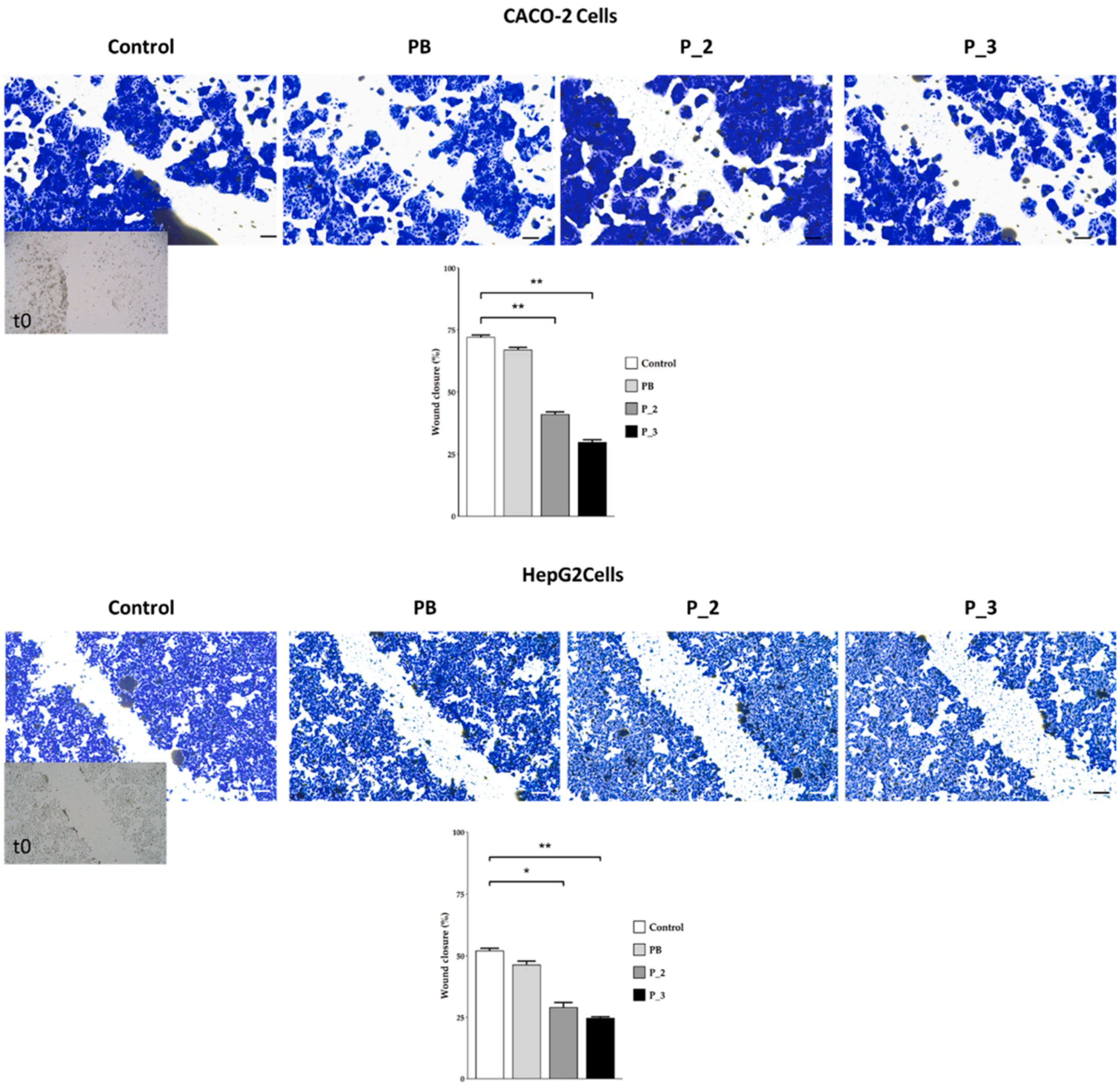

**Fig. 2.** A wound-healing assay on Caco-2 and HepG2 cells after treatment with of PB, P_2, and P_3 (50 μg $mL^{-1}$). The percentage of closure was quantified by ImageJ after 24 h. Scale bars: 100 $\mu$m, * $p < 0.01$; ** $p < 0.005$ treated *vs.* control. [Copyright: The Authors. Reproduced from Carullo et al., 2022, Creative Commons CC BY 4.0 Attribution License].

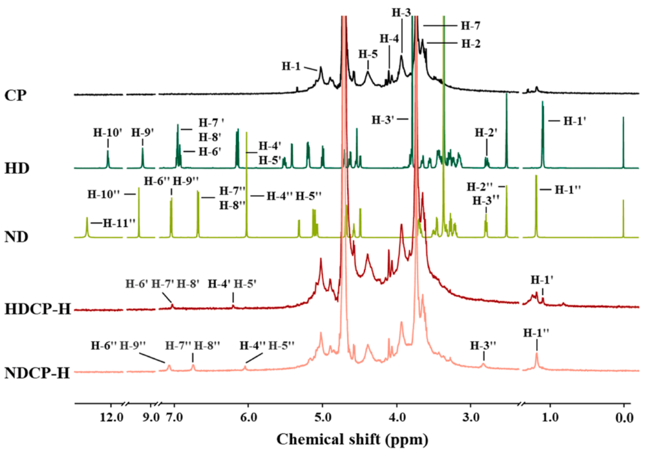

**Fig. 3.** 1. H NMR spectra of citrus pectin, flavonoids, and flavonoid-pectin conjugates. [Copyright 2025, Elsevier. Reproduced from Liu et al., 2025, with kind permission].

emulsifying properties thanks to lower interfacial tension at the oil-water interface between corn oil and water in the presence of flavonoid-pectin conjugates (from 20.6 to 18.4 mN/m) and increased adsorbed layer thickness (from 11.8 to 37.8 nm). Plots of the interfacial tensions show that all tensions gradually decreased with increasing adsorption time.

The conjugate with the highest amount of hesperetin HDCP-H most effectively reduced interfacial tension, followed by NDCP-H, HDCP-M, NDCP-M, and CP. Thanks to the higher number of methoxy groups in the benzene rings of HD compared to ND, reduction in interfacial tension afforded by HDCP was larger than that of NDCP leading to even smaller droplet size and better emulsion stability.

As mentioned above, the team was first to identify and report the presence of natural flavonoid-pectin conjugates in the peel of mandarin (*Citrus reticulata*). The analysis used alkaline hydrolysis combined with ultra-high performance liquid chromatography. In detail, the team undertook alkaline hydrolysis of citrus peel powder dissolved in 80 % (v/v) methanol and sonicated for 15 min, followed by centrifugation. This process was repeated three times to remove free flavonoids. Natural flavanoid peel was extracted from the residue into 0.8 % (w/v) $(NH_4)_2C_2O_4$ at 80 °C for 1 h, followed by precipitation with 95 % ethanol. The precipitate was dried by freeze-drying. Out of the nine substances identified, HD and ND were the main components in natural flavonoid peel, respectively with 2.64 and 3.14 mg/g.

One month before the publication of the aforementioned experimental work in which natural flavonoid-pectin conjugates were isolated from the peel of mandarin (Liu et al., 2025), the teams of Butera and Pagliaro reported in a study in preprint form the outcomes of density functional theory (DFT) calculations of modeling IntegroPectin derived via hydrodynamic or acoustic cavitation of citrus industrial processing waste from the main citrus fruits (orange, lemon and grapefruit) (Butera et al., 2024). IntegroPectin was modeled as a dimer in which one galacturonic unit is linked to a rhamnose molecule representing the RG-I hairy regions thereby forming the "Pec-RGI" structure selecting for each citrus fruit IntegroPectin the most abundant flavonoid (naringenin for grapefruit, hesperidin for orange, eriocitrin for lemon).

Formation of IntegroPectin-flavonoid (Pec-RGI-Fla) bioconjugates was found to be endergonic for all the three bioconjugates. The values of $\Delta G_{form}$ are low, ranging from 3.3 to 4.9 kcal/mol. Accordingly, the large amount of energy released upon the implosion of the cavitation bubbles during the natural product extraction is largely enough to overcome the slightly positive $\Delta G_{form}$ required for the formation of said conjugates.

The optimized structures of the corresponding conjugates in Fig. 4, show that for both naringenin and eriocitrin conjugates, the pectin derivates are rotated to form a hydrogen bond between the O1 oxygen of the RGI monomer and the H1 of the flavonoid units, whose calculated distances are identical, amounting to 2.24 Å. On the other hand, Fig. 4c shows that in Pec-RGI-Hes the O1 of the RGI monomer establishes a hydrogen bond of 2.22 Å with H2 of hesperidin (Butera et al., 2025).

### 2.5 Cavitation

In 2019, the teams of Pagliaro and Meneguzzo reported the isolation of a new citrus pectin sourced from red orange processing waste (the agro-industrial waste residue of citrus juice production) via hydrodynamic cavitation conducted in water only directly on semi-industrial scale (Meneguzzo et al., 2019). Colored in orange, the resulting pectin is a low methoxyl (LM) pectin with degree of methylation of about 17 %. The subsequent year, the team reported the exceptional antioxidant, non-cytotoxic activity of a pectin sourced using the same process from industrial lemon processing waste (Nuzzo et al., 2020). This new class of citrus pectins sourced via cavitation of CPW conducted in water only was thus named "IntegroPectin".

Subsequent *in vitro* and *in vivo* investigations will show that IntegroPectin sourced from all main citrus (orange, red orange, bitter orange, lemon, grapefruit, and mandarin) fruits both via hydrodynamic or acoustic cavitation of CPW in water only (Ciriminna et al., 2024), has broad scope bioactivity, including antioxidant, anti-inflammatory, cardioprotective, anti-apoptotic, immunomodulatory, neuroprotective, mitoprotective, antimicrobial and anticancer properties (R. Ciriminna et al., 2025).

Numerous studies suggest a synergistic mode of action for which enhanced, broad scope bioactivity would be due to: *i*) the low degree of methylation 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) (R. Ciriminna et al., 2025). A photograph of bitter orange (*Citrus aurantium*) peel and red orange (*Citrus sinensis*) IntegroPectin after extraction with aqueous EtOH under sonication for 15 min shows (Fig. 5) that both IntegroPectin samples retained their orange color even after the ultrasound-assisted extraction, allowing to visualize that these pectins are bioconjugates with high loads of chemically bound flavonoids (Scurria et al., 2022).

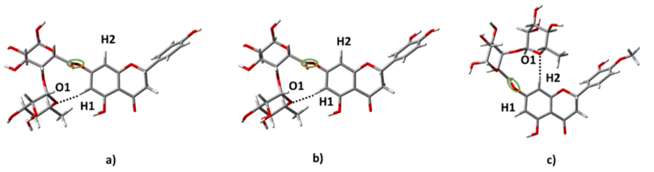

**Fig. 4.** Optimized structures of pectin conjugates with naringenin (a), eriocitrin (b) and hesperidin (c). [Copyright: The Authors, 2025. Reproduced from Butera et al., 2025, Creative Commons CC BY-NC—ND 4.0 License].

Concerning the mechanism of conjugation, it is relevant that both hydrodynamic and acoustic cavitation of water are well-known to form hydroxyl (OH) radicals (De-Nasri et al., 2022), that in the presence of ascorbic acid and citric acid abundant in citrus processing waste (CPW) likely give place to a radical conjugation mechanism analogous to that based on ascorbic acid and $H_2O_2$ to functionalize polysaccharides with polyphenols introduced by Liu and co-workers in 2013 (Liu et al., 2013).

The DRIFT spectra in Fig. 6 (500–2000 cm$^{-1}$ region) show the typical absorption peaks of pectin, with differences in the relative intensities and frequencies of some fingerprint bands. The 950–1200 cm-$^{1}$ region contains a set of very intense bands typical of pectin, partially overlapped, assigned to skeletal stretching modes ($\nu$C—C and $\nu$C—O-C) of the pyranose rings and of the glycosidic bonds, and to a combination of the $\nu$C—OH and $\nu$C—C modes from the pyranose rings.

The 500–950 cm$^{-1}$ region contains the bands related to the external deformation vibrations of methyl, methylene, and methine groups ($\rho CH_x$ and $\delta_C$—H). Both IntegroPectin conjugates are rich in smooth HG regions, with bitter orange IntegroPectin having a substantially higher share of HG regions (71 % *vs.* 63 %). This points to higher quality of red ("blood") orange IntegroPectin not only because the latter is a valued LM pectin able to form gels electrostatically, in the absence of sugar and in a broad pH range (2–7) in the presence of a small amount of divalent cations such as Ca$^{2}$, but also because pectin molecules richer in hairy, branched RG-I chains have significantly higher bioactivity (Niu et al., 2023).

**Fig. 5.** Bitter orange IntegroPectin (left) and red orange IntegroPectin (right) after flavonoid extraction with aqueous EtOH at room temperature. [Copyright: The Authors, 2022. Reproduced from Scurria et al., 2022, Creative Commons CC BY 4.0 Attribution License].

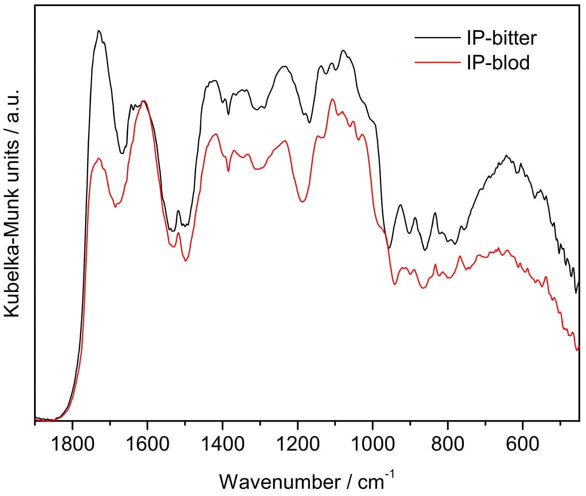

**Fig. 6.** DRIFT spectra of red orange and bitter orange IntegroPectin samples in the 500–2000 cm$^{-1}$ region, normalized to the $\nu_{as}COO^{-}$ band carboxylate groups, at ~1610 cm$^{-1}$. [Copyright: The Authors. Reproduced from Scurria et al., 2022, Creative Commons CC BY 4.0 Attribution License].

## 3. Practical aspects

Reviewing in 2021 the structure and properties of novel pectin-based materials obtained by chemical modifications of pectin via covalent linkage in the previous decade, Heinze and co-workers concluded that pectin is a very important yet underestimated biopolymer resource (Würfel et al., 2021). Things, however, are rapidly changing with the advent of new green extraction methods generally afford pectin of vastly enhanced quality (lesser degradation) with full valorization of citrus processing waste (Satari & Karimi, 2018).

Said new green extraction routes such as microwave-assisted extraction (Ciriminna et al., 2016), and cavitation (acoustic or hydrodynamic) of CPW conducted in water only (the CytroCav process, Ciriminna et al., 2024), are suitable to afford standardized pectin-based extracts processing each time the same amount of CPW. In the latter case, this was recently shown using the same process parameters (amount of CPW, amount of water, temperature, applied energy, extraction time, etc.), including dyring process conditions, from different batches of freshly harvested and processed red orange fruits (Vesci et al., 2025).

Besides new green extraction routes, also the pectin conjugation routes have been substantially improved and made sustainable. Conjugation syntheses have evolved from the use of highly toxic epichlorohydrin (Ahn et al., 2017), to the use of environmentally friendly reactants such as aqueous $H_2O_2$ and vitamin C (Carullo et al., 2022; Ren et al., 2023). Said methods allow extensive derivatization of pectin's hydroxyls with flavonoids enhancing the low oral bioavailability of flavonoids (Chen et al., 2022), mitigating the detrimental effect on bioavailability due to the larger molecular structure.

Finally, the large availability of citrus fruits widely 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, ensures similarly large abundance of both citrus pectin and citrus flavonoids (Zhong & Nicolosi, 2020). This makes the cost of producing pectin-flavonoid bioconjugates largely reliant on the route employed to afford them. Both the radical route employing ascorbic acid and aqueous hydrogen peroxide (Carullo et al., 2022; Ren et al., 2023), and the cavitation-based "CytroCav" process afford high yields of said bioconjugates and lack of toxic effluent formation.

## 4. Conclusions

In conclusion, reviewing research on citrus flavonoid-pectin conjugates reveals that the synthesis of citrus flavonoid-pectin bioconjugates may employ synthetic organic chemistry, enzymatic, free radical or cavitation-based routes affording in each case bioconjugates whose bioactivity largely exceeds that of unmodified citrus pectin. In general, furthermore, the binding of flavonoids to pectin polysaccharide chain partially disrupts the inter- and intra-molecular hydrogen bonds of pectin, affording amorphous flavonoid-pectin conjugates of pronounced water solubility, and amphiphilicity.

This study, the first to review a class of relatively new bioconjugate compounds, will hopefully be useful to inform forthcoming pre-clinical and clinical studies aimed at evaluating the effect of newly developed citrus flavonoid-pectin conjugates in the treatment and prevention of a number of ailments, including cancer, neurodegenerative and metabolic diseases, and atherosclerosis.

## Funding

R.C. and M.P. thank the Ministero dell'Università e della Ricerca for funding, Progetto "FutuRaw", Le materie prime del futuro da fonti non-critiche, residuali e rinnovabili, Fondo Ordinario Enti di Ricerca, 2022, CNR (CUP B53C23008390005). Work of G.L.P. was financially supported by the Made in Italy - Circular and Sustainable (MICS) Extended Partnership funded by the European Union NextGenerationEU (PNRR - Missione 4, Componente 2, Investimento 1.3 – D.D. 1551.11–10–2022, PE00000004). Work of G.A. was financially supported by the SAMOTHRACE (Sicilian Micro and Nano Technology Research and Innovation Center) Innovation Ecosystem funded by the European Union NextGenerationEU (PNRR – Mission 4 Component 2 - Investment 1.5 (ECS00000022) - CUPB63C22000620005).

## Ethical statement

No animal or human subjects were involved in this review study.

## CRediT authorship contribution statement

**Caterina Di Sano:** Writing – review & editing, Methodology, Conceptualization. **Claudia D'Anna:** Writing – review & editing, Methodology, Conceptualization. **Giovanna Li Petri:** Validation, Investigation, Data curation. **Giuseppe Angellotti:** Validation, Investigation, Data curation. **Francesco Meneguzzo:** Writing – review & editing, Visualization, Conceptualization. **Rosaria Ciriminna:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Mario Pagliaro:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The Authors declare no competing financial interest.

## Data availability

No data was used for the research described in the article.

## References

Alam, F., Mohammadin, K., Shafique, Z., Amjad, S. T., & Hassan bin Asad, M. H. (2022). Citrus flavonoids as potential therapeutic agents: A review. *Phytotherapy Research, 36*, 1417. https://doi.org/10.1002/ptr.7261

Ahn, S., Halake, K., & Lee, J. (2017). Antioxidant and ion-induced gelation functions of pectins enabled by polyphenol conjugation. *International Journal of Biological Macromolecules, 101*, 776–782. https://doi.org/10.1016/j.ijbiomac.2017.03.173

Assini, J. M., Mulvihill, E. E., & Huff, M. W. (2013). Citrus flavonoids and lipid metabolism. *Current Opinion in Lipidology, 24*, 34–40. https://doi.org/10.1097/mol.0b013e32835c07fd

Butera, V., Ciriminna, R., Valenza, C., Li Petri, G., Angellotti, G., Barone, G., Meneguzzo, F., Di Liberto, V., Bonura, A., & Pagliaro, M. (2024). Citrus IntegroPectin: A computational insight. *ChemRxiv*. https://doi.org/10.26434/chemrxiv-2024-hs24g

Butera, V., Ciriminna, R., Valenza, C., Li Petri, G., Angellotti, G., Barone, G., Meneguzzo, F., Di Liberto, V., Bonura, A., & Pagliaro, M. (2025). Citrus IntegroPectin: A computational insight. *Discover Molecules, 2*, 6. https://doi.org/10.1007/s44345-025-00013-z

Bou Daher, F., & Braybrook Siobhan, A. (2015). How to let go: Pectin and plant cell adhesion. *Frontiers in Plant Science, 6*, 523. https://doi.org/10.3389/fpls.2015.00523

Carullo, G., Spizzirri, G., Malivindi, R., Rago, V., Motta, M. F., Lofaro, D., Restuccia, D., & Aiello, F. (2022). Development of quercetin-DHA ester-based pectin conjugates as new functional supplement. Effects on cell viability and migration. *Nutraceuticals, 2*, 278–288. https://doi.org/10.3390/nutraceuticals2040021

Chen, L., Cao, H., Huang, Q., Xiao, J., & Teng, H. (2022). Absorption, metabolism and bioavailability of flavonoids: A review. *Critical Reviews in Food Science and Nutrition, 62*, 7730–7742. https://doi.org/10.1080/10408398.2021.1917508

Ciriminna, R., Fidalgo, A., Scurria, A., Ilharco, L. M., & Pagliaro, M. (2022). Pectin: New science and forthcoming applications of the most valued hydrocolloid. *Food Hydrocolloids, 127*, Article 107483. https://doi.org/10.1016/j.foodhyd.2022.107483

Ciriminna, R., Li Petri, G., Angellotti, G., Luque, R., Fabiano Tixier, A.-S., Meneguzzo, F., & Pagliaro, M. (2025a). Citrus flavonoids as antimicrobials. *Chemistry & Biodiversity, 22*, Article e202403210. https://doi.org/10.1002/cbdv.202403210

Ciriminna, R., Angellotti, G., Li Petri, G., Meneguzzo, F., Riccucci, C., Di Carlo, G., & Pagliaro, M. (2024). Cavitation as a zero-waste circular economy process to convert citrus processing waste into biopolymers in high demand. *Journal of Bioresources and Bioproducts, 9*, 486. https://doi.org/10.1016/j.jobab.2024.09.002

Ciriminna, R., Di Liberto, V., Valenza, C., Li Petri, G., Angellotti, G., Albanese, L., Meneguzzo, F., & Pagliaro, M. (2025b). Citrus IntegroPectin: A multifunctional bioactive phytocomplex with large therapeutic potential. *Chem Food Chem 1.* , Article e00014. https://doi.org/10.1002/cfch.202500014

Ciriminna, R., Fidalgo, A., Carnaroglio, D., Cravotto, G., Grillo, G., Tamburino, A., Ilharco, L. M., & Pagliaro, M. (2016). Eco-friendly extraction of pectin and essential oils from orange and lemon peels. *ACS Sustainable Chemistry & Engineering, 4*, 2243–2251. https://doi.org/10.1021/acssuschemeng.5b01716

David, N. (2023). Pectin in tissue engineering. In R. Jayakumar, & V. P. Murali (Eds.), *Natural biopolymers in drug delivery and tissue engineering* (pp. 609–626). Sawston (Great Britain): Woodhead Publishing. https://doi.org/10.1016/B978-0-323-98827-8.00003-5. Ed.s.

De-Nasri, S. J., Sarvothaman, V. P., Nagarajan, A., Manesiotis, P., Robertson, P. K. J., & Ranade, V. V. (2022). Quantifying OH radical generation in hydrodynamic cavitation via coumarin dosimetry: Influence of operating parameters and cavitation devices. *Ultrasonics Sonochemistry, 90*, Article 106207. https://doi.org/10.1016/j.ultsonch.2022.106207

Ebrahimi, F., Mahdi Ghazimoradi, M., Fatima, G., & Bahramsoltani, R. (2023). Citrus flavonoids and adhesion molecules: Potential role in the management of atherosclerosis. *Heliyon, 9*, Article e21849. https://doi.org/10.1016/j.heliyon.2023.e21849

Elmeligy, S., Hathout, R. M., Khalifa, S. A. M., El-Seedi, H. R., & Farag, M. A. (2021). Pharmaceutical manipulation of citrus flavonoids towards improvement of its bioavailability and stability. A mini review and a meta-analysis study. *Food Bioscience, 44*, Article 101428. https://doi.org/10.1016/j.fbio.2021.101428

Google Scholar. (2025). earch conducted at scholar.Google.Com using the boolean query "citrus flavonoid" + pectin + conjugate. S. August 2025.

Gupta, A., Jamal, A., Jamil, D. A., & Al-Aubaidy, H. A. (2023). A systematic review exploring the mechanisms by which citrus bioflavonoid supplementation benefits blood glucose levels and metabolic complications in type 2 diabetes mellitus. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 17*, Article 102884. https://doi.org/10.1016/j.dsx.2023.102884

Hu, W., Chen, S., Wu, D., Zhu, K., & Ye, X. (2021). Physicochemical and macromolecule properties of RG-I enriched pectin from citrus wastes by manosonication extraction. *International Journal of Biological Macromolecules, 176*, 332–341. https://doi.org/10.1016/j.ijbiomac.2021.01.216

Jalili, F., Moradi, S., Talebi, S., Mehrabani, S., Ghoreishy, S. M., Wong, A., Jalalvand, A. R., Kermani, M. A. H., Jalili, C., & Jalili, F. (2024). The effects of citrus flavonoids supplementation on endothelial function: A systematic review and dose–response meta-analysis of randomized clinical trials. *Phytotherapy Research, 38*, 2847–2859. https://doi.org/10.1002/ptr.8190

Liu, J., Lu, J.-f., Kan, J., & Jin, C.-h. (2013). Synthesis of chitosan-gallic acid conjugate: Structure characterization and *in vitro* anti-diabetic potential. *International Journal of Biological Macromolecules, 62*, 321–329. https://doi.org/10.1016/j.ijbiomac.2013.09.032

Liu, X., Wang, F., Wang, J., Liu, T., Zhao, C., & Zheng, J. (2025). Citrus flavonoid-pectin conjugates with enhanced emulsifying properties. *Food Hdrocolloids, 159*, Article 110675. https://doi.org/10.1016/j.foodhyd.2024.110675

Meneguzzo, F., Brunetti, C., Fidalgo, A., Ciriminna, R., Delisi, R., Albanese, L., Zabini, F., Gori, A., dos Santos Nascimento, L. B., De Carlo, A., Ferrini, F., Ilharco, L. M., & Pagliaro, M. (2019). Real-scale integral valorization of waste orange peel via hydrodynamic cavitation. *Processes, 7*, 581. https://doi.org/10.3390/pr7090581

Muhidinov, Z. K., Ikromi, K. I., Jonmurodov, A. S., Nasriddinov, A. S., Usmanova, S. R., Bobokalonov, J. T., Strahan, G. D., & Liu, L. (2021). Structural characterization of pectin obtained by different purification methods. *International Journal of Biological Macromolecules, 183*, 2227–2237. https://doi.org/10.1016/j.ijbiomac.2021.05.094

Musumeci, L., Maugeri, A., Russo, C., Lombardo, G. E., Cirmi, S., & Navarra, M. (2023). Citrus flavonoids and autoimmune diseases: A systematic review of clinical studies. *Current Medicinal Chemistryt, 30*, 2191–2204. https://doi.org/10.2174/0929867329666220629144744

Mundlia, J., Ahuja, M., Kumar, P., & Pillay, V. (2019). Improved antioxidant, antimicrobial and anticancer activity of naringenin on conjugation with pectin. *3 Biotech, 9*, 312. https://doi.org/10.1007/s13205-019-1835-0

Niu, H., Dou, Z., Hou, K., Wang, W., Chen, X., Chen, X., Chen, H., & Fu, X. (2023). A critical review of RG-I pectin: Sources, extraction methods, structure, and applications. *Critical Reviews in Food Science and Nutrition, 64*, 8911–8931. https://doi.org/10.1080/10408398.2023.2204509

Nuzzo, D., Cristaldi, L., Sciortino, M., Albanese, L., Scurria, A., Zabini, F., Lino, C., Pagliaro, M., Meneguzzo, F., Di Carlo, M., & Ciriminna, R. (2020). Exceptional antioxidant, non-cytotoxic activity of integral lemon pectin from hydrodynamic cavitation. *ChemistrySelect, 5*, 5066–5071. https://doi.org/10.1002/slct.202000375

Qiu, M., Wei, W., Zhang, J., Wang, H., Bai, Y., & Guo, D.-a. (2023). A scientometric study to a critical review on promising anticancer and neuroprotective compounds: Citrus flavonoids. *Antioxidants, 12*, 669. https://doi.org/10.3390/antiox12030669

Ropartz, D., & Ralet, M. C. (2020). Pectin structure. In V. Kontogiorgos (Ed.), *Pectin: Technological and physiological properties* (pp. 17–36). Cham (Switzerland): Springer. https://doi.org/10.1007/978-3-030-53421-9_2.

Ren, Y., Yu, D., Wu, J., Mao, S., Chen, P., Chen, S., Gao, Q., Ye, X., & Tian, J. (2023). Preparation and physicochemical properties characterization of hesperetin-grafted pectin conjugate. *International Journal of Biological Macromolecules, 243*, Article 124887. https://doi.org/10.1016/j.ijbiomac.2023.124887

Rubio-Senent, F., Bermúdez-Oria, A., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., & Fernández-Bolaños, J. (2025). Structural and antioxidant properties of hydroxytyrosol-pectin conjugates: Comparative analysis of adsorption and free radical methods and their impact on *in vitro* gastrointestinal process. *Food Hydrocolloids, 162*, Article 110954. https://doi.org/10.1016/j.foodhyd.2024.110954

Saffer, A. M. (2018). Expanding roles for pectins in plant development. *Journal of Integrative Plant Biology, 60*, 910–923. https://doi.org/10.1111/jipb.12662

Satari, B., & Karimi, K. (2018). Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. *Resources, Conservation and Recycling, 129*, 153–167. https://doi.org/10.1016/j.resconrec.2017.10.032

Scurria, A., Sciortino, M., Rosa Garcia, A., Pagliaro, M., Avellone, G., Fidalgo, A., … Ilharco, L. M. (2022). Red orange and bitter orange IntegroPectin: Structure and main functional compounds. *Molecules, 27*, 3243. https://doi.org/10.3390/molecules27103243

Seisun, D., & Zalesny, N. (2021). Strides in food texture and hydrocolloids. *Food Hydrocolloids, 117*, Article 106575. https://doi.org/10.1016/j.foodhyd.2020.106575

Shen, S., Cheng, H., Liu, Y., Chen, Y., Chen, S., Liu, D., Ye, X., & Chen, J. (2021). New electrolyte beverages prepared by the citrus canning processing water through chemical improvement. *Food Chem X, 12*, Article 100155. https://doi.org/10.1016/j.fochx.2021.100155

Vesci, L., Martinelli, G., Liu, Y., Tagliavento, L., Dell'Agli, M., Wu, Y., Soldi, S., Sagheddu, V., Piazza, S., Sangiovanni, E., & Meneguzzo, F. (2025). The new phytocomplex AL0042 extracted from red orange by-products inhibits the minimalhepatic encephalopathy in mice induced by thioacetamide. *Biomedicines, 13*, 686. https://doi.org/10.3390/biomedicines13030686

Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Ding, T., Ye, X., & Liu, D. (2016). Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids, 61*, 730. https://doi.org/10.1016/j.foodhyd.2016.06.019

Weber, A. M., Pascale, N., Gu, F., Ryan, E. P., & Respondek, F. (2025). Nutrition and health effects of pectin: A systematic scoping review of human intervention studies. *Nutrition Research Reviews, 38*, 306–323. https://doi.org/10.1017/S0954422424000180

Würfel, H., Geitel, K., Qi, H., & Heinze, T. (2021). Chemical modification of pectin and polygalacturonic acid: A critical review. *BioResources, 16*, 8457–8488. https://bioresources.cnr.ncsu.edu/resources/chemical-modification-of-pectin-and-polygalacturonic-acid-a-critical-review/.

Zdunek, A., Pieczywek, P. M., & Cybulska, J. (2021). The primary, secondary and structures of higher levels of pectin polysaccharides. *Comprehensive Reviews in Food Science and Food Safety, 20*, 1101. https://doi.org/10.1111/1541-4337.12689

Zhong, G., & Nicolosi, E. (2020). Citrus origin, diffusion, and economic importance. In A. Gentile, S. La Malfa, & Z. Deng (Eds.), *The citrus genome* (pp. 5–21). Cham: Springer. https://doi.org/10.1007/978-3-030-15308-3_2. Ed.s.