Online conference

15-30

October

# IntegroPectin: a new citrus pectin with uniquely high biological activity

The 2nd International Electronic Conference on Foods - "Future Foods and Food Technologies for a Sustainable World"
15-30 October 2021

**Mario Pagliaro**
CNR, Palermo, Italy

# Mario Pagliaro

- ✓ Research director at Italy's Research Council

- ✓ In 2021 elected ordinary member of Academia Europaea, in the chemical sciences class




mario.pagliaro@cnr.it
www.qualitas1998.net

# Research at our Lab is highly collaborative

- ✓ Research Group based in Sicily's Palermo collaborates with leading researchers in >20 countries
- ✓ All papers self-archived and freely available on our Lab website: qualitas1998.net


qualitas1998.net → Mario Pagliaro → Publications

**Mario Pagliaro's Lab Publications**

**Submissions to peer-reviewed journals and preprints in arXiv**

317. Rosaria Ciriminna, Mina Ghahremani, Babak Karimi, Mario Pagliaro, "Waste-free oxidation of alcohols at the surface of catalytic electrodes: what is required for industrial uptake?" *submitted* (2021).
316. Domenico Nuzzo, Antonino Scurria, Pasquale Picone, Alessandro Guiducci, Mario Pagliaro, Giuseppe Pantaleo, Lorenzo Albanese, Francesco Meneguzzo, Rosaria Ciriminna, "A gluten-free biscuit fortified with lemon IntegroPectin", *Research Square* (2021), https://doi.org/10.21203/rs.3.rs-752972/v1
315. Rosaria Ciriminna, Mina Ghahremani, Babak Karimi, Mario Pagliaro, "Emerging green routes to nanocellulose", *submitted* (2021).
314. Samar Al Jitan, Antonino Scurria, Lorenzo Albanese, Mario Pagliaro, Francesco Meneguzzo, Federica Zabini, Reem Al Sakkaf, Ahmed Yusuf, Giovanni Palmisano, Rosaria Ciriminna, "Nanocellulose from citrus processin using water and electricity only", *submitted* (2021). Preprint: https://doi.org/10.21203/rs.3.rs-534535/v1
313. Antonino Scurria, Marzia Sciortino, Lorenzo Albanese, Domenico Nuzzo, Federica Zabini, Francesco Meneguzzo, Rosa V. Alduina, Alessandro Presentato, Mario Pagliaro, Giuseppe Avellone, Rosaria Ciriminna, "Flavon Lemon and Grapefruit IntegroPectin", *submitted* (2021). Preprint: https://doi.org/10.20944/preprints202102.0620.v1
312. Mario Pagliaro, "Du snobisme: Alle radici della caduta dell'uomo moderno", *submitted* (2021). Preprint: https://doi.org/10.13140/rg.2.2.31127.19363
311. Francesco Meneguzzo, Lorenzo Albanese, Mario Pagliaro, "A novel greenhouse heating technology matching the mature stage photovoltaic era", *submitted* (2021).
310. Antonino Scurria, Pietro Genco, Mario Pagliaro, "Sicilian Cannoli of Enhanced Stability", *submitted* (2021). Preprint: https://doi.org/10.13140/rg.2.2.19766.86085
309. Babak Karimi, Mina Ghahremani, Rosaria Ciriminna, Mario Pagliaro, "Enhancing the activity of ABNO@PMO-IL-Br catalyzed the aerobic oxidation of alcohols and Self-Esterification of Primary Aliphatic Alcohols through Synergistic Relay Mechanism under Metal-Free Conditions", *submitted* (2021).
308. Francesco Meneguzzo, Rosaria Ciriminna, Lorenzo Albanese, Mario Pagliaro, "Italy 100% Renewable: A Suitable Energy Transition Roadmap", *arXiv*:1609.08380 [physics.soc-ph] http://arxiv.org/abs/1609.08380 Se at the URL: https://doi.org/10.13140/rg.2.2.14168.65285
307. Francesco Meneguzzo, Rosaria Ciriminna, Lorenzo Albanese, Mario Pagliaro, "The energy-population conundrum and its possible solution", *arXiv* (2016) 1610.07298. See also at the URL: http://doi.org/10.13140/rg.2.2.11717.60643

**Articles in peer-reviewed journals (Check it out also the journal covers) - Articles self-archived in "green" open access**

306. Domenico Nuzzo, Miriana Scordino, Antonino Scurria, Costanza Giardina, Francesco Giordano, Francesco Meneguzzo, Giuseppa Mudò, Mario Pagliaro, Pasquale Picone, Alessandro Attanzio, Stefania Raimondo, Rosar Ciriminna, Valentina Di Liberto, "Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells ", *International Journal of Molecular Sciences 22* (2021) 9368. https://doi.org/10.3390/ijms22179368
305. Rosaria Ciriminna, Antonino Scurria, Mario Pagliaro, "Microbial production of hyaluronic acid: the case of an emergent technology in the bioeconomy", *Biofuels, Bioproducts & Biorefining* 15 (2021) xx. in production https://doi.org/10.1002/bbb.2285
304. Babak Karimi, Mina Ghahremani, Hojatollah Vali, Rosaria Ciriminna, Mario Pagliaro, "Aerobic oxidation and oxidative esterification of alcohols through cooperative catalysis under metal-free conditions", *Chemical Communications* 57 (2021) xx https://doi.org/10.1039/d1cc02937a

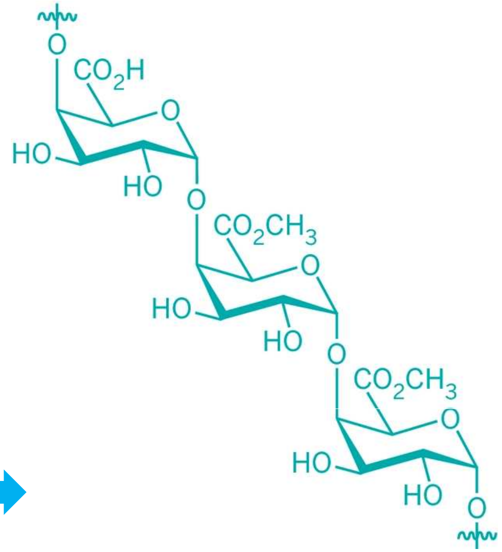


mario.pagliaro@cnr.it
www.qualitas1998.net

# Pectin: heteropolysaccharide ubiquitous in plant and fruit cell wall

- ✓ The structurally most complex polysaccharide from a structural point, plays a key role in ensuring cell adhesion

- ✓ Since the most abundant polysaccharide, about 65% of pectin, is the homogalacturonan (HG) chain made of galacturonic acid monomers linked with α-1,4 glycosidic bond and partially methyl esterified to the carboxylic C-6, the structure is often represented as in Figure




https://cen.acs.org/articles/91/i29/Pushing-Pectin.html

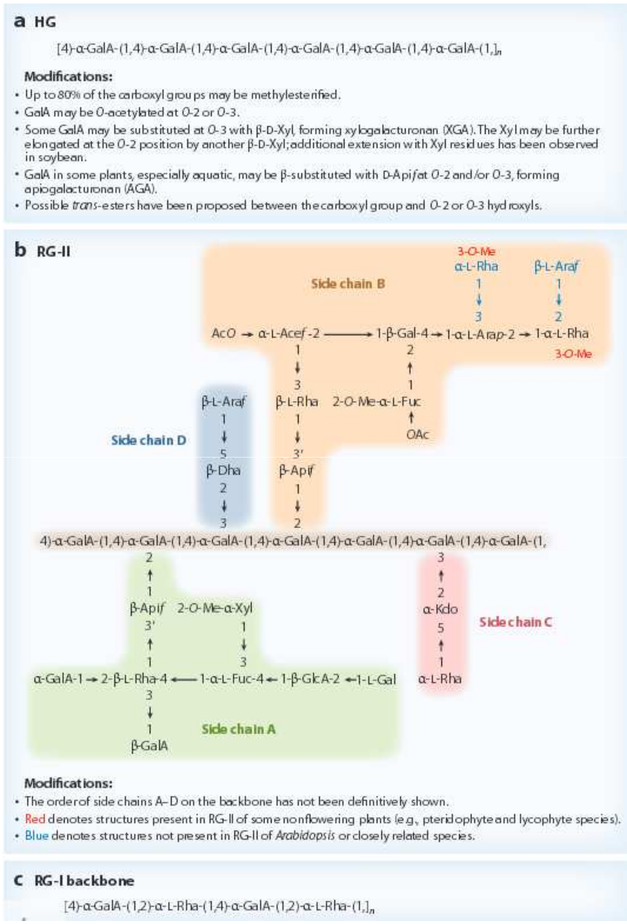



mario.pagliaro@cnr.it
www.qualitas1998.net

# Yet, pectin structure includes at least three bound polysaccharides

- ✓ Along with the HG chains alternate branched α(1−2)-l-rhamnosyl-α(1−4)-d-galacturonosyl chains substituted with side chains of mainly α-l-arabinofuranose and α-d-galactopyranose (known as rhamnogalacturonan-I chains, RG-I, about 20−35%) and rhamnogalacturonan-II (RG-II, 2-10%)

- ✓ The RG-II chain has a highly substituted HG skeleton with side chains A, B, C, D, containing 12 different sugars, with more than 20 different bonds .




Atmodjo, M. A., Hao, Z., and Mohnen, D. Evolving views of pectin biosynthesis. *Annu. Rev. Plant Biol.* 64 (2013) 747−779.

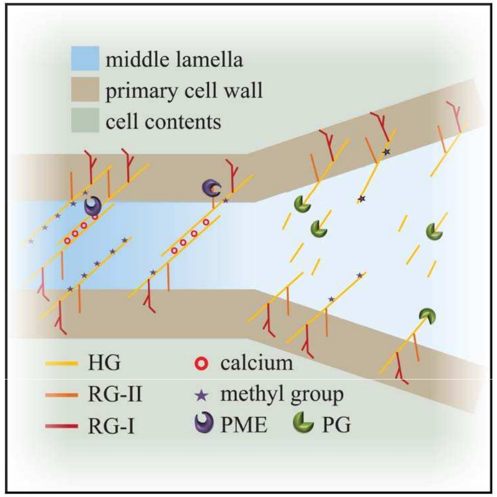



mario.pagliaro@cnr.it
www.qualitas1998.net

# The RG-I and RG-II chains play a more complex role than the HG chain

- ✓ **The role of the RG-I and RG-II chains in cell adhesion, much less studied, clearly suggests "specificities in pectin-mediated cell adhesion that extend beyond a simple story where pectin-equals-glue".**

- ✓ **The RG-II chain plays a critical role in plant growth and development.**




Daher Firas Bou, Braybrook Siobhan A., How to let go: pectin and plant cell adhesion , Frontiers in Plant 6 (2015) 523



mario.pagliaro@cnr.it
www.qualitas1998.net

# Natural hydrocolloid marketed since 1908

- ✓ Anne Frank's father Otto ran the Opekta factory in Amsterdam


Bottiglie Opekta in una mostra speciale su Anna Frank al Museo ebraico di Basilea




mario.pagliaro@cnr.it
www.qualitas1998.net

# The most expensive and versatile natural food-grade hydrocolloid

- ✓ According to recent estimates by Seisun, the price that in 2020 reached $ 18 / kg and production approached 70,000 tons

- ✓ The food industry cannot do without it, for a variety of products starting from drinkable yoghurt

**Food Hydrocolloid Market 2020**

| Hydrocolloid | '000 TPA | $ Million | $/kg |
|---|---|---|---|
| Gelatin | 219 | 1,592 | 7.26 |
| Starches | 1,666 | 1,400 | 0.84 |
| Pectin | 70 | 1,252 | 17.82 |

Dennis Seisun, Nesha Zalesny,, Strides in food texture and hydrocolloids, *Food Hydrocolloids*, 117 (2021) 106575,



mario.pagliaro@cnr.it
www.qualitas1998.net

# Market structure

- ✓ **Strong growth of the $ 1.3 billion market. Price more than doubled in 10 years. Historic oligopoly dominated by 7 companies from Western Europe and North America**

- ✓ **The first plant based on acid hydrolysis has been located in Redon in France since 1941. Still operating today. In the former socialist countries it was produced in Poland and in the USSR**

FUNCTIONAL INGREDIENTS



ROSARIA CIRIMINNA[1], ALEXANDRA FIDALGO[2], RICCARDO DELISI[1], LAURA M. ILHARCO[2], MARIO PAGLIARO*[1]

*Corresponding author

1. Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy

2. Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal


## Pectin production and global market


KEYWORDS: Pectin, waste citrus peel, biopolymer, biorefinery, bioeconomy.



Abstract Pectin is a valued hydrocollid with multiple functional properties applied in the food, cosmetic and pharmaceutical industries. In this study we describe pectin, its properties and sources, and identify the main market trends, drivers, and open opportunities.


Rosaria Ciriminna, Alexandra Fidalgo, Riccardo Delisi, Laura M. Ilharco, Mario Pagliaro, "Pectin Production and Global Market", *Agro FOOD Industry Hi Tech* 27(5) (2016) 17-20

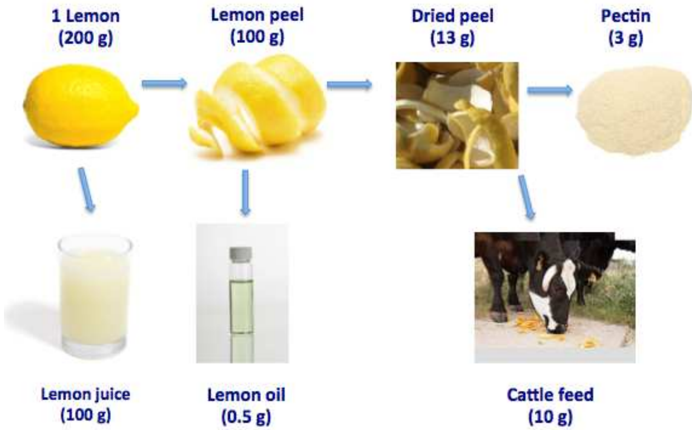



mario.pagliaro@cnr.it
www.qualitas1998.net

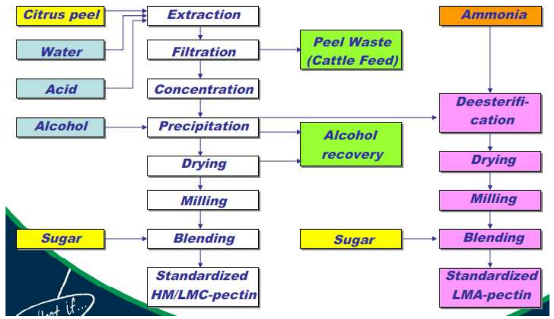
# Production process

- ✓ Produced from dried or fresh lemon, orange and lime peels (and, to a lesser extent, from apple pomace). Industrial process based on prolonged acid hydrolysis with mineral acid in hot water (70-80 C) followed by precipitation with isopropyl alcohol

- ✓ In addition to generating large amounts of acidic water, the process strongly degrades the natural structure of pectin




Adapted from J. Staunstrup (CP Kelko), Citrus Pectin Production and World Market, The International Citrus & Beverage Conference, September 17, 2009.

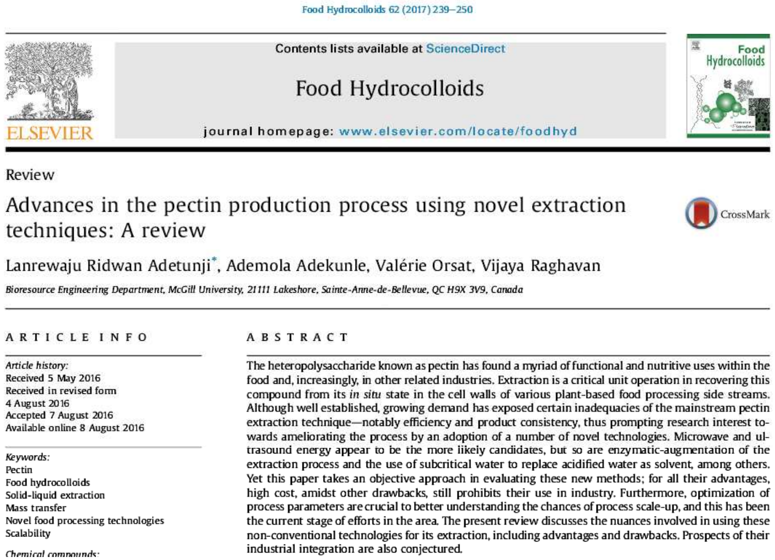





mario.pagliaro@cnr.it
www.qualitas1998.net

# Tens of alternative production methods proposed

- ✓ "Numerous alternative technologies have been proposed for pectin extraction, but none have been commercialized"


Food Hydrocolloids 62 (2017) 239–250

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Review

Advances in the pectin production process using novel extraction techniques: A review

Lanrewaju Ridwan Adetunji*, Ademola Adekunle, Valérie Orsat, Vijaya Raghavan

*Bioresource Engineering Department, McGill University, 21111 Lakeshore, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada*

ARTICLE INFO

*Article history:*
Received 5 May 2016
Received in revised form
4 August 2016
Accepted 7 August 2016
Available online 8 August 2016

*Keywords:*
Pectin
Food hydrocolloids
Solid-liquid extraction
Mass transfer
Novel food processing technologies
Scalability

*Chemical compounds:*

ABSTRACT

The heteropolysaccharide known as pectin has found a myriad of functional and nutritive uses within the food and, increasingly, in other related industries. Extraction is a critical unit operation in recovering this compound from its *in situ* state in the cell walls of various plant-based food processing side streams. Although well established, growing demand has exposed certain inadequacies of the mainstream pectin extraction technique—notably efficiency and product consistency, thus prompting research interest towards ameliorating the process by an adoption of a number of novel technologies. Microwave and ultrasound energy appear to be the more likely candidates, but so are enzymatic-augmentation of the extraction process and the use of subcritical water to replace acidified water as solvent, among others. Yet this paper takes an objective approach in evaluating these new methods; for all their advantages, high cost, amidst other drawbacks, still prohibits their use in industry. Furthermore, optimization of process parameters are crucial to better understanding the chances of process scale-up, and this has been the current stage of efforts in the area. The present review discusses the nuances involved in using these non-conventional technologies for its extraction, including advantages and drawbacks. Prospects of their industrial integration are also conjectured.

Figure adapted and Map reproduced from J. Staunstrup (CP Kelko), Citrus Pectin Production and World Market, The International Citrus & Beverage Conference, September 17, 2009.



mario.pagliaro@cnr.it
www.qualitas1998.net

# 2019: introduction of Orange IntegroPectin

- ✓ In collaboration with Francesco Meneguzzo's team at CNR in Florence in 2019 we reported the first application of hydrodynamic cavitation to over 30 kg of residues from the industrial production of orange juices using only water (120 L) and electricity

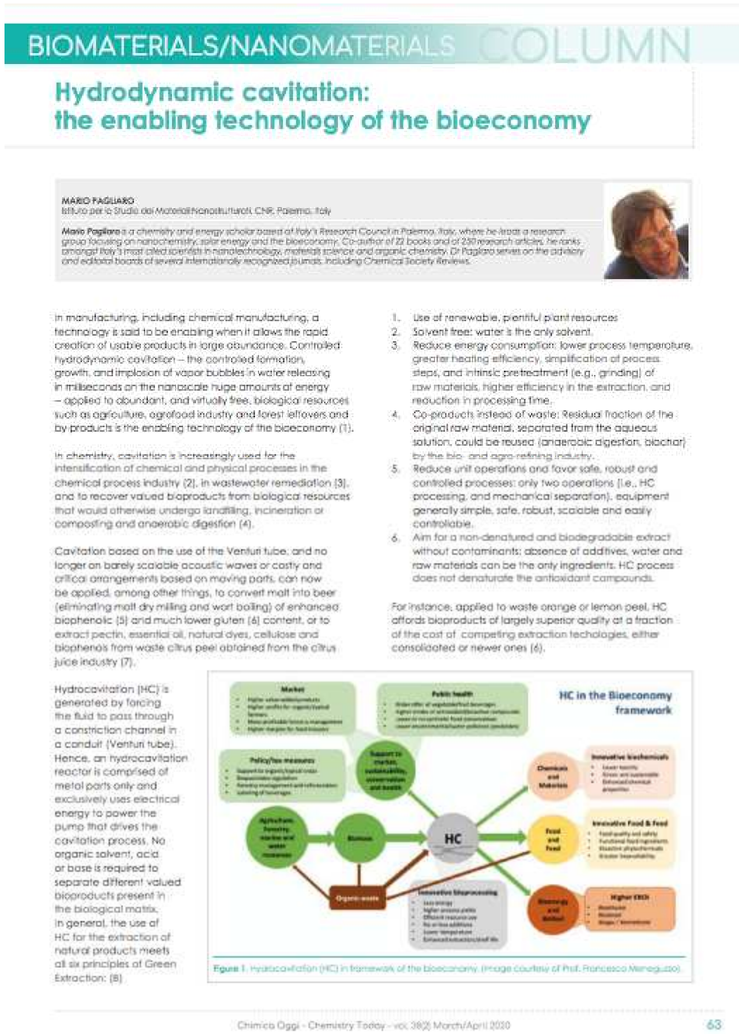
- ✓ It was immediately clear that it was a different pectin from the conventional one .

F. Meneguzzo, R. Ciriminna, et al., "Real-Scale Integral Valorization of Waste Orange Peel via Hydrodynamic Cavitation", *Processes* 7 (2019) 581.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Hydrodynamic cavitation: the enabling technology of the bioeconomy



BIOMATERIALS/NANOMATERIALS COLUMN

## Hydrodynamic cavitation: the enabling technology of the bioeconomy

MARIO PAGLIARO
Istituto per lo Studio dei Materiali Nanostrutturati, CNR, Palermo, Italy

*Mario Pagliaro is a chemistry and energy scholar based at Italy's Research Council in Palermo, Italy, where he leads a research group focusing on nanochemistry, solar energy and the bioeconomy. Co-author of 22 books and of 230 research articles, he ranks amongst Italy's most cited scientists in nanotechnology, materials science and organic chemistry. Dr Pagliaro serves on the advisory and editorial boards of several internationally recognized journals, including Chemical Society Reviews.*

In manufacturing, including chemical manufacturing, a technology is said to be enabling when it allows the rapid creation of usable products in large abundance. Controlled hydrodynamic cavitation – the controlled formation, growth, and implosion of vapor bubbles in water releasing in milliseconds on the nanoscale huge amounts of energy – applied to abundant, and virtually free, biological resources such as agriculture, agrofood industry and forest leftovers and by-products is the enabling technology of the bioeconomy (1).

In chemistry, cavitation is increasingly used for the intensification of chemical and physical processes in the chemical process industry (2), in wastewater remediation (3), and to recover valued bioproducts from biological resources that would otherwise undergo landfilling, incineration or composting and anaerobic digestion (4).

Cavitation based on the use of the Venturi tube, and no longer on barely scalable acoustic waves or costly and critical arrangements based on moving parts, can now be applied, among other things, to convert malt into beer (eliminating malt dry milling and wort boiling) of enhanced biophenolic (5) and much lower gluten (6) content, or to extract pectin, essential oil, natural dyes, cellulose and biophenols from waste citrus peel obtained from the citrus juice industry (7).

Hydrocavitation (HC) is generated by forcing the fluid to pass through a constriction channel in a conduit (Venturi tube). Hence, an hydrocavitation reactor is comprised of metal parts only and exclusively uses electrical energy to power the pump that drives the cavitation process. No organic solvent, acid or base is required to separate different valued bioproducts present in the biological matrix. In general, the use of HC for the extraction of natural products meets all six principles of Green Extraction: (8)

1. Use of renewable, plentiful plant resources
2. Solvent free: water is the only solvent.
3. Reduce energy consumption: lower process temperature, greater heating efficiency, simplification of process steps, and intrinsic pretreatment (e.g., grinding) of raw materials, higher efficiency in the extraction, and reduction in processing time.
4. Co-products instead of waste: Residual fraction of the original raw material, separated from the aqueous solution, could be reused (anaerobic digestion, biochar) by the bio- and agro-refining industry.
5. Reduce unit operations and favor safe, robust and controlled processes: only two operations (i.e., HC processing, and mechanical separation), equipment generally simple, safe, robust, scalable and easily controllable.
6. Aim for a non-denatured and biodegradable extract without contaminants: absence of additives, water and raw materials can be the only ingredients. HC process does not denaturate the antioxidant compounds.

For instance, applied to waste orange or lemon peel, HC affords bioproducts of largely superior quality at a fraction of the cost of competing extraction techologies, either consolidated or newer ones (6).



Figure 1. Hydrocavitation (HC) in framework of the bioeconomy. (Image courtesy of Prof. Francesco Meneguzzo)



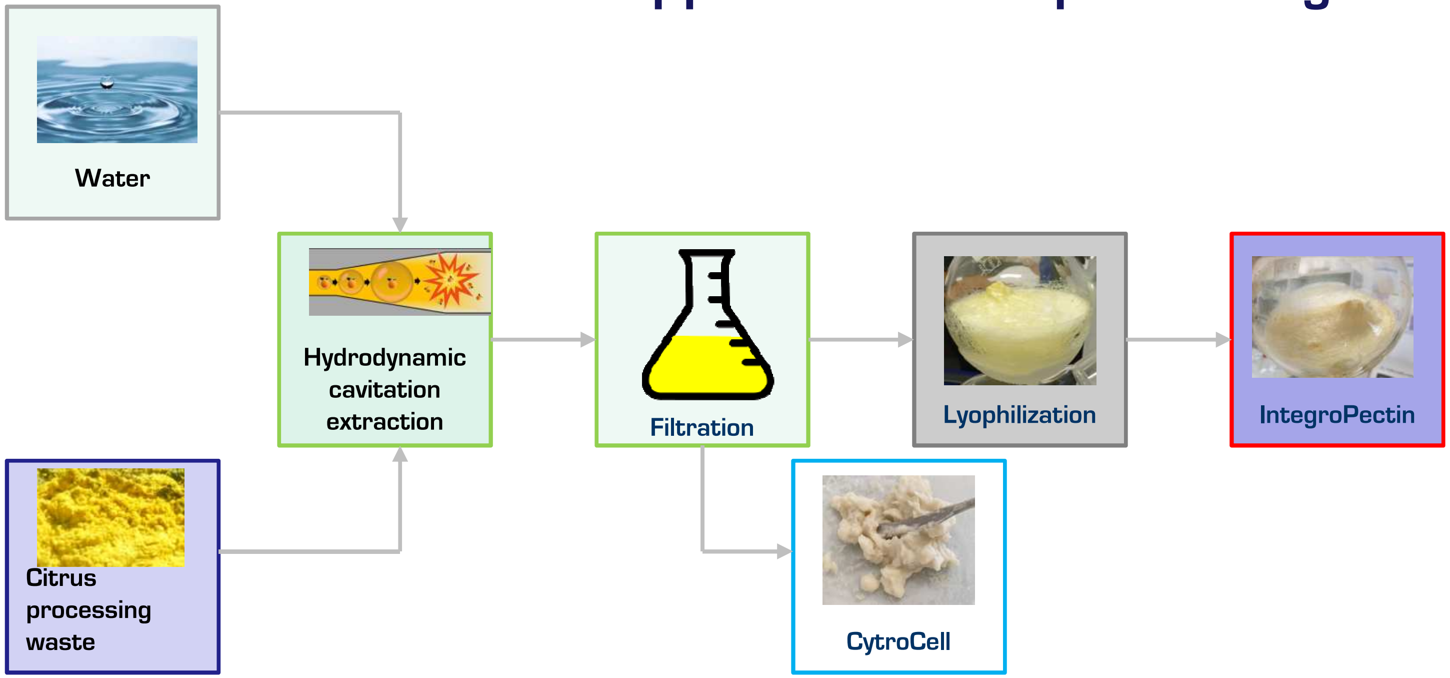
HCT agrifood lab

http://hct.fi.ibimet.cnr.it/



mario.pagliaro@cnr.it
www.qualitas1998.net

# HC applied to Citrus processing waste






mario.pagliaro@cnr.it
www.qualitas1998.net

# CytroCell, too, is an exceptional biomateral

molecules

MDPI

*Article*

## CytroCell: Valued Cellulose from Citrus Processing Waste

Antonino Scurria [1], Lorenzo Albanese [2], Mario Pagliaro [1], Federica Zabini [2], Francesco Giordano [1], Francesco Meneguzzo [2,*] and Rosaria Ciriminna [1,*]

[1] Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy; antonino.scurria@ismn.cnr.it (A.S.); mario.pagliaro@cnr.it (M.P.); francesco.giordano@cnr.it (F.G.)

[2] Istituto per la Bioeconomia, CNR, via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy; lorenzo.albanese@cnr.it (L.A.); federica.zabini@cnr.it (F.Z.)

* Correspondence: francesco.meneguzzo@cnr.it (F.M.); rosaria.ciriminna@cnr.it (R.C.)

**Abstract:** Isolating cellulose from citrus processing waste without employing chemicals has so far been an unfulfilled goal of chemical research applied to the valorization of a widely available biowaste, annually totaling >100 million tonnes. We have applied hydrodynamic cavitation using a Venturi-type reactor for the extraction of all valued bioproducts of industrial citrus processing waste in water only, directly on a semi-industrial scale. After reporting the discovery of IntegroPectin in the soluble fraction of the aqueous extract, we now report the isolation of a cellulosic material in the water-insoluble fraction of cavitated lemon and grapefruit processing waste. Named "CytroCell", the material is cellulose of low crystallinity, high porosity, good water holding capacity and good dispersibility in water. These properties open the route to mass-scale production of a useful functional material from a cheap and abundant biowaste.



**Keywords:** cellulose; lemon; grapefruit; citrus processing waste; hydrodynamic cavitation; bioeconomy

# Orange IntegroPectin: very low degree of esterification

- ✓ Using the Ilharco-Fidalgo equation "it was possible to determine a rather low degree of esterification for this pectin, namely 17.05 ± 0.60%"

**Table 4.** Decomposition results of the 1800–1550 $cm^{-1}$ region of the DRIFT spectra: Centers (C), full width at half maxima (FWHM), integrated areas (A) of the $\nu C = O$ and $\nu_{as}COO^-$ band areas, and degree of esterification (DE).

| Sample | Band Areas | C ($cm^{-1}$) | FWHM ($cm^{-1}$) | A(a.u.) | DE |
|---|---|---|---|---|---|
| P2 | $\nu C = O_{ester}$ | 1741 | 47 | 28.03 | 0.1786 |
| | $\nu C = O_{acid}$ | 1648 | 18 | 3.37 | |
| | $\nu_{as}COO^-$ | 1608 | 137 | 125.50 | |
| P3 | $\nu C = O_{ester}$ | 1740 | 50 | 28.67 | 0.1715 |
| | $\nu C = O_{acid}$ | 1649 | 19 | 3.04 | |
| | $\nu_{as}COO^-$ | 1609 | 143 | 135.42 | |
| P4 | $\nu C = O_{ester}$ | 1741 | 48 | 28.66 | 0.1664 |
| | $\nu C = O_{acid}$ | 1648 | 18 | 3.05 | |
| | $\nu_{as}COO^-$ | 1610 | 148 | 140.55 | |
| P5 | $\nu C = O_{ester}$ | 1741 | 47 | 28.55 | 0.1655 |
| | $\nu C = O_{acid}$ | 1648 | 19 | 3.09 | |
| | $\nu_{as}COO^-$ | 1610 | 149 | 140.87 | |

F. Meneguzzo, R. Ciriminna, et al., "Real-Scale Integral Valorization of Waste Orange Peel via Hydrodynamic Cavitation", *Processes* 7 (2019) 581.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Commercial citrus pectin is highly esterified (> 65%)

- ✓ Conventional commercial pectin has a DE> 50%, costs $ 18-20 / kg, and forms hydrogels with an acid pH (<3.5) in the presence of a high concentration of sugar
- ✓ The pectin industry also markets pectin with a low degree of esterification (DE <50%). Costs> $ 25 / kg and forms gels that contain up to 70g of water per g in the presence of Ca2 + without the need to add sugar over a wide range of pH values

Perspective

Biofpr Biofuels Bioproducts & Biorefining

## Pectin: A new perspective from the biorefinery standpoint

**Rosaria Ciriminna,** Istituto per lo Studio dei Materiali Nanostrutturati, CNR, Palermo, Italy
**Norberto Chavarría-Hernández, Adriana Inés Rodríguez Hernández,**
Universidad Autónoma del Estado de Hidalgo, Mexico
**Mario Pagliaro,** Istituto per lo Studio dei Materiali Nanostrutturati, CNR, Palermo, Italy

Received February 5, 2015; revised March 4, 2015, and accepted March 4, 2015
View online April 13, 2015 at Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.1551; *Biofuels, Bioprod. Bioref.* 9:368–377 (2015)

Abstract: Pectin is a natural product of central importance in the emerging biorefinery that uses fruit waste as a raw material. Generally obtained from lemon peel or from apple pomace, pectin is mainly used as a thickener and a stabilizer in the food industry. Due to its unique chemical properties and biochemical activity, however, its use is growing in many other sectors. Providing a unified view of the main research and utilization trends, this study identifies open opportunities toward larger scale production of a valued biopolymer that, we argue, will shortly emerge as a central product of the biorefinery, and of the citrus-based biorefinery in particular. © 2015 Society of Chemical Industry and John Wiley & Sons, Ltd

Rosaria Ciriminna, Norberto Chavarría-Hernández, Adriana Rodríguez Hernández, Mario Pagliaro, "Pectin: A New Perspective from the Biorefinery Standpoint", *Biofuels, Bioproducts & Biorefining* 9 (2015) 368–377.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Demand for low methoxyl pectin is growing steadily

- ✓ **LMP pectin is successfully used to give structure (mouthfeel) to all sugar-free beverages, including sparkling ones**

- ✓ **And as a fat replacer and stabilizer in a growing number of products, from ice cream to baked goods**

## Fat Replacer: Pectin Still Going Strong

October 10, 2012
Robby Gardner

*As a fat replacer, pectin's proven its value.*

*As a fat replacer, pectin's proven its value.*

https://www.nutritionaloutlook.com/view/fat-replacer-pectin-still-going-strong



mario.pagliaro@cnr.it
www.qualitas1998.net

# Pectin: “a universal medicine”

- ✓ “A number of studies demonstrate that pectin polysaccharides are physiologically active substances with immunomodulating properties, including anti-inflammatory activity, they lower cholesterol and triglyceride in the blood serum, normalize glucose metabolism, bind and remove toxins and radionuclides from the body, regulate work and provide protection of the gastrointestinal tract, have anticarcinogenic and antimetastatic effects”.

ELSEVIER

Fitoterapia
Volume 146, October 2020, 104676

Review

## Pectins as a universal medicine

Oksana Zaitseva, Andrey Khudyakov, Marta Sergushkina, Olga Solomina, Tatyana Polezhaeva

Institute of Physiology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences, FRC Komi SC UB RAS, 50 Pervomayskaya str., 167982 Komi Republic, Syktyvkar, Russia

Received 2 March 2020, Revised 19 May 2020, Accepted 10 June 2020, Available online 16 June 2020.

# A long-neglected antibacterial

- ✓ First reported in the late 1930s and partly explained in 1970, the antibacterial activity of pectin remained almost ignored until the late 1990s
- ✓ The concomitant emergence of research on natural antibacterials and new usages of pectic polysaccharides, including those in medicine widely researched in Russia, has led to a renaissance of research into the physiological properties of this uniquely versatile polysaccharide

ChemMedChem | Concepts
doi.org/10.1002/cmdc.202000518

Special Collection

## Pectin: A Long-Neglected Broad-Spectrum Antibacterial

Rosaria Ciriminna,[a] Alexandra Fidalgo,[b] Francesco Meneguzzo,[c] Alessandro Presentato,[d] Antonino Scurria,[a] Domenico Nuzzo,[e] Rosa Alduina,[d] Laura M. Ilharco,*[b] and Mario Pagliaro*[a]

*This article is dedicated to Professor Mohamed A. el-Nakeeb (University of Alexandria, Egypt) for his pioneering research on the antimicrobial activity of pectin*

First reported in the late 1930s and partly explained in 1970, the antibacterial activity of pectin remained almost ignored until the late 1990s. The concomitant emergence of research on natural antibacterials and new usages of pectin polysaccharides, including those in medicine widely researched in Russia, has led to a renaissance of research into the physiological properties of this uniquely versatile polysaccharide ubiquitous in plants and fruits. By collecting scattered information, this study provides an updated overview of the subtle factors affecting the behaviour of pectin as an antimicrobial. Less-degraded pectin extracted by acid-free routes, we argue in the conclusions, will soon find applications from new treatments for polymicrobial infections to use as an implantable biomaterial in tissue and bone engineering.

### 1. Introduction

In 1937 Edith Haynes and co-workers at Indiana University Medical School reported a surprising discovery: apple pectin added in 2% weight to a highly nutritious liquid medium (hearth infusion broth) inoculated with *Escherichia coli* killed all or 98% of the Gram-negative bacterial strain within 48 h.[1] The addition of pectin decreased the pH of the broth from 7.6 to 5.0–5.4. The team also reported that the bactericidal action was lost above pH 5.5.

Furthermore, in their brief communication the scholars reported the successful treatment of deep and superficial wounds, with coltural studies of the wounds healed showing "a marked decrease or complete disappearance of streptococci and a more gradual diminution of staphylococci".[1]

[a] Dr. R. Ciriminna, Dr. A. Scurria, Dr. M. Pagliaro
Istituto per lo Studio dei Materiali Nanostrutturati, CNR
via U. La Malfa 153, 90146 Palermo (Italy)
E-mail: mario.pagliaro@cnr.it

Two years later, researchers from a pediatric nutrition company reported in the same journal that broths containing 4 diverse commercial pectins whose pH ranged from 4.1 to 3.9 quickly killed inoculated *E. coli* cells, but that broths containing pectin at pH >4.9 were inactive, concluding that the "H-ion concentration is the factor responsible"[2] for the decreases in bacterial cell counts.

Given the relevance of these findings and taking into account the wide use of pectin in the food industry since the early 1900s when pectin started to be manufactured on and industrial scale in Germany and North America extracting it from apple pomace,[3] one would expect to see quick and significant growth of research on pectin as antibacterial.

Yet it is enough to conduct a search with the keywords "pectin" and "antibacterial" on a research database to learn that at the time of writing (mid-2020), only 238 documents have been published in the scientific literature (196 articles, 27 reviews, 10 conference papers and reviews, and 4 book chapters).[4]

This finding is even more surprising when learning that the 1937 discovery of pectin as an antimicrobial was confirmed and

# 2017: Citrus pectin obtained via microwave hydrodiffusion and gravity

## 3. CONCLUSIONS

Pectins extracted with microwaves from outer skin, peel, and waste of red orange, lemon, and grapefruit harvested in Sicily are low-methoxyl pectins, in which the degree of esterification increases in the order waste < peel < skin for red orange pectin, and inverts for lemon. The pectin extracted from lemon exocarp has not only a minimum degree of esterification (DE) but also a minimum content in smooth regions, whereas the pectin extracted from red orange waste (exo-, meso-, and endocarp) combines low methoxyl content with high HG content. The analytical method based on vibrational spectroscopy has been applied to samples of commercial citrus pectins. Remarkably, the results obtained with the new method are within the DE range reported by pectin manufacturers. These findings may open the route to high-quality nutraceutical- and pharmaceutical-grade citrus pectins obtained via an intrinsically clean process, employing pure water as the only extraction medium, in which the fruit and its region are chosen as a source of pectin according to the intended DE. We are currently studying and will shortly report the antioxidant and antimicrobial role imparted to these new pectins by the soluble flavonoid biophenols contained in the orange,[23] lemon,[24] and grapefruit[25] exocarp. It is also of relevance, in conclusion, that a similar versatility enabling to selectively adjust the essential oil composition has been recently reported also when microwave hydrodiffusion and gravity extraction were applied to waste lemon peel carried out on semi-industrial scale.[26]

ACS OMEGA

Article

Cite This: ACS Omega 2017, 2, 7991-7995

http://pubs.acs.org/journal/acsodf

### Controlling the Degree of Esterification of Citrus Pectin for Demanding Applications by Selection of the Source

Rosaria Ciriminna,[†] Alexandra Fidalgo,[‡] Riccardo Delisi,[†] Alfredo Tamburino,[⊥] Diego Carnaroglio,[§,∥] Giancarlo Cravotto,[§] Laura M. Ilharco,*[,‡] and Mario Pagliaro*[,†]

[†]Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy

[‡]Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal

[§]Dipartimento di Scienza e Tecnologia del Farmaco and Centre for Nanostructured Interfaces and Surfaces, Università degli Studi di Torino, via P. Giuria 9, 10125 Torino, Italy

[∥]Milestone, via Fabenefratelli, 1-5, 24010 Sorisole, Bergamo, Italy

[⊥]Dipartimento Agricoltura, Legambiente Sicilia, via Tripoli 3, 90138 Palermo, Italy

*Supporting Information*

ABSTRACT: Analyzed by a quantitative method based on diffuse reflectance infrared Fourier transform spectroscopy, pectins extracted from different regions (outer skin, peel, and waste) of citrus fruits (red orange, lemon, and grapefruit) via microwave-assisted hydrodiffusion show significant variations. All polymers obtained are low-methoxyl pectins, with high contents in galacturonic acid regions. The degree of esterification (DE) of pectin extracted from different regions increases in the order waste < peel < outer skin for red orange, inverting for lemon. Thus, the pectins with the lowest DE are those extracted from red orange waste and lemon outer skin (~25%). These findings

# Lemon IntegroPectin, much more effective and efficient antibacterial than commercial pectin

ChemistryOpen

Communications
doi.org/10.1002/open.202000076

Chemistry Europe
European Chemical Societies Publishing

## Superior Antibacterial Activity of Integral Lemon Pectin Extracted via Hydrodynamic Cavitation

Alessandro Presentato,[a] Antonino Scurria,[b] Lorenzo Albanese,[c] Claudia Lino,[b] Marzia Sciortino,[b] Mario Pagliaro,[b] Federica Zabini,[c] Francesco Meneguzzo,[c] Rosa Alduina,*[a] Domenico Nuzzo,*[d] and Rosaria Ciriminna*[b]

Pectin extracted via hydrodynamic cavitation in water only from waste lemon peel and further isolated via freeze drying displays significant antibacterial activity against *Staphylococcus aureus*, a Gram positive pathogen which easily contaminates food. The antibacterial effect of the new IntegroPectin is largely superior to that of commercial citrus pectin, opening the way to advanced applications of a new bioproduct now obtainable in large amounts and at low cost from citrus juice industry's waste.

processing environments, including hydrophobic coatings preventing or minimizing condensation which creates a number of hazards in food manufacturing facilities.[8]

Amid natural antibacterials, the surprising (in vitro) antibacterial properties of pectin were first reported by Russian scholars in the late 1990s.[9] The team found that amid four food fibers (wheat bran, soya isolate, soybean flour, and pectin), pectin is the only food fiber showing bactericidal activity on the most widely distributed pathogenic and opportunistic microorganisms, inactivating therapeutic bacteriophages at high concentration (>2 per cent), and decreasing the antimicrobial activity of antibiotic penicillins.[9]



mario.pagliaro@cnr.it
www.qualitas1998.net

# Grapefruit IntegroPectin, powerful bactericidal vs. Gram-positive and Gram-negative

antibiotics

MDPI

Article

A New Water-Soluble Bactericidal Agent for the Treatment of Infections Caused by Gram-Positive and Gram-Negative Bacterial Strains

Alessandro Presentato [1], Elena Piacenza [1,2], Antonino Scurria [3], Lorenzo Albanese [4], Federica Zabini [4], Francesco Meneguzzo [4], Domenico Nuzzo [5], Mario Pagliaro [3,*], Delia Chillura Martino [1,2], Rosa Alduina [1,*] and Rosaria Ciriminna [3]

**Abstract:** Grapefruit and lemon pectin obtained from the respective waste citrus peels via hydrodynamic cavitation in water only are powerful, broad-scope antimicrobials against Gram-negative and -positive bacteria. Dubbed IntegroPectin, these pectic polymers functionalized with citrus flavonoids and terpenes show superior antimicrobial activity when compared to commercial citrus pectin. Similar to commercial pectin, lemon IntegroPectin determined ca. 3-log reduction in *Staphylococcus aureus* cells, while an enhanced activity of commercial citrus pectin was detected in the case of *Pseudomonas aeruginosa* cells with a minimal bactericidal concentration (MBC) of 15 mg $mL^{-1}$. Although grapefruit and lemon IntegroPectin share equal MBC in the case of *P. aeruginosa* cells, grapefruit IntegroPectin shows boosted activity upon exposure of *S. aureus* cells with a 40 mg $mL^{-1}$ biopolymer concentration affording complete killing of the bacterial cells. Insights into the mechanism of action of these biocompatible antimicrobials and their effect on bacterial cells, at the morphological level, were obtained indirectly through Fourier Transform Infrared spectroscopy and directly through scanning electron microscopy. In the era of antimicrobial resistance, these results are of great societal and sanitary relevance since citrus IntegroPectin biomaterials are also devoid of cytotoxic activity, as already shown for lemon IntegroPectin, opening the route to the development of new medical treatments of polymicrobial infections unlikely to develop drug resistance.



mario.pagliaro@cnr.it
www.qualitas1998.net

# *Pseudomonas aeruginosa* in the presence of lemon and grapefruit IntegroPectin

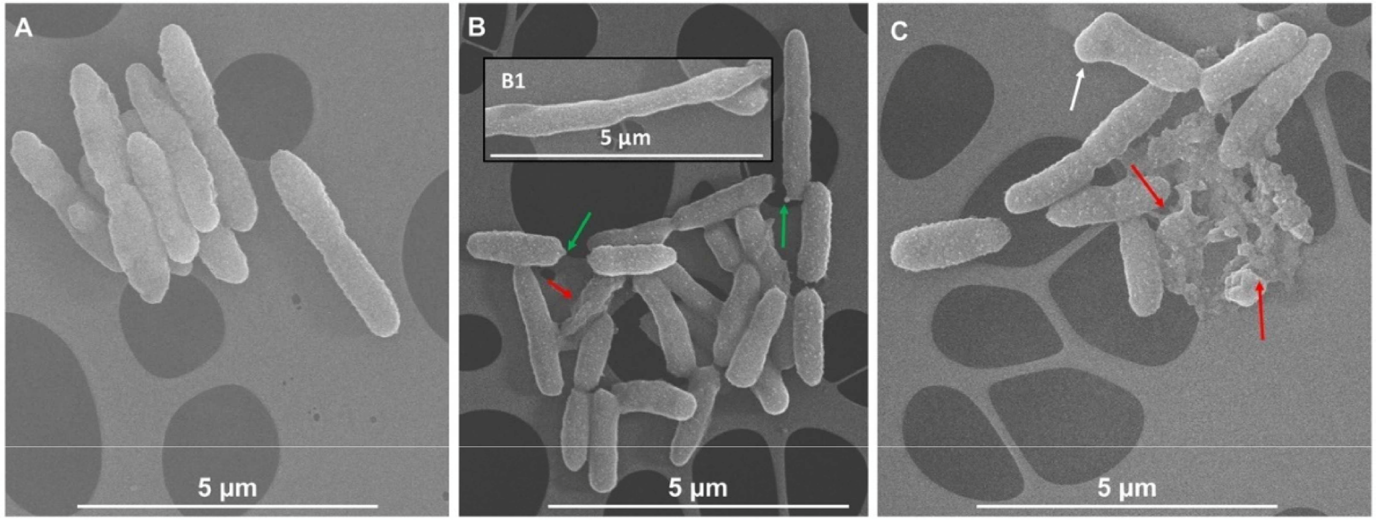



**Figure 5.** Scanning electron micrographs of unchallenged (**A**) or challenged *P. aeruginosa* cells with lemon (**B**) and/or grapefruit (**C**) IntegroPectins. The inlet **B1** shows an elongated and undivided cell. Nonorthodox cell morphology is indicated by white arrow; cell death events are underlined by red arrows, while green arrows highlight the presence of blebs of the cell envelope.



mario.pagliaro@cnr.it
www.qualitas1998.net

# *Staphylococcus aureus* in the presence of lemon and grapefruit IntegroPectin

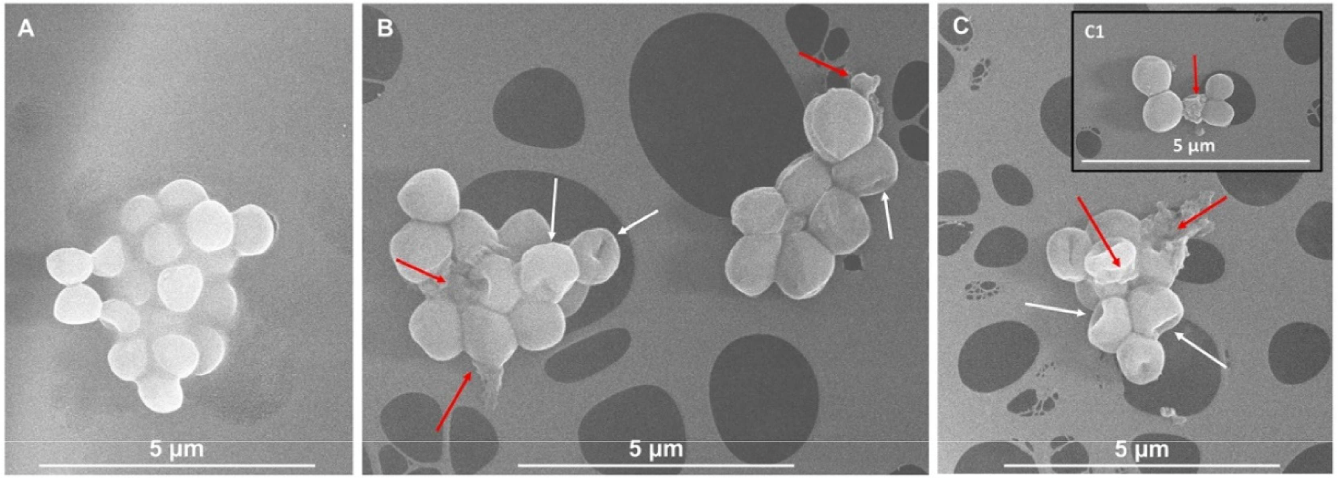



**Figure 6.** Scanning electron micrographs of unchallenged (**A**) or challenged *S. aureus* cells with lemon (**B**) and/or grapefruit (**C**) IntegroPectins. The inlet **C1** shows a collapsed coccus cell. Nonorthodox cell morphologies are indicated by white arrows; cell death events are underlined by red arrows.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Lemon IntegroPectin, a powerful antioxidant, devoid of cytotoxic activity

- ✓ Il valore di ORAC per la IntegroPectin di limone è di 122.200 µmol TE/100 g.
- ✓ Le acque di vegetazione dei frantoi liofilizzate hanno un valore di ORAC di 201.100 µmol TE/100 g, mentre il lampone nero ha un valore di ORAC di 16210 µmol TE/100 g (su base materiale essiccato).

Domenico Nuzzo, Laura Cristaldi, Marzia Sciortino, Lorenzo Albanese, Antonino Scurria, Federica Zabini, Claudia Lino, Mario Pagliaro, Francesco Meneguzzo, Marta Di Carlo, Rosaria Ciriminna, "Exceptional antioxidant, non-cytotoxic activity of integral lemon pectin from hydrodynamic cavitation", *ChemistrySelect* 5 (2020) 5066-5071.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Epithelial human lung protective agent

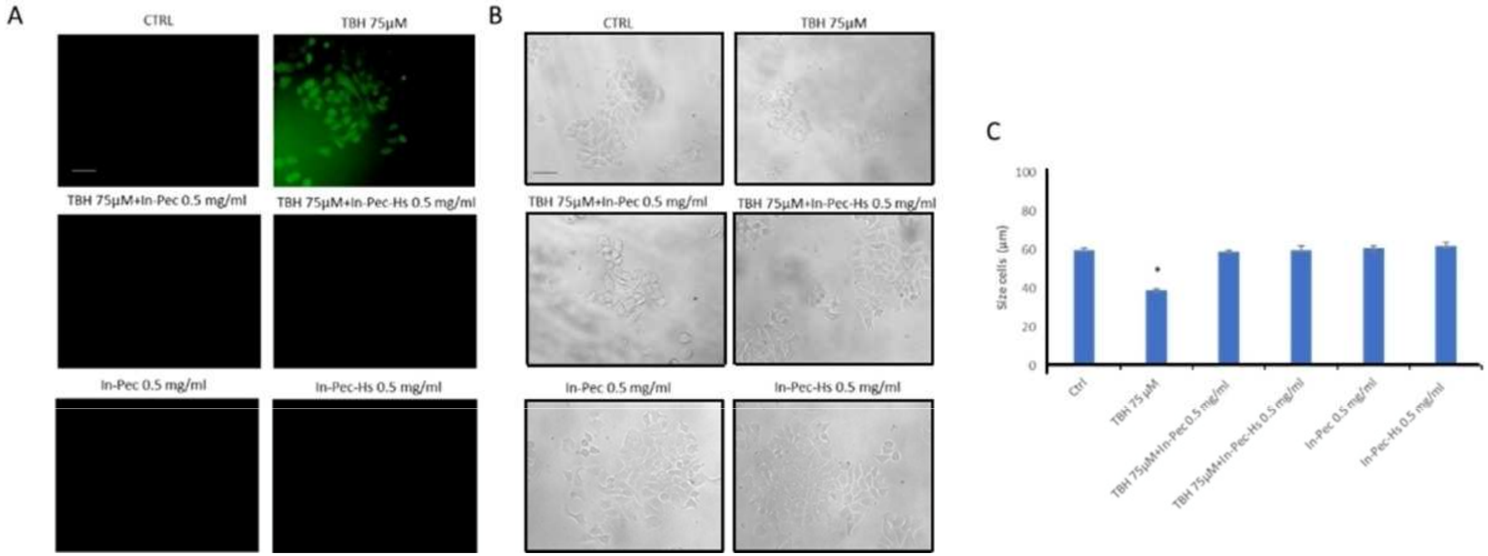


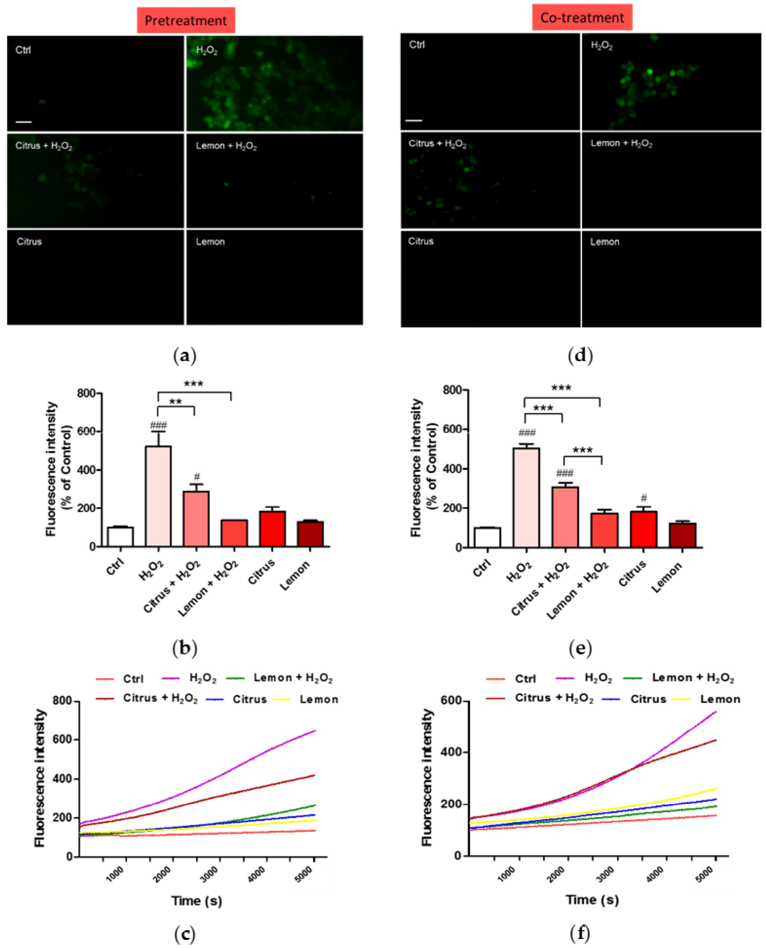

**Figure 6.** TBH-induced stress on A549 human epithelial cells (A), and stress inhibition due to both integral lemon pectin Int-Pec (A, *middle*) and heat-stressed lemon pectin Int-Pec-Hs (A, *bottom*) in 0.5 mg/mL concentration; microscopic observations (B, Bar = 100 μm) and cell size of the TBH-induced altered cells following addition of each pectin in 0.5 mg/mL concentration (C).

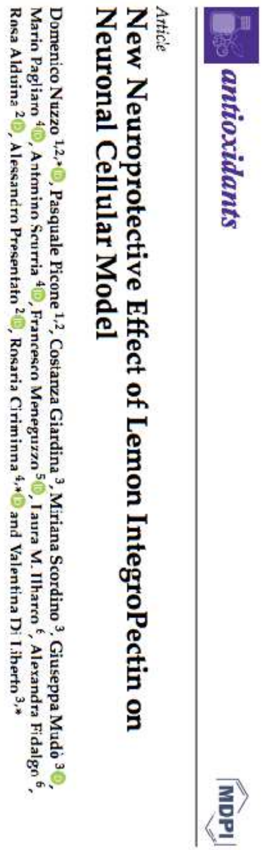
Domenico Nuzzo, Laura Cristaldi, Marzia Sciortino, Lorenzo Albanese, Antonino Scurria, Federica Zabini, Claudia Lino, Mario Pagliaro, Francesco Meneguzzo, Marta Di Carlo, Rosaria Ciriminna, "Exceptional antioxidant, non-cytotoxic activity of integral lemon pectin from hydrodynamic cavitation", *ChemistrySelect* 5 (2020) 5066-5071.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Neuronal cell protective agent





antioxidants

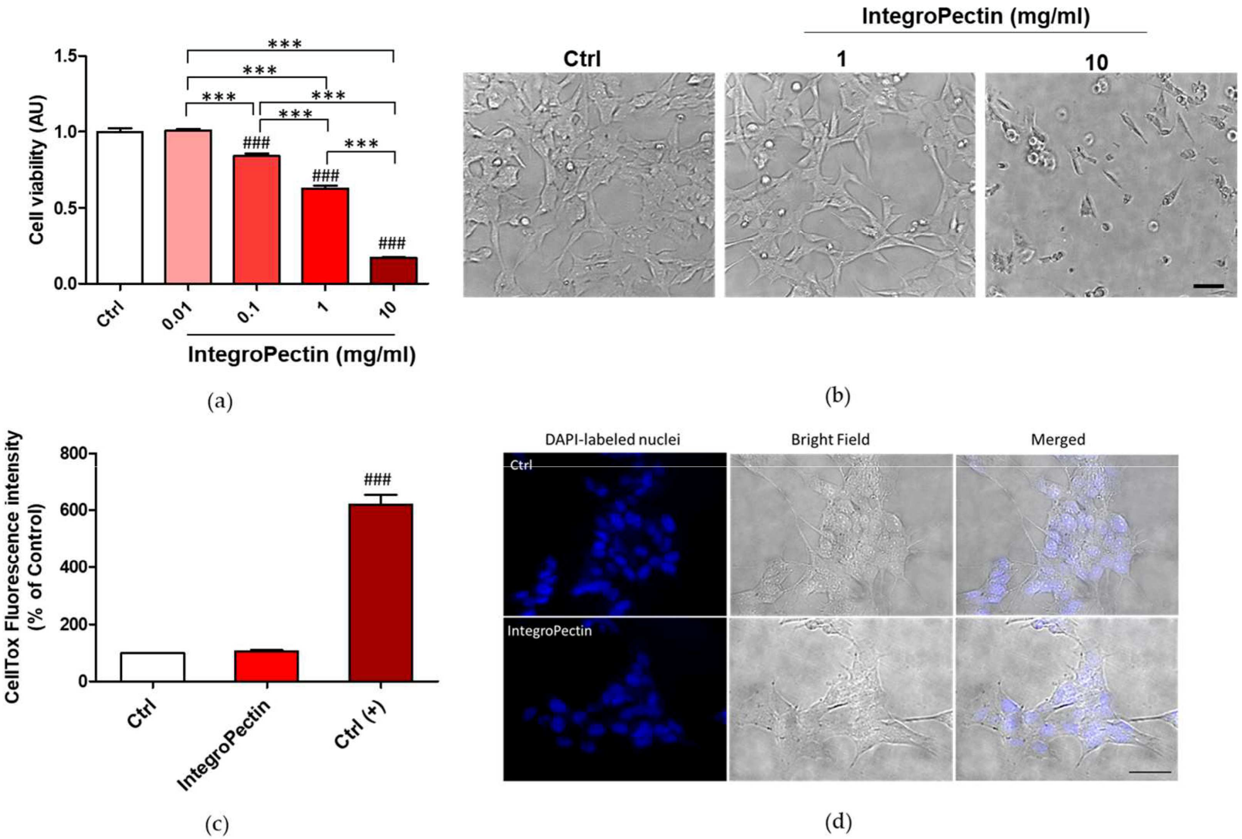
Article

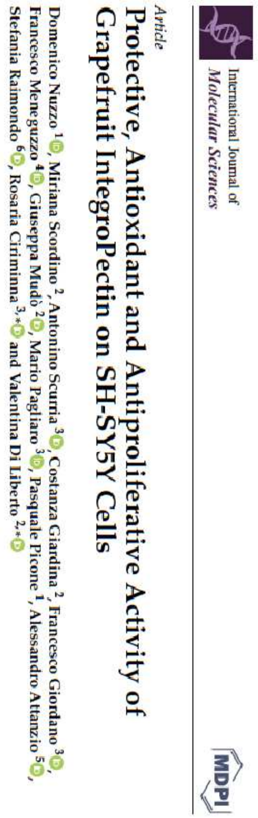
**New Neuroprotective Effect of Lemon IntegroPectin on Neuronal Cellular Model**

Domenico Nuzzo [1,2,*], Pasquale Picone [1,2], Costanza Giardina [3], Miriana Scordino [3], Giuseppa Mudò [3], Mario Pagliaro [4], Antonino Scurria [4], Francesco Meneguzzo [5], Laura M. Ilharco [6], Alexandra Fidalgo [6], Rosa Alduina [2], Alessandro Presentato [2], Rosaria Ciriminna [4,*] and Valentina Di Liberto [3,*]

MDPI

# Grapefruit IntegroPectin, powerful antiproliferative agent





International Journal of *Molecular Sciences*

*Article*

**Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells**

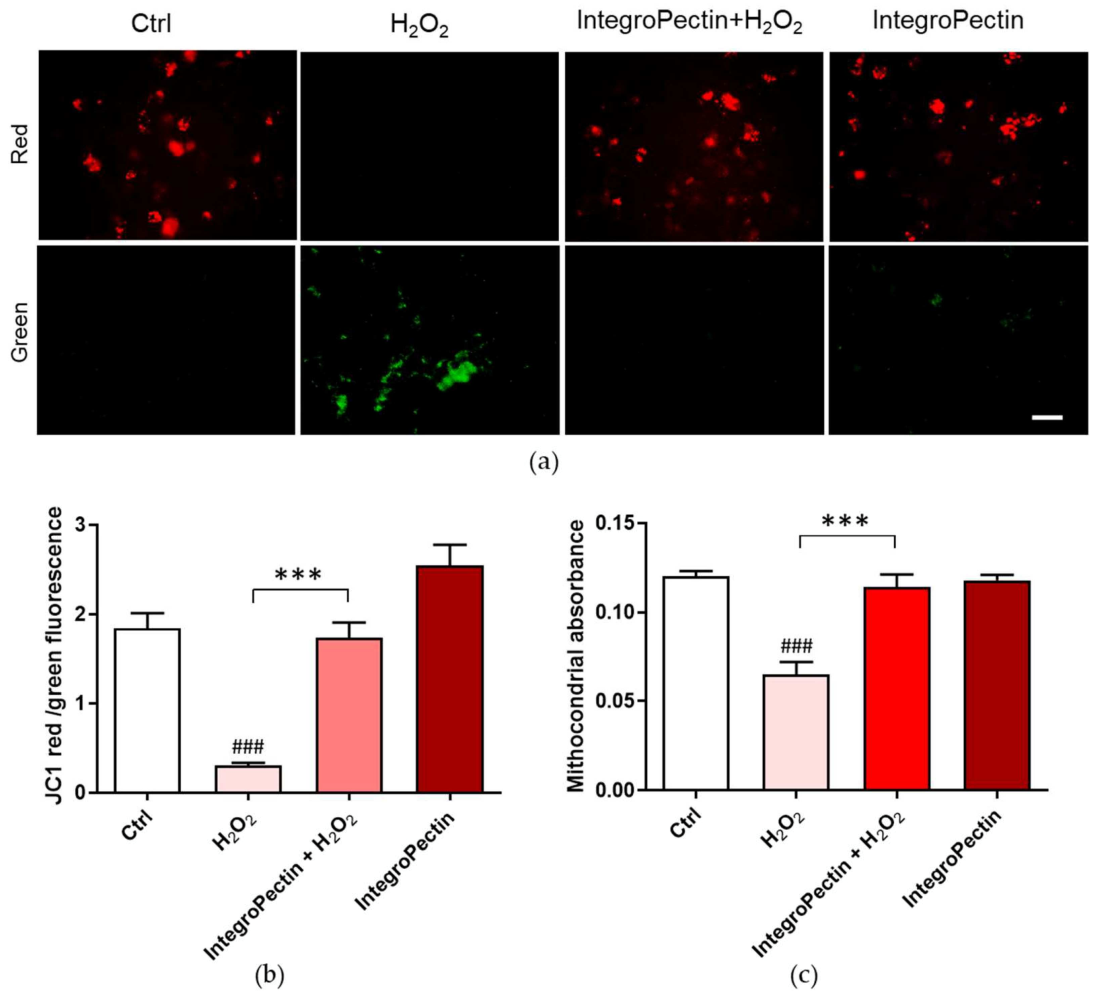
Domenico Nuzzo [1], Miriana Scordino [2], Antonino Scurria [3], Costanza Giardina [2], Francesco Giordano [3], Francesco Meneguzzo [4], Giuseppa Mudò [2], Mario Pagliaro [3], Pasquale Picone [1], Alessandro Attanzio [5], Stefania Raimondo [6], Rosaria Ciriminna [3,*] and Valentina Di Liberto [2,*]

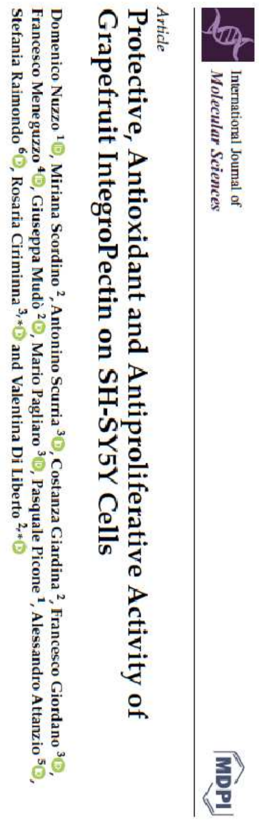
MDPI



mario.pagliaro@cnr.it
www.qualitas1998.net

# Grapefruit IntegroPectin, powerful mitoprotective agent



International Journal of *Molecular Sciences*

*Article*

**Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells**

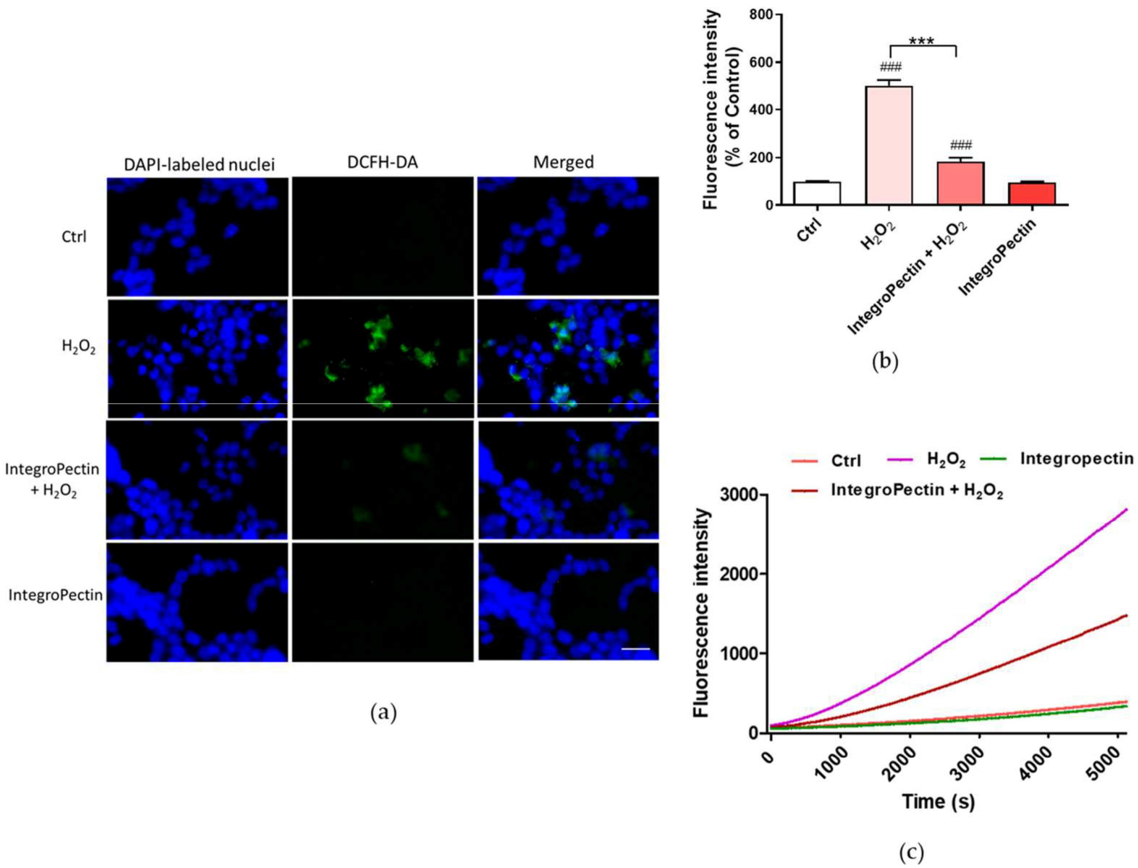
Domenico Nuzzo [1], Miriana Scordino [2], Antonino Scurria [3], Costanza Giardina [2], Francesco Giordano [3], Francesco Meneguzzo [4], Giuseppa Mudò [2], Mario Pagliaro [3], Pasquale Picone [1], Alessandro Attanzio [5], Stefania Raimondo [6], Rosaria Ciriminna [3,*] and Valentina Di Liberto [2,*]

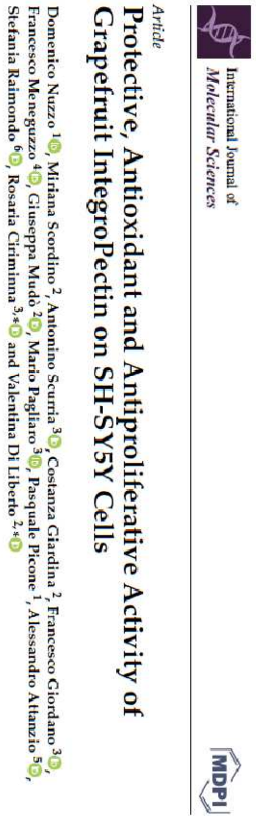
MDPI



mario.pagliaro@cnr.it
www.qualitas1998.net

# Grapefruit IntegroPectin, powerful antioxidant protecting neuronal model cells





International Journal of *Molecular Sciences*

*Article*

**Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells**

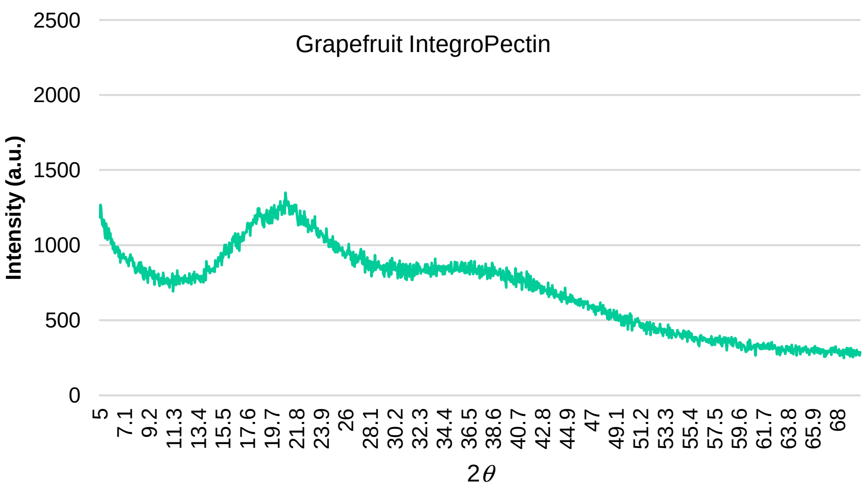
Domenico Nuzzo [1], Miriana Scordino [2], Antonino Scurria [3], Costanza Giardina [2], Francesco Giordano [3], Francesco Meneguzzo [4], Giuseppa Mudò [2], Mario Pagliaro [3], Pasquale Picone [1], Alessandro Attanzio [5], Stefania Raimondo [6], Rosaria Ciriminna [3,*] and Valentina Di Liberto [2,*]

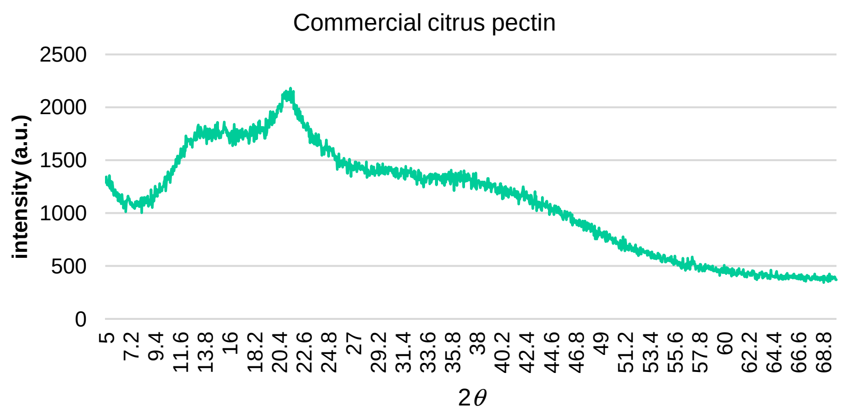
MDPI



mario.pagliaro@cnr.it
www.qualitas1998.net

# Why is IntegroPectin so much more bioactive than conventional pectin?




✓ Exactly as it happens with grapefruit pectin obtained via cavitation driven by ultrasounds, the diffraction peaks characteristic of the commercial citrus pectin at 12.4°, 14.3°, 21.0°, 28.9°, 31.5°, 32.2° and 40.2° shift to higher 2θ values, with several sharp peaks disappearing



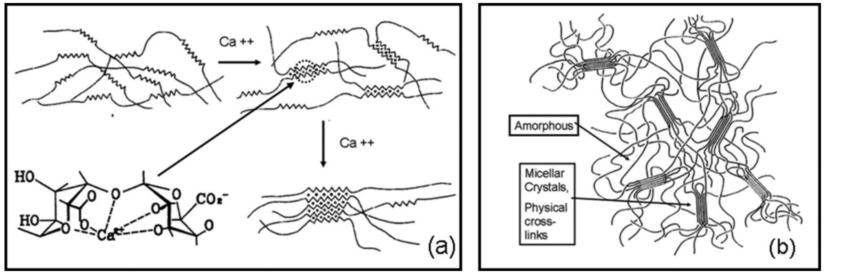
mario.pagliaro@cnr.it
www.qualitas1998.net

# Nearly complete decrystallization of homogalacturonan (HG) chains

- ✓ **This indicates that the hydrodynamic cavitation of grapefruit biowaste induces the nearly complete decrystallization of the homogalacturonan (HG) chains of the grapefruit pectin, following which it crystallizes in a hexagonal closest packing arrangement that ensures the closest packing of the chains (we remind readers that the diffraction from the HG regions is the only contributing to the XRD pattern).**

- ✓ **These findings confirm that cavitation, no matter if acoustic or hydrodynamic, destroys the “fringed-micellar” structure of the crystalline regions of the semicrystalline pectin biopolymer.**

- ✓ **Along with the substantially higher number of RG-I regions and very low DE, this also explains the significantly larger solubility of the IntegroPectin in water at room temperature when compared to the poorly soluble commercial citrus pectin.**




Gohil, R.M. Synergistic blends of natural polymers, pectin and sodium alginate. *J. Appl. Polym. Sci.* 2010, 120, 2324–2336.



mario.pagliaro@cnr.it
www.qualitas1998.net

# Flavonoids adsorbed and concentrated on the surface

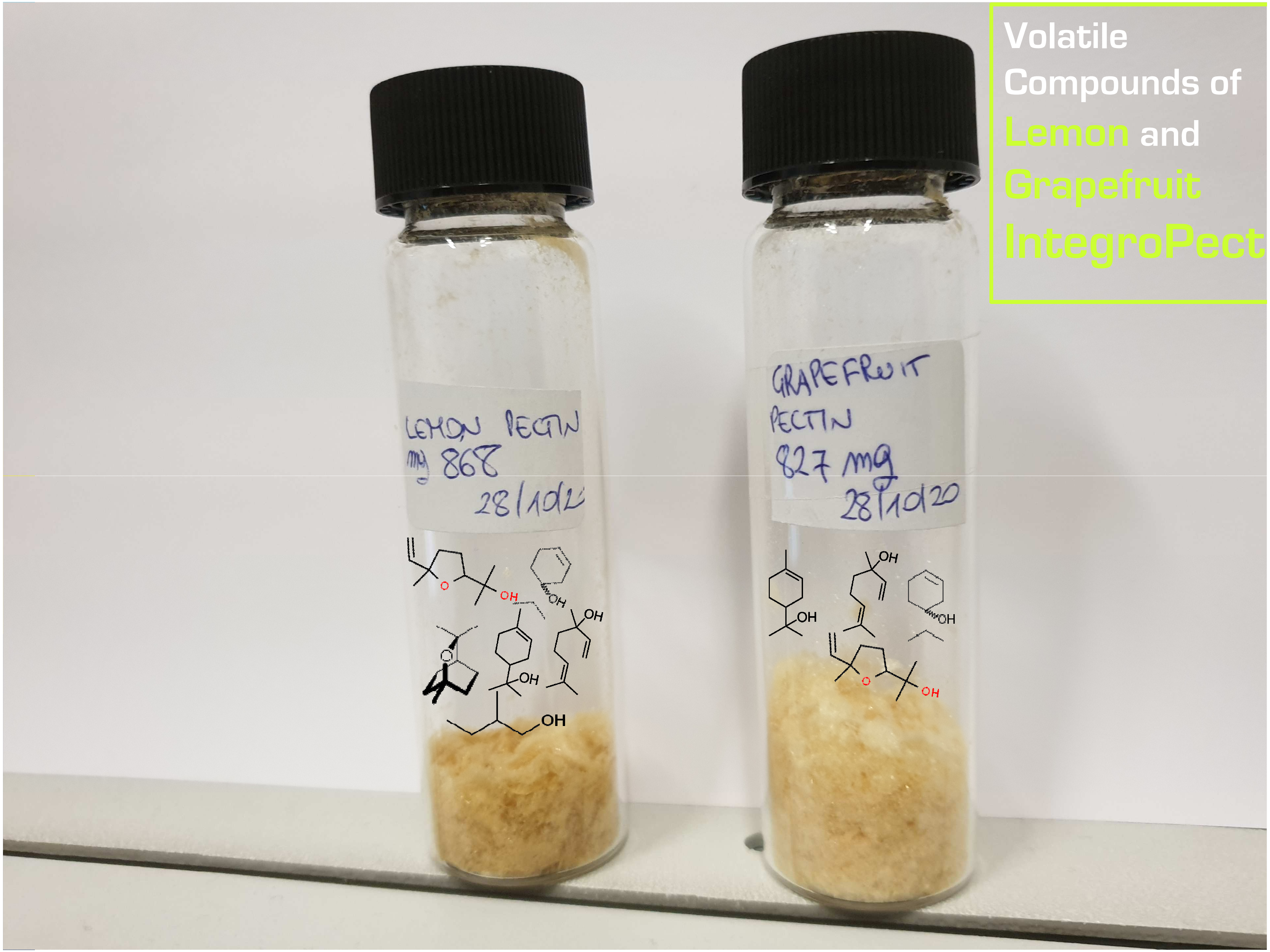
**Table 1.** Flavonoids in lemon and grapefruit IntegroPectin identified by HPLC-MS.

| Biophenol | Lemon IntegroPectin (mg/g) | Grapefruit IntegroPectin (mg/g) |
|---|---|---|
| Naringenin | - | 0.34 |
| Naringin | 0.11 | 73.66 |
| Hesperidin | 0.60 | 0.60 |
| Eriocitrin | 3.35 | - |
| Kaempeferol | 0.26 | - |
| Kaempferol-7-*O*- glucuronide | 0.26 | 0.26 |
| *p*-Coumaric acid | 0.28 | |
| Gallic acid | 0.56 | 0.31 |

Antonino Scurria, Marzia Sciortino, Lorenzo Albanese, Domenico Nuzzo, Federica Zabini, Francesco Meneguzzo, Rosa V. Alduina, Alessandro Presentato, Mario Pagliaro, Giuseppe Avellone, Rosaria Ciriminna, "Flavonoids in Lemon and Grapefruit IntegroPectin", *Preprints* (2021). 2021020620



mario.pagliaro@cnr.it
www.qualitas1998.net


Volatile Compounds of Lemon and Grapefruit IntegroPect
LEMON PECTIN
mg 868
GRAPEFRUIT PECTIN
827 mg
28/10/20

# Terpenes adsorbed and concentrated on the surface (lemon)

**Table 1.** Volatile organic compounds in the lemon IntegroPectin.

| Entry | Compound | Apex RT (min) | Area (%) |
|---|---|---|---|
| 1 | 3-Methyl-2-buten-2-ol (prenol) | 7.34 | 1.08 |
| 2 | 1-Butanol, 3-methyl acetate (isoamyl acetate) | 8.27 | 0.73 |
| 3 | Limonene | 8.33 | 3.39 |
| 4 | 2-Methyl-1-butanol | 8.54 | 17.11 |
| 5 | 3-Methyl-1-butanol | 8.6 | 12.43 |
| 6 | Eucalyptol | 8.98 | 2.32 |
| 7 | 1-Hexanol | 9.95 | 2.52 |
| 8 | 2-Hexen-1-ol | 10.33 | 2.33 |
| 9 | 6-Hepten-1-ol, 2-methyl | 10.88 | 1.52 |
| 10 | α-Linalool | 11.73 | 4.94 |
| 11 | Terpinen-4-ol | 12.88 | 21.85 |
| 12 | α-Citral | 13.62 | 0.58 |
| 13 | α-Terpineol | 13.72 | 27.42 |
| 14 | Safranal | 14.15 | 1.11 |
| 15 | 2,4-Cyclohexadiene-1-methanol, α,α-4-trimethyl | 14.88 | 0.65 |

Antonino Scurria, Marzia Sciortino, Alessandro Presentato, Claudia Lino, Elena Piacenza, Lorenzo Albanese, Federica Zabini, Francesco Meneguzzo, Domenico Nuzzo, Mario Pagliaro, Delia Francesca Chillura Martino, Rosa Alduina, Giuseppe Avellone, Rosaria Ciriminna, "Volatile Compounds of Lemon and Grapefruit IntegroPectin", *Molecules* 26 (2021) 51.

S.-H. Jun, et al.
Polymers 11 (2019) 1044



mario.pagliaro@cnr.it
www.qualitas1998.net

# Terpenes adsorbed and concentrated on the surface (grapefruit)

**Table 2.** Volatile organic compounds in grapefruit IntegroPectin.

| Entry | Compound | RT (min) | %Area |
|---|---|---|---|
| 1 | 1-Butanol, 3-methyl-acetate (isoamyl acetate) | 8.26 | 0.67 |
| 2 | Limonene | 8.31 | 11.77 |
| 3 | 2-Methyl-1-butanol | 8.54 | 12.91 |
| 4 | 3-Methyl-1-butanol | 8.6 | 15.17 |
| 5 | 1-Hexanol | 9.94 | 3.24 |
| 6 | 3-Hexen-1-ol | 10.33 | 1.28 |
| 7 | *cis*-Linalool oxide | 11.26 | 14.4 |
| 8 | *trans*-Linalool oxide | 11.66 | 1.76 |
| 9 | α-Linalool | 11.71 | 12.01 |
| 10 | Terpinen-4-ol | 12.88 | 9.37 |
| 11 | α-Terpineol | 13.73 | 17.4 |

Antonino Scurria, Marzia Sciortino, Alessandro Presentato, Claudia Lino, Elena Piacenza, Lorenzo Albanese, Federica Zabini, Francesco Meneguzzo, Domenico Nuzzo, Mario Pagliaro, Delia Francesca Chillura Martino, Rosa Alduina, Giuseppe Avellone, Rosaria Ciriminna, "Volatile Compounds of Lemon and Grapefruit IntegroPectin", *Molecules* 26 (2021) 51.

S.-H. Jun, et al.
Polymers 11 (2019) 1044

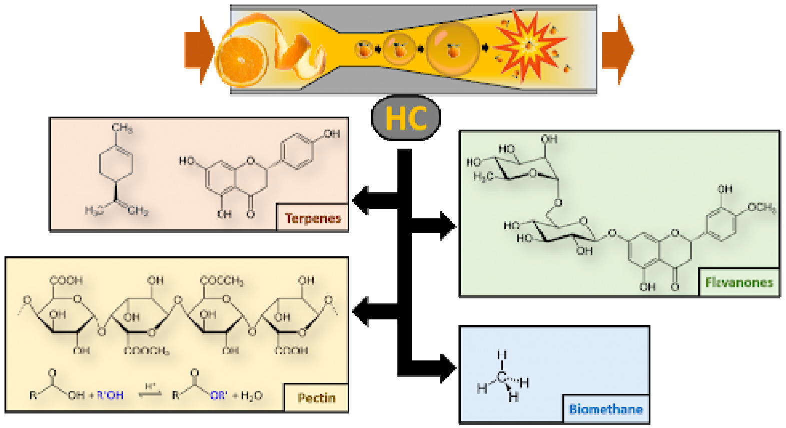


mario.pagliaro@cnr.it
www.qualitas1998.net

# Take home messages

- ✓ The application of hydrodynamic cavitation to citrus biowaste directly on a pre-industrial scale provides a pectin with exceptional biological properties, already verified in vitro for lemon and grapefruit waste, called IntegroPectin, using electricity and water only

- ✓ The biological activity is synergistically related to the special structure of the new pectin, and to the highly bioactive molecules (flavonoids and terpenes) adsorbed and concentrated on the surface of the IntegroPectin after freeze-drying the aqueous extract






mario.pagliaro@cnr.it
www.qualitas1998.net

# Thank you!

Rosaria Ciriminna
(Italy)

Laura M. Ilharco
(Portugal)

Alexandra Fidalgo
(Portugal)

Marzia Sciortino
(Italy)

Alessandro Presentato
(Italy)

Rosa Valeria Alduina
(Italy)

Domenico Nuzzo (Italy)

Antonino Scurria (Italy)

Beppe Avellone
(Italy)

Lorenzo Albanese e Francesco Meneguzzo
(Italy)

Valentina Di Liberto (Italy)



mario.pagliaro@cnr.it
www.qualitas1998.net




mario.pagliaro@cnr.it
www.qualitas1998.net