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Pectin: A Long-Neglected Broad-Spectrum Antibacterial

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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]

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 expanded in 1970 by el-Nakeeb and Yousef,[5] who proposed the first explanation for the antibacterial properties of pectin.[6]

Based at the University of Alexandria, Egypt, these scholars published their thoroughly researched findings in English in one of the leading international journals in the field of medicinal plants and natural products, even including a summary in German for each paper published.

Still, the fact that pectin is a broad-spectrum antibacterial remained almost ignored until the late 1990s when scholars in Russia reported in a journal published in Russian only that pectin, amid all food fibres, is the only one showing bactericidal activity on the most widely distributed pathogenic and opportunistic microorganisms.[7]

Also these findings generated little scientific progress. We had to wait until 2011 to record the publication of more than

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10 papers (12) on the antibacterial activity of pectin.[4] In 2019, the number of research articles in the field tripled to 33, and only in the first half of 2020, we record 23 research reports on antibacterial pectin.[4]

The interest of today's life scientists for pectin is unveiled by a search with "pectin" as a search query in the main preprint servers used by life science scholars (bioRxiv), which returned 467 preprints having pectin or its derivatives as research object.[8]

Blossoming research in the biological properties of pectin is due to flourishing research in natural antimicrobials driven by the emergence of drug-resistant bacteria and fungi,[9] to the concomitant emergence of several new uses of pectin,[10] as well as to its recently discovered anti-inflammatory properties chiefly due to the galacturonan chain of the biopolymer.[11]

We briefly remind that the molecular structure of pectin consists of homopolymeric partially 6-methylated and 2- and/or 3-acetylated poly-α(1–4)-D-galacturonic acid residues (the homogalacturonan, HG, "smooth" regions), alternating with 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, RG–I, "hairy" regions), whose relative proportion determines the rheological properties of the polymer dissolved in water.[10]

Collecting scattered information, this study provides an updated overview of the subtle factors affecting the behavior of pectin as an antimicrobial. We conclude providing arguments for which pectin extracted via acid-free routes replacing the one-century old production route based on the hydrolysis of fruit peels with mineral acids and subsequent precipitation with alcohol, might soon find high-value applications spanning from new treatments for polymicrobial infections through use as implantable biomaterial in tissue and bone engineering.

2. A Broad-Spectrum Antimicrobial

The first major advances towards understanding the antibacterial action of pectin were reported in 1970 by Yousef and el-Nakeeb.[5,6] The two scholars first showed that i) active bacterial growth is not a prerequisite for pectin to exert its action since incubation of *E. coli* cells with 1% apple pectin either in nutrient broth or in distilled water gave comparable bactericidal results; that ii) the majority of the cells are inhibited by 0.5% pectin while the remaining number requires levels higher than 2%, as shown by the two linear segments with different slopes and an inflection point at 0.5% pectin in the logarithmic plot of the number of survivors *vs.* the concentration of pectin; and finally that iii) the antibacterial activity of pectin is entirely due to its undissociated acid form.[6]

In closer detail, confirming the 1937 findings of Haynes for which activity ceased at pH 5.0, they showed that maximum activity against *E. coli* was observed when the cell suspension was treated with 1% pectin solution at its "natural" pH, namely 2.65, while the bactericidal activity was already greatly reduced at pH 3.5 and completely ceased to occur at pH 5 and higher. Titration curve of 1% pectin in water shows that the fractions of the apple pectin neutralized at pH 3.5 and 4.5 were 49.3 and 87.5%, respectively.

The aforementioned low pH (2.65) of 1% pectin solution was termed "natural" because, following extensive alcoholic washing of pectin and treatment with either ion exchange resin or Sephadex G-25 resulting in neither change of the pH nor in the decrease of the viscosity of the pectin solution, showed that the pectin solution acidity was not due "to any inorganic acid radicals absorbed during the commercial preparation of pectin".[6]

In the same year, the Egypt-based scholars reported several new findings that make the subsequent prolonged absence of research even more surprising. In detail, they reported the bactericidal effect of a 1% pectin solution on 9 different Gram-negative bacteria, yeasts and filamentous fungi.[5] Pectin killed within the first 15 min of contact more than 90% of the Gram-negative bacteria (*Shigella vulgaris*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhimurium*, *Klebsiella aerogenes*, *E. coli*, *Proteus vulgaris*, *Bordetella bronchiseptica* and *Pseudomonas aeruginosa*) and by the end of the second hour practically none had survived.

The bactericidal action of the same 1% pectin solution towards Gram-positive bacteria was slower. *Streptococcus pyogenes* was killed within 15 min of contact, while *Staphylococcus lactis* and *Corynebacterium hofmannii* were killed in 2 h. The killing of *Staphylococcus aureus* required 4 h of contact, similar to acid-resistant *Lactobacillus acidophilus* whose cells mostly survived during the first hour, but then were rapidly killed in the subsequent 3 h. Finally, the spore-forming Gram-positive *Bacillus subtilis* was virtually unaffected by contact of pectin with modest inhibition even after several days of treatment.[5]

In addition, the scholars reported the antifungal activity of pectin in solution against *Candida albicans* and *Saccharomyces cerevisiae* treated with 1% pectin remained unaffected during the first 2 h but then were mostly killed in the subsequent 22 h. After 24 h, less than 10% of the initial yeast cells were recovered and virtually no *C. albicans* cells. On the other hand, the scientists confirmed the already known pectolytic activity of filamentous fungi such as *Aspergillus niger* and *Penicillium italicum* that in contact with 1% pectin in a mycological broth showed a reduction in mycelial weight during the first 2 days, followed by a rapid and large increase pointing to pectin degradation by the fungi using it as a carbon source which explains "why pectin solutions become heavily contaminated with molds when exposed to air".[5]

As mentioned in the introductory section, these findings remained virtually ignored until the late 1990s when scholars in Russia led by Men'shikov reported that pectin is the only food fiber showing bactericidal activity on the most widely distributed pathogenic and opportunistic microorganisms, with concentrations of pectin > 2% having an inactivating effect on therapeutic bacteriophages.[7]

Ten years later, the same team tested *in vitro* the influence of a 2% solution of pectin (red beet, apple, citrus, citrus high- and low-etherified pectins, and nutraceutical 'Pecto' product) on the growth of staphylococci and production by them of type

A and B enterotoxins, amid the most potent bacterial superantigens leading the immune system to stimulate cytokine release and inflammation.[12]

The Russian scholars thereby reported that the aforementioned pectin solutions were able to inhibit the synthesis of types A and B staphylococcal enterotoxins, the most effective being red beet, apple, citrus low-methoxy pectins and the biologically active food supplement.[13]

Affiliated with Moscow's Sklifosovsky Clinical and Research Institute for Emergency Medicine, Men'shikov, a microbiologist, and Popova, head of the laboratory of experimental pathology, had a prolonged clinical interest in the therapeutic uses of pectin.

The team in 2002 demonstrated that oral administration of pectins for prophylaxis and treatment of purulent septic complications in patients with burns had a lower frequency of bacteremia, intoxication, and infectious complications, with the use of pectin resulting in the accelerated healing of burn wounds.[14]

Remarkably, the microbial profile of feces demonstrated enhanced bacterial microflora (bifidobacteria and lactobacilli) and diminished opportunistic bacteria.

In another study published in the same year in the same Russian journal, the team reported that topical treatment of burned wounds with 1–2% apple or beet pectin solutions (in the form of wet gauze dressings or collagen-pectin coatings on the area of burn wounds) inhibited inflammation, and reduced bacterial contamination of the wound (particularly of *P. aeruginosa*) resulting in acceleration of epithelization of burn wounds of II–IIIA degree, allowing to diminish the preliminary period before auto-dermoplastic operation (class IIIB burns).[15]

The best results were obtained when the application started the first day after the burn; clinical evaluation demonstrated excellent pectin tolerability (absence of side effects and complications).

The experimental finding that pectin does not harm human microbiota, but kills pathogenic bacteria, was exploited in the first successful experience of small intestine transplantation at the aforementioned Russian clinical institute when supplementation with a 2% solution of sugar beet pectin and probiotics resulted in the elimination of pathobionts (defined as a symbiont that is able to promote pathology only when specific genetic or environmental conditions are altered in the host) and increase of gastrointestinal beneficial bacterial symbionts (bifidobacteria and lactobacilli).[16]

Lazareva, a medical microbiologist on the Russian team pioneering the therapeutic uses of the polysaccharide, continued research on the use of pectin for the treatment of surgical and burn patients. Asked to comment for the present account she emphasized how:

"*In vitro* studies in the microbiology laboratory showed high antibacterial activity of apple and beet pectins, citrus pectin did not have a bactericidal effect.

"In the clinic, 1–2% pectin solutions (apple or beet) were used in patients of the burn center and surgical patients. The use of pectin normalized the motor activity of the gastrointestinal tract. The bactericidal action of pectin improved the microbial landscape of the intestine. At the same time, apple pectin contributed to the growth and reproduction of their own lactobacilli and bifidobacteria. We did not reveal the fungicidal action of pectin. In patients receiving pectin, the antioxidant activity of blood serum increased in patients.

"As a result of the use of a pectin solution, the frequency of bacteremia, infectious complications and mortality decreased. We have shown that pectin therapy should be carried out from an early stage and throughout the acute period of a burn disease, while clinical indicators of a scoring of Systemic Inflammatory Response Syndrome are recorded, there are prerequisites for impaired barrier function of the intestinal wall, the risk of developing dysbiosis and increased translocation of bacteria and toxins from the intestine.

"Our research took place in the early 2000s. In recent years, our suppliers have had big problems with the production of pectins, which deprived us of the possibility of further studies."[17]

Indeed, pectin production was established in the former USSR but ceased with its end in the early 1990s. Subsequently, Russia satisfied the large and increasing demand for pectin by importing more than 90 million dollars' worth on a monthly basis, with imports reaching 2503 tonnes of pectin by 2010.[18]

A major advance towards understanding the antimicrobial mechanism of action of pectin, though, was reported again by Russian scholars in 2017 describing the powerful antibacterial properties of pectin solution against both *Vibrio cholerae* and related biofilms.[19]

Using transmission electron microscopy (TEM), the team showed evidence that a 5.0% pectin solution in contact for 1 h with a *V. cholerae* biofilm caused complete destruction of cholera vibrio cells (Figure 1) otherwise unchanged when left exposed to air.[19]

As mentioned above, in the early 2010s research on the antimicrobial activity of pectin restarted across the world. In 2013, scholars in Lebanon reported the significant antibacterial activity of citrus pectin against all 16 clinical isolates and 2 reference strains of Gram-negative *Helicobacter pylori*, a common human pathogen and public health problem that causes gastritis and peptic ulcers.[20]

Once again, the highest antibacterial activity was observed at pH 5.0 and lower activity at higher pH values, with a minimum inhibitory concentration (MIC) of 0.162 mg/mL and minimum bactericidal concentration (MBC) of 0.325 mg/mL.

Two years later, the same team reported that optimal antibacterial activity against *S. aureus* and *E. coli* was observed at pH 6, with MIC values against *S. aureus* ranging between 0.39 mg/mL and 3.125 mg/mL and minimum bactericidal concentration (MBC) varying in the ample range 3.125–12.5 mg/mL.[21] A lower antibacterial activity was observed against *E. coli* with MICs of 25 and 50 mg/mL and MBC values ranging between 25 and 50 mg/mL.

The same team shortly afterwards showed that citrus pectin exerts a moderate cytotoxic and significant anti-proliferative

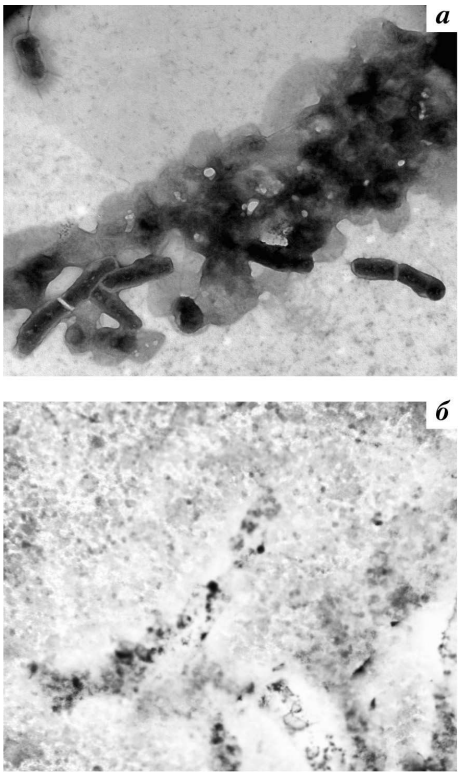

Figure 1. TEM images of *V. cholerae* El-Tor P-5879 biofilm a) without exposure to pectin and b) exposed to a 5.0% solution of pectin for 1 h. Reproduced from ref. [19] under Creative Commons Attribution 4.0 License. Copyright: 2017, the authors.

activity on human cancer cells, and also that the polysaccharide is an antioxidant exerting scavenging free radicals with a dose-dependent antioxidant activity up to 4 mg/mL (after which it starts to gel).[22] In a subsequent study devoted to the immunomodulatory activity of citrus pectin in mice, the scholars led by Abdel-Massih noted how:

"The mechanisms of actions of both pectin and its modified forms are still not understood. The complexity of the structure, the differences in extraction methods, different sources, and different fragmentation techniques make it hard to determine the active molecule(s)."[23]

The same problem was addressed by developing modified citrus pectin (MCP), namely a low molecular weight and low degree of esterification version of pectin, allowing absorption from the small intestinal epithelium into the circulation. Commenting on the results of a study using non-standardized MCP of high molecular weight, the researcher who developed standardized, low molecular mass MCP later commercialized (M-CMP in the following) after showing positive effects in multiple studies on the reduction of cardiovascular disease, fibrosis, and inflammation, wrote to the editor of a journal that had published clinical outcomes with a non-standardized version of modified citrus pectin:

"MCP is not a defined term, and the source of the material used in this study does not meet the same specifications of low molecular (<13 kDA) and esterification (<5%) molecular composition found in P-MCP. The MCP used in the Nguyen study claims an average molecular mass of 30 kDa. P-MCP has been shown to have positive health effects preclinically and clinically on cancer progression, cardiovascular disease, organ fibrosis, inflammation, heavy metal detoxification, immune modulation, and prebiotic and antibiotic properties. In the authors' conclusion, they state, 'the reason for the lack of efficacy of MCP in this DCM model remains unclear.'

"The answer could simply be that it is not the same compound as the highly standardized, low-molecular mass P-MCP."[24]

By the same token, the need to use pectin at pH not higher than 5.0 to observe bactericidal (and not bacteriostatic) activity entirely due to the galacturonic acid residues of pectin ($-C_6OOH$) was clearly explained in 1970,[5,6] confirming findings dating back to 1937.[1] Yet, in 2014 a lack of antibacterial activity of citrus pectin against *P. aeruginosa*, *Salmonella typhimurium*, *S. aureus* and *Listeria monocytogenes* was reported by scholars in Taiwan.[25] Indeed, the citrus pectin used in the latter work had high (60%) or very high (90–93%) degree of esterification, and the pH was not specified.

Not only is pectin a broad-spectrum antimicrobial agent whose *in vitro*[1,5,6,7,12,19,20] and *in vivo*[14,15,16] activity has long been established in thorough laboratory and clinical trials, but low methoxyl pectin is also a biomaterial whose exceptional biocompatible nature and superior rheological properties make it ideally suited as an injectable cell vehicle for bone tissue regeneration.[26] In the latter case, a low degree of esterification is required because the galacturonic acid groups are functionalized with an RGD-containing oligopeptide (RGD, Arg-Gly-Asp).

Similar to what subsequently noted by Eliaz, reviewing in the early 2010s the biomedical applications of pectin including tissue engineering, wound healing and drug and gene delivery, scholars in Italy emphasized how:

"Extraction procedures other than the industrial ones… to obtain controlled structures and chemical properties… acquire great importance for the biomedical research where higher cost products can be accepted to some extent to obtain specific properties."[27]

Such routes invoked in 2012 have subsequently been developed, and they do not lead to higher but rather to significantly lower pectin production costs.

3. Acid-Free Routes to Pectin

Entirely carried out in water with no addition of acid or of organic solvent, two methods for the production of high-quality pectin from suitable natural sources such as the peels of lemon, orange, grapefruit or *Opuntia-ficus indica* (OFI) are ready for scale-up and industrialization: microwave assisted extraction (MAE) and hydrodynamic cavitation.

Applied for example in the advanced version of microwave distillation, hydrodiffusion and gravity followed by freeze-drying (MHG-FD) directly on a semi-industrial scale to > 20 kg of waste lemon or waste orange peel, the former method affords low-methoxy pectin, with increased fraction in the relative content in galacturonic acid (HG) regions, leading to a more aggregated structure.[28]

Visual inspection of the pectin obtained (Figure 2) and subsequent analyses showed evidence that this newly extracted pectin contained both citrus terpenes and flavanones responsible also for the yellow color of the lemon peel incorporated in the structure of the biopolymer.

Applied to the milled peel of *Opuntia-ficus indica* (OFI) the green extraction method followed by dialysis and lyophilization affords highly pure pectin with a high degree of crystallinity (Figure 3) and intermediate degree of esterification (DE) of 53% with a larger percentage (compared to citrus pectin extracted with the same process) of hairy RG regions that promote the formation of more entangled structures, playing a gel-stabilizing role.[29]

The method has opened the route to nutraceutical- and pharmaceutical-grade low methoxy pectins from citrus, in which the source fruit and its regions are chosen according to the desired DE.[30] The DE of pectin extracted from different regions of citrus fruits (red orange, lemon, and grapefruit) by microwave-assisted hydrodiffusion increases in the order waste < peel < outer skin for red orange, inverting for lemon, with pectins from lemon waste, red orange (*Citrus sinensis*) peel, and grapefruit peel (34% DE) being the richest in galacturonic acid regions.

Compared to low-methoxyl (29% DE) pectin obtained from waste orange peel by MHG-FD,[28] pectin obtained on semi-industrial scale from processing 42 kg of waste orange peel in 120 L of water only via controlled hydrodynamic cavitation using an aptly developed device comprising a Venturi-shaped cavitation reactor has a low degree of esterification of 17%.[31] Entirely carried out in water, with no addition of acid or of organic solvent, this method is a new route to the integral valorization of this by-product, based on simple equipment, speed, effectiveness and efficiency, scalability, and compliance with green extraction principles.

Dubbed IntegroPectin for the concomitant presence of orange flavonoids (flavanones and hydroxycinnamic acid derivatives) and terpenes (mainly *d*-limonene) adsorbed at its surface (Figure 4), this pectin showed the first evidence of antimicrobial properties as did not degrade for 18 months, stored at room temperature and exposed to air, between its production by extraction and lyophilization and its analysis.

Pectolytic enzymes abundant in many fungi including *A. niger*, el-Nakeeb and Yousef reported in 1970,[5] confirming findings going back to 1964, drive rapid degradation of pectin powder and pectin solutions which become heavily contaminated with molds when they were exposed to air at room temperature.

The method was thus applied to extract IntegroPectin from waste lemon peel obtained from a citrus company in Sicily processing only organically grown lemons, carrying out the extraction in a closed hydrocavitation reactor in order to prevent evaporation of terpenes and other volatile compounds.[32] The outcomes in terms of antimicrobial properties of the lemon IntegroPectin thereby obtained were remarkable.

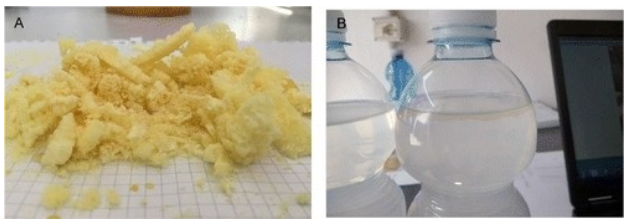

Figure 2. A) Freeze dried pectin from microwave distillation, hydrodiffusion and gravity applied to waste lemon peel. B) Supernatant essential oil obtained from the extraction of 20 kg of waste lemon peel using a commercial (MAC-75) microwave extractor. Reproduced from ref. [28], with kind permission. Copyright: 2016, American Chemical Society.

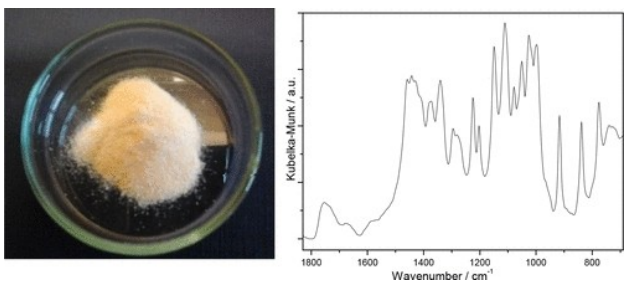

Figure 3. Pectin powder from the peel of red/green *Opuntia-ficus indica* fruit and DRIFT spectrum in the fingerprint region. Reproduced from ref. [29] with kind permission. Copyright: 2019, American Chemical Society.

4. Antimicrobial activity of lemon IntegroPectin

Lemon IntegroPectin obtained by hydrodynamic cavitation of waste lemon peel in water only shows high antibacterial activity against both Gram-positive bacterial strains of *S. aureus*[33] and

Figure 4. Sample of lyophilized orange IntegroPectin powder before (right), and after (left) grinding in a quartz mortar. Reproduced from ref. [31] under Creative Commons Attribution (CC BY) license.

against Gram-negative bacteria such as *P. aeruginosa* and *E. coli*.[34]

The antibacterial effect of the new IntegroPectin was found to be largely superior to that of commercial citrus pectin. For example, both commercial citrus pectin and lemon IntegroPectin inhibit *S. aureus* growth (Figure 5), with a decrease in the number of viable cells in the range 1 to 2 log units, respectively, when the concentration was 3 mg mL^{-1}. In closer detail, the $\log_{10}$ (CFU) went from 8.3 for the control to 6.8 and 7.2 for IntegroPectin and commercial citrus pectin, respectively.[33]

A higher difference in antibacterial activity of commercial pectin and lemon IntegroPectin was noted when cultures were challenged with 6 mg mL^{-1}. Indeed, in the presence of 6 mg mL^{-1} of commercial pectin the $\log_{10}$ (CFU) remained almost unvaried (from 7.2 to 7.1). Doubling the concentration of lemon IntegroPectin, the viable bacterial count decreased from 6.8 to 6.2, thus highlighting the greater antimicrobial power of lemon IntegroPectin when compared to commercial citrus pectin.[33]

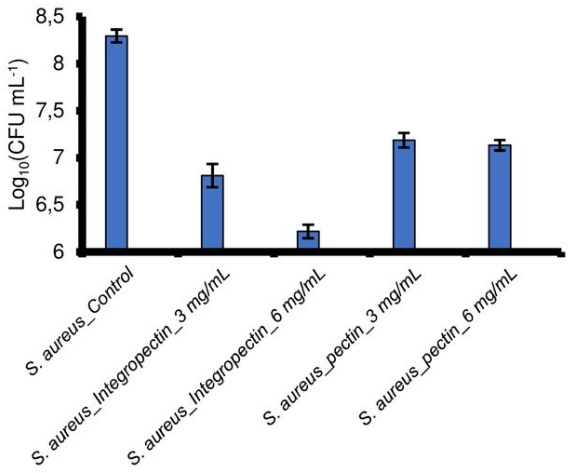

Figure 5. Viable cells of *S. aureus* ATCC 25923 in the presence of lemon IntegroPectin and of commercial citrus pectin. Reproduced from ref. [33] with kind permission. Copyright: 2020, the authors.

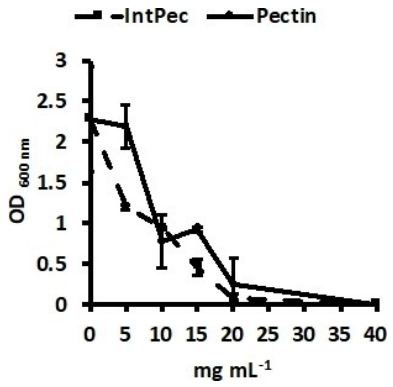

Figure 6. Optical density at 600 nm due to viable cells of *S. aureus* ATCC 25923 in the presence of increasing concentrations of lemon IntegroPectin and of commercial citrus pectin.

Evaluated in terms of the minimal inhibitory concentration (MIC, Figure 6) the antibacterial effect of IntegroPectin against the ubiquitous pathogen *S. aureus* was twice larger (MIC = 20 mg mL^{-1}) than that of commercial citrus pectin (40 mg mL^{-1}).

The same ratio holds for the antibacterial effect against the ubiquitous pathogen *P. aeruginosa* with MIC = 10 mg mL^{-1} for lemon IntegroPectin and 20 mg mL^{-1} for commercial citrus pectin.[34] However, the minimal bactericidal concentration (MBC, the concentration value for obtaining a total killing effect) of the lemon IntegroPectin against *P. aeruginosa* is 15 mg mL^{-1}, whereas that of citrus pectin is 40 mg mL^{-1}, almost three times higher.

Preliminary insight on the antibacterial mechanism of IntegroPectin from investigating its inhibitory activity against *E. coli* suggests (Figure 7) that lemon IntPec at 1.0 mg/mL already after 16 min (~1000 s) the amount of reactive oxygen species (ROS) is higher than that generated by concentrated hydrogen peroxide, to eventually almost double the oxidative stress exerted by H_2O_2 0.5 M 1.7 h after the addition of the new pectic substance.[34]

Dissolved in 1.0 mg/mL concentration this new form of lemon pectin is readily adsorbed at the surface of the microbes where it rapidly drives (after ~1000 s) the formation of reactive oxygen species (ROS) in higher amount than that generated by 0.5 M hydrogen peroxide, to eventually almost double the oxidative stress exerted by the latter strong oxidant 1.7 h since the addition of the new pectic substance.[34]

In other words, the very same non-cytotoxic pectin showing exceptionally high antioxidant activity expressed by an oxygen radical absorbance capacity (ORAC) value of 122 200 μmol TE/100 g (μmol of Trolox equivalents per 100 g of pectin)[32] adsorbed at the surface of a typical Gram-negative bacterium behaves as a strong oxidizer driving formation of high amounts of ROS.

Regardless of its high and rapid oxidant capacity against *E. coli* bacterial cells, however, the same lemon IntegroPectin shows exceptionally high antioxidant activity expressed by its ORAC value of 122 200 μmol TE/100 g (μmol of Trolox equiv-

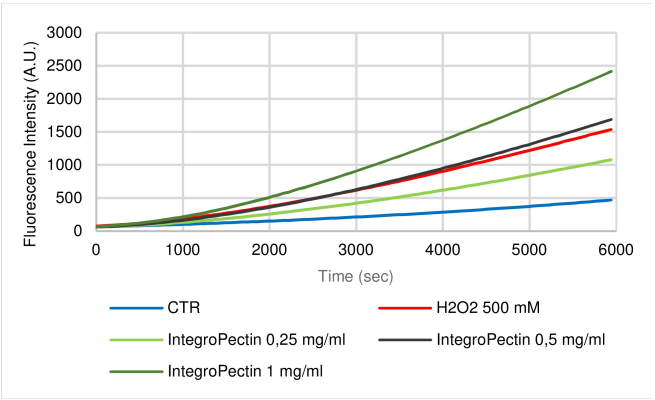

Figure 7. *E. coli* oxidation kinetics driven by aqueous H_2O_2 0.5 M and by increasing concentrations of lemon IntPec. CTR stands for control sample. Reproduced from ref. [34] under CC-BY-ND 4.0 International license. Copyright: 2020, the authors.

alents per 100 g of pectin), and it is completely devoid of cytotoxic activity towards human epithelial (lung) cells up to high concentrations of 1 mg/mL.[32]

Given the non-cytotoxic[32] nature of citrus IntegroPectin and the ease of its reproducible production in large amounts,[31] the route is open to the industrial development of a new antimicrobial treatment against polymicrobial infections, unlikely to develop drug resistance, based on a new form of natural, unmodified citrus pectin now obtainable in large amounts and at low cost from citrus juice industry's waste. Strains of Gram-positive *S. aureus*, indeed, are often present in conjunction with *P. aeruginosa* forming hazardous polymicrobial complex communities particularly resistant to antibiotics.[35]

5. Summary and Outlook

Pectin is a broad-spectrum antimicrobial capable to kill Gram-negative bacteria, yeasts and nonfilamentous fungi. Its mechanism of action likely involves the binding action of the carboxylic acid groups in the main backbone of the biopolymer. Optimal antibacterial activity is generally observed at acid pH, with a threshold identified around pH 5–6 since the early studies dating back to the late 1930s and 1940s.[1,5,6]

Surprisingly, research on the antibacterial action of pectin polysaccharides first reported in 1937[1] lagged for decades even after fully demonstration, in 1970, of the biocidal action of citrus pectin against Gram-negative strains (and inhibitory activity against Gram-positive bacteria), as well as against nonfilamentous fungi.[5,6]

Research restarted in the late 1990s chiefly in Russia[7] where it continued until, in 2017, the first evidence that the antimicrobial activity of pectin dissolved in aqueous solution lies in its ability to bind and destroy the outer membrane of microbes was reported.[19]

Most research on the antimicrobial activity of pectins carried out in the last decade in countries besides Russia has been devoted to the uses of pectin as a carrier for well-known antimicrobials such as silver or essential oils. For example, scholars in Italy reported in 2017 the excellent antibacterial and wound healing activity of citrus pectin embedding Ag nanoparticles against both Gram-positive *Staphylococcus epidermidis* and Gram-negative *E. coli* bacterial strains.[36] Similarly, researchers in Thailand showed in 2018 how mango-derived pectin loaded with 3% orange oil forms an antibacterial film highly active against *S. aureus*.[37]

A noticeable exception was the series of studies carried out by scholars in Lebanon reporting first the significant antibacterial activity of citrus pectin against Gram-negative *H. pylori*;[20] and then also against *S. aureus* and *E. coli*.[21]

Using a new form of citrus pectin extracted and isolated from waste citrus peel under acid-free conditions by hydrodynamic cavitation in water followed by freeze drying,freeze-drying scholars in Italy first confirmed the broad spectrum activity of pectin against both Gram-positive and Gram-negative ubiquitous pathogens such as *S. aureus*[33] and and *P. aeruginosa*.[34] Later, they extended the approach to grapefruit IntegroPectin providing the first insight on the antibacterial mechanism of this new form of pectic material interacting with the cell membrane of *S. aureus* and *P. aeruginosa* strains.[38]

Due to its proven immunomodulating, anti-inflammatory, antioxidant, hypolipidemic, antidiabetic, anticarcinogenic, antitussive, gastroprotective, and wound-healing properties, pectin has recently been termed "a universal medicine"[39] by scholars in Russia reviewing the medical uses of pectin in medicine, both as therapeutic substance as well as a biomaterial for regenerative medicine and biomedical engineering.

In the 2020s and subsequent decades, several new biomedical uses of pectin polysaccharides will emerge, including the use of pectin-based new-generation antimicrobials. These novel uses require to replace the one-century old industrial extraction method based on the hydrolysis of dried citrus peels or apple pomace with mineral acids followed by precipitation of the degraded polymer with isopropyl alcohol,[3] with acid-free and organic solvent-free processes affording new forms of pectin polymers devoid of the degradation occurring during the conventional extraction (hydrolysis and loss of the ramified rhamnogalacturonan chains).

Along with less and different molecular degradation, processes such as microwave hydrodiffusion and gravity[28] and hydrodynamic cavitation carried out in an easily scalable Venturi tube[31] or by ultrasound[40] applied to the wet peels of different fruits (apple, citrus, *Opuntia-ficus indica* etc.) suspended in water only, followed by freeze-drying, afford pectins embedding bioactive phytochemicals such as polyphenols, flavonoids, terpenes and phenolic acids. The resulting pectins offer a large potential in terms of synergistic physiological and biological activity[38,41] which remains largely untapped and represents a new and highly promising area of research in the life sciences and in medicine.

Now that the aforementioned green extraction methods have been developed on industrial and semi-industrial scales, new important medical and bioengineering usages of this uniquely versatile biopolymer will follow within a few years, including those as tissue engineering biomaterial.[26] Adding to the fully biocompatible nature of pectin, its long-neglected antimicrobial properties summarized in this study will accelerate the practical uptake of this versatile polymer in the biomedical industry of the 21st century.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: antibacterial agents, antimicrobial agents · citrus pectin · pectin · polysaccharides

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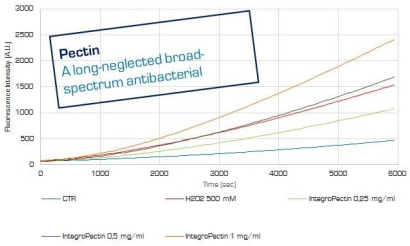
CONCEPTS

Pectin, antimicrobial of the near future: Known since 1937, the antibacterial activity of pectin remained almost ignored until the late 1990s. This study provides an updated overview of pectin as antimicrobial. Less-degraded pectin extracted by green extraction routes will, we conclude, find applications spanning from treatment of polymicrobial infections through implantable biomaterial in tissue and bone engineering.



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Pectin: A Long-Neglected Broad-Spectrum Antibacterial

Special Collection