

■ Biological Chemistry & Chemical Biology

A Gluten-Free Biscuit Fortified with Lemon IntegroPectin

Domenico Nuzzo,^{*[a]} Antonino Scurria,^[b] Pasquale Picone,^[a] Alessandro Guiducci,^[c] Mario Pagliaro,^{*[b]} Giuseppe Pantaleo,^[b] Lorenzo Albanese,^[d] Francesco Meneguzzo,^[d] and Rosaria Ciriminna^{*[b]}

We report the first outcomes of producing a gluten-free biscuit by replacing 2.5 wt% of the rice flour used in the preparation of a commercial gluten-free cookie with lemon IntegroPectin, a new citrus pectin obtained from lemon processing waste via hydrodynamic cavitation. The cookie's friability, palate adhesion, flavor persistency and compactness remain virtually unchanged, whereas sapidity and color improve. Only the

sweetness and the smell (flavor) of the functionalized cookie are lower than those of the commercial biscuit. The enhanced heat resistance and unique structure of this new citrus pectin unveiled by XRD and TGA structural and thermal analyses make it suitable for the straightforward functionalization of bakery products with numerous potential health benefits.

Introduction

Plentiful nutrition research carried out in the last five decades suggests that a balanced diet low in calories and rich of phytonutrients prevents chronic disease, including pathologies linked to aging.^[1] For example, coupled to physical exercise a phytonutrient-rich dietary pattern, rich in fresh fruit and vegetables with low intake of meat, refined grains, sugar, saturated fat, and salt prevents the development of diabetes and metabolic syndrome,^[2,3] and neurodegeneration.^[4]

Biscuits are among the most widely and increasingly consumed food in economically developed countries. For instance, in 2020 virtually all (99.5%) households in Great Britain purchased biscuits, with sales approaching £3 billion (7.2% annual growth in sales), and sweet biscuits accounting for 81% of sales.^[5] Due to high sugar content and widespread utilization of palmitic oil, most biscuit and bakery products are

often rich in saturated fats and calories. Furthermore, the reformulation of these products begun in the early 2000s to lower (or even eliminate altogether) the hazardous *trans* fatty acids formed upon catalytic hydrogenation of edible fats, has occurred at expenses of increasing the amount of less toxic but still unhealthy saturated fatty acids.^[6]

Besides using healthier (and far more expensive) fats such as olive oil,^[7] an approach to reduce calories and produce healthier bakery products now common in industry is the use of carbohydrate hydrocolloids and proteins. The former act as fat mimetics entrapping the vegetable oil within a gel network, retaining good sensory properties and palatability.^[8,9]

Carbohydrates such as β -glucan, partially hydrolysed guar gum, polydextrose and inulin, and proteins such as those of whey, today are used as fat replacers in a large number of food products, including cookies.^[10]

Pectin, the most valued food hydrocolloid ingredient,^[11] has long been used as a water binder and fat replacer in low fat food products. Driven by its performance (the hydrocolloid mimics the mouthfeel of fats) and by the increasing consumer demand for healthy food ingredients, pectin is widely used as a fat replacer in low-fat dressings, mayonnaise, beverages, ice creams, yoghurts and milk drinks.^[12] A further benefit is that pectin is an effective satiety inducer due to its ability to not lose its hydrogel structure under gastric and intestinal conditions.

Employed to produce low fat biscuits, however, citrus-derived pectin significantly increases bitterness,^[13] an undesirable property of products which are chiefly sold as sweet foodstuff. Good results in terms of texture were obtained in the early 2010s using pectin derived from apple pomace.^[14] Replacing shortening up to a level of 30% produced more tender cookies. The resulting taste, however, was not reported.

Following a recent study in which a biscuit containing algae extracts rich in polyphenols was used to produce a functional cookie for the prevention of metabolic and age-related diseases,^[15] now we report the outcomes of producing a

[a] Dr. D. Nuzzo, Dr. P. Picone

Istituto per la Ricerca e l'Innovazione Biomedica, CNR,
via U. La Malfa 153, 90146
Palermo, Italy
E-mail: domenico.nuzzo@cnr.it

[b] Dr. A. Scurria, Dr. M. Pagliaro, Dr. G. Pantaleo, Dr. R. Ciriminna

Istituto per lo Studio dei Materiali Nanostrutturati,
CNR, via U. La Malfa 153,
90146 Palermo, Italy
E-mail: rosaria.ciriminna@cnr.it
mario.pagliaro@cnr.it


[c] Dr. A. Guiducci

Istituto Euro Mediterraneo di Scienza e Tecnologia, via M. Miraglia 20,
90139 Palermo, Italy

[d] Dr. L. Albanese, Dr. F. Meneguzzo

Istituto per la Bioeconomia, CNR, via Madonna del Piano 10, 50019 Sesto
Fiorentino, Italy

 Supporting information for this article is available on the WWW under
<https://doi.org/10.1002/slct.202104247>

 © 2022 The Authors. ChemistrySelect published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

gluten-free biscuit using lemon IntegroPectin, namely a new citrus pectin obtained from lemon processing waste via hydrodynamic cavitation. Production of biscuits with this new pectin showing exceptional antioxidant^[16] and (*in vitro*) neuroprotective^[17] activity, might result not only in a gluten-free and low-calorie cookie but also in a fortified biscuit capable to aid in the prevention of chronic disease.

Results and discussion

As mentioned above, the cookies were produced by a local company specialized in food manufacture for celiac patients adding IntegroPectin at 2.5 wt% at the expenses of rice flour. Figure 1 shows that cooking the functionalized and the reference dough in an oven at 180 °C for 15 min resulted in an even brighter and more pleasant yellow color of the resulting cookie.

Twenty volunteer subjects were enrolled for the sensory characterization of the IntegroPectin cookie formulations. Plot in Figure 2 shows that the panel perception of friability, palate

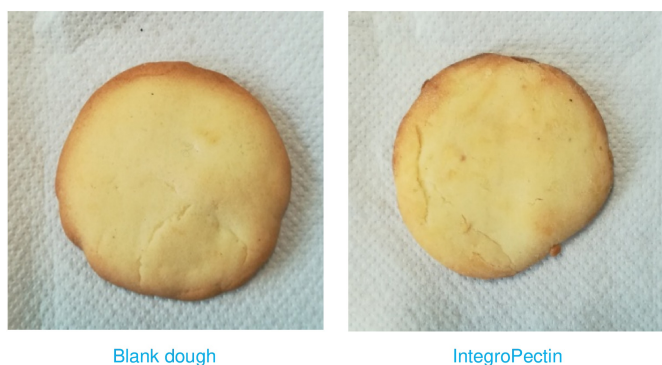


Figure 1. Unmodified rice-based cookie (left) and cookie modified with 2.5 wt% lemon IntegroPectin (right).

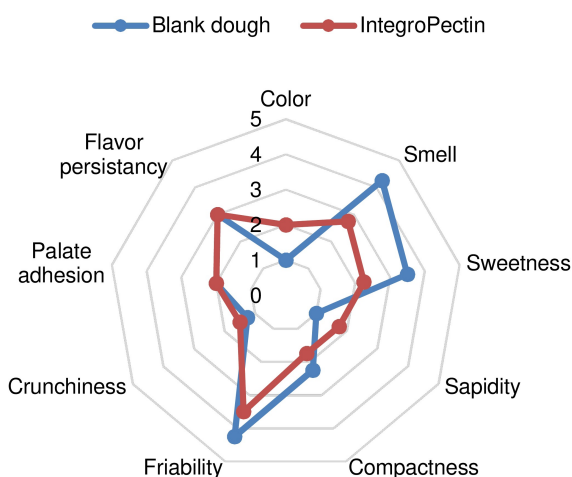


Figure 2. Sensory plot with respect to nine different descriptors of rice-based cookie (blue line) and cookie functionalized with 2.5 wt% lemon IntegroPectin (red line) evaluated by a panel of 20 subjects.

adhesion, flavor persistency and compactness remained virtually unchanged.

Only the sweetness and the smell (flavor) of the functionalized cookie turned out to be lower than those of the commercial biscuit. In detail, sweetness nearly halved from 3.8 to 2.1, whereas the smell went from 4.1 to 2.9.

Figure 3 shows that the viability of human colorectal cells (Caco-2) cultured *in vitro* after 4 h and after 24 h in the presence of increasing dosage from 10 to 40 µg/mL of IntegroPectin or heat-treated IntegroPectin (180 °C for 20 min) even increased when compared to the control group (Control) receiving an equal volume of PBS buffer solution. The significant heat treatment of this new citrus pectin did not alter its ability to exert its cytoprotective activity. This finding is in agreement with the fact that the ORAC (Oxygen Radical Absorbance Capacity) value of lemon IntegroPectin after heat treatment at 200 °C for 5 min is even higher (126,800 µmol TE/100 g) when compared to the non heat-stressed pectin (122,200 µmol TE/100 g).^[16]

Besides the intrinsic antioxidant activity of hydroxyl-rich pectin polysaccharide,^[18] the exceptionally high antioxidant activity of lemon IntegroPectin is due to the large amounts of citrus flavonoids and phenolic acids adsorbed at its surface, particularly eriocitrin (3.35 mg/g), hesperidin (0.60 mg/g), and gallic acid (0.56 mg/g).^[19]

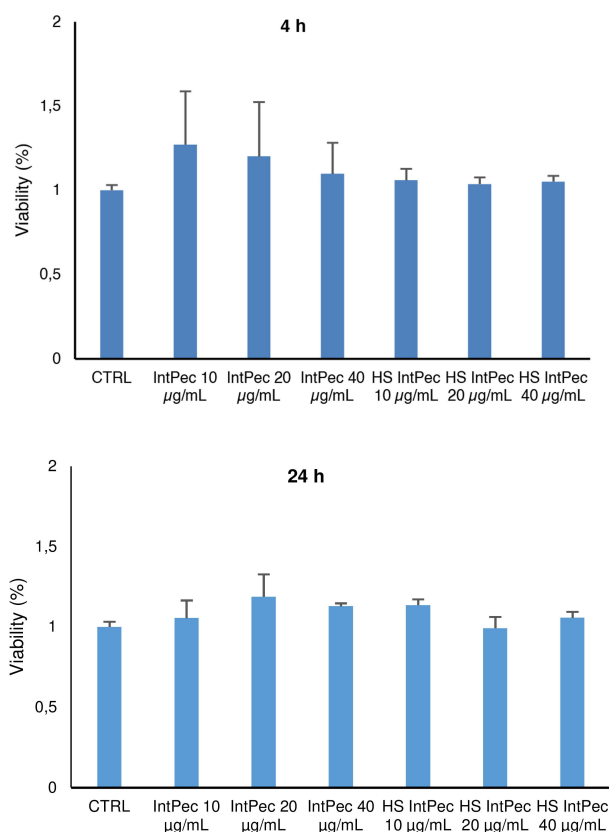


Figure 3. Viability of Caco-2 human colorectal cells cultured *in vitro* after 4 h (top) and 24 h (bottom) in the presence of increasing dosages of lemon IntegroPectin or heat-treated lemon IntegroPectin.

Figure 4 shows optical microscopy evidence that the treatment of the cells with increasing dosages of both IntegroPectin and heat-stressed IntegroPectin did not alter the cell morphology and viability in the aqueous dispersion.

From a structural viewpoint, lemon IntegroPectin is very different when compared to commercial citrus pectin extracted via conventional hydrolytic extraction in hot acidic water followed by precipitation with alcohol. The X-ray diffraction (XRD) pattern of lemon IntegroPectin (Figure 5) shows that most diffraction peaks characteristic of lemon pectin at 12.36,

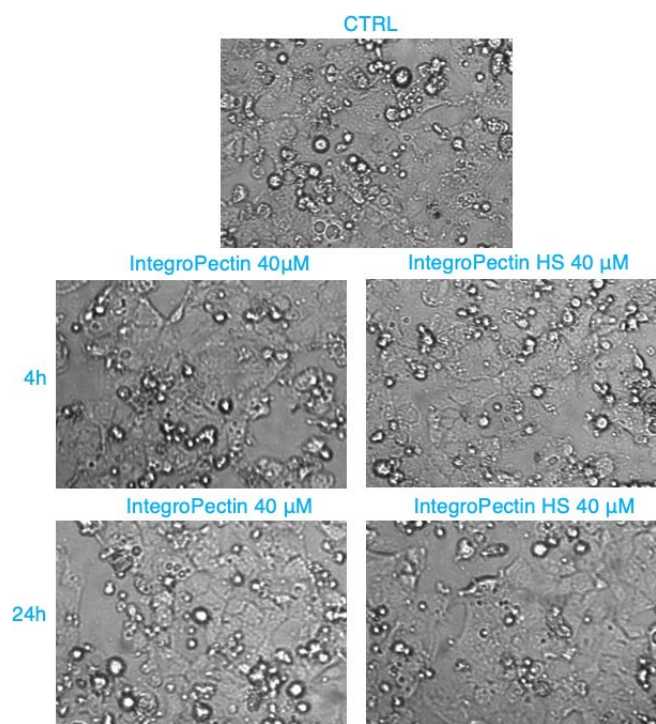


Figure 4. Optical microscopy photographs of Caco-2 human colorectal cells in the presence of increasing dosage of lemon IntegroPectin and heat-treated lemon IntegroPectin.

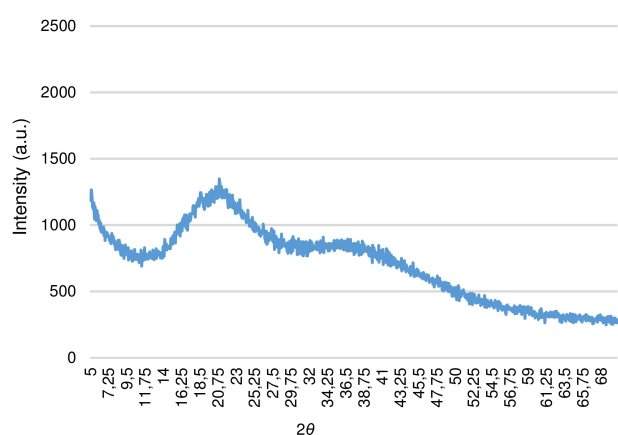


Figure 5. X-ray diffraction pattern of lemon IntegroPectin.

13.96, 14.91, 19.61, 18.91, 21.36, 32.46 and 36.66° (2θ) due to the crystalline regions of semicrystalline lemon pectin^[20] disappear, with the remaining peaks shifting to higher 2θ values.

This indicates that hydrodynamic cavitation (HC) of lemon biowaste induces nearly complete decrystallization of the homogalacturonan (HG) chains of lemon pectin (industrially obtained from dried lemon peel via acid hydrolysis in hot water)^[21] crystallizing in hexagonal closest packing arrangement.^[22] Cavitation, in other words, seems to destroy the “fringed-micellar” structure of the crystalline regions of semicrystalline pectin.^[23]

This finding, along with the low degree of esterification (lemon IntegroPectin is a low-methoxyl citrus pectin with DE = 27%)^[17] explains also the significantly larger solubility of IntegroPectin, which readily dissolves in water at room temperature when compared to the poorly soluble commercial conventional citrus pectin (having DE = 70%), requiring prolonged mechanical stirring at higher temperature.

We briefly remind that high methoxyl pectins (DE > 50%) are stabilised by hydrophobic interactions between ester groups and hydrogen bonds (with participation of the free carboxylic groups), and destabilised by the electrostatic repulsion between negatively charged carboxylate groups, and steric hindrances of the side chains.^[24] Both carboxylate and rhamnogalacturonan I (RG-I) lateral chains, indeed, are particularly abundant in the newly obtained lemon (and grapefruit) IntegroPectin.^[25]

The thermogravimetric analysis (TGA) of the new IntegroPectin and of commercial citrus pectin provided further structural information. In agreement with previous results,^[26,27] the TGA profiles (Figure 6) are similar showing three regions at 50–200 °C, 200–400 °C and 400–600 °C, though in the present case somewhat shifted to 50–250 °C, 250–500 °C and above 500 °C.

The first step corresponds to loss of water adsorbed at the surface of the hydrophilic regions of the biopolymer. Likewise, to what happens with grapefruit pectin obtained via acoustic cavitation,^[27] the water content of the commercial citrus pectin is higher than that in lemon IntegroPectin, with a 39.10%

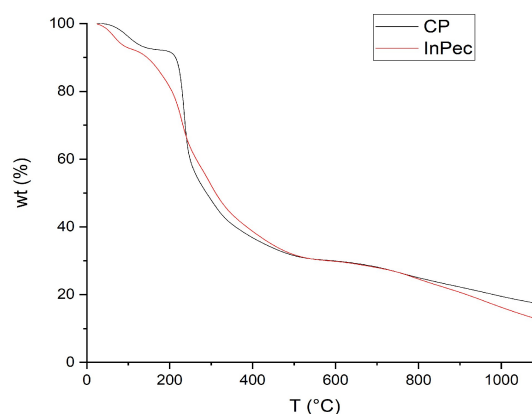


Figure 6. TGA curves of lemon IntegroPectin and commercial citrus pectin.

weight loss in the latter compared to 41.80% in the conventional pectin.

The second step between 200 and 400 °C corresponding to the polysaccharide pyrolytic decomposition consisting in a primary and secondary decarboxylation (involving the acid side group and a carbon in the ring),^[26] and further water loss due to cleavage of hydroxyl groups in pectin lateral chains, takes place at slower pace for lemon IntegroPectin. The higher mass loss for lemon IntegroPectin (43.50%) compared to commercial citrus pectin (39.05%) in this region is likely due to loss of adsorbed terpenes^[28] and flavonoids^[19] present in the former whole pectin, but not in commercial citrus pectin which undergoes extensive purification after precipitation with alcohol.

The third region above 400 °C reveals a slow mass loss due to thermal decomposition of the solid biochar containing polyaromatic structures that are slowly degraded with formation of compact polyaromatic stacks.^[29]

The differential scanning calorimetry (DSC) curves (not shown) have a similar shape, but whereas an exothermic transition was recently observed at the same temperature (233 °C) for both citrus pectin extracted with the conventional hydrolytic process and pectin obtained via acoustic cavitation (pointing to decomposition of pectin at this temperature),^[18] no exothermic transition peak was noted for the lemon IntegroPectin. This shows further evidence of the enhanced thermal resistance of this new pectin, suggesting its widespread use in bakery products.

Conclusions

A fortified biscuit was produced by replacing 2.5% of the rice-based flour with the new citrus pectin "IntegroPectin" derived from lemon processing waste via hydrodynamic cavitation.

The organoleptic features of the food are well preserved, but for smell and sweetness, avoiding the off-flavour classically due to limonene and other terpenes mixed with this new citrus pectin.^[28]

In detail, the fortified cookie's friability, palate adhesion, flavor persistency and compactness remain virtually unchanged, whereas sapidity and colour, in the Integropectin-functionalized cookie, are even improved.

Only the sweetness and the smell (flavor) of the functionalized cookie were lower than those of the commercial biscuit (sweetness nearly halved from 3.8 to 2.1, whereas the smell went from 4.1 to 2.9). Rich in type I rhamnogalacturonan regions preserved during the HC-based extraction from lemon processing waste, the new pectin is likely to provide further benefits. The RG-I region, indeed, is far more bioactive than the homogalacturonan portion of pectin, which explains some of the numerous health benefits associated to fruit and vegetable consumption.^[30]

Indeed, optical microscopy investigation shows evidence that the treatment of human colorectal cells (Caco-2) cultured *in vitro* in the presence of increasing dosages (from 10 mg/L to 40 mg/L) of IntegroPectin even increased the cell viability with no sign of cell morphology alteration.

Production of biscuits with this new pectin showing exceptional antioxidant^[16] and (*in vitro*) neuroprotective^[17] activity, might therefore result in a functional cookie capable to aid in the prevention of chronic disease. Studies aimed at investigating the physiological effects of regular assumption of this and related fortified bakery products produced using these new citrus pectins are in progress.

Alongside with showing that the substitution of more expensive ingredients by food-waste derived ingredients improves the cyclic economy approach and when used on industrial scale avoids notable organic pollution into aquatic and land ecosystems, this fundamental study shows how the enhanced heat resistance and higher solubility of lemon IntegroPectin (compared to commercial citrus pectin) make it suitable for the straightforward functionalization of bakery products with numerous potential health benefits.

Acknowledgements

We thank OPAC Campisi Società Cooperativa Agricola (Siracusa, Italy) for a generous gift of waste lemon peel from which the IntegroPectin was extracted. Thanks to Dr Francesco Giordano, Istituto per lo Studio dei Materiali Nanostrutturati, CNR, for the XRD measurements. We are indebted to Gloria Bosco, "Le Farine dei Nostri Sacchi", Palermo, Italy, for her kind willingness to comply with our requests concerning this research. Open Access Funding provided by Consiglio Nazionale delle Ricerche within the CRUI-CARE Agreement. This research was partly supported by Programma ENI CT Italia-Tunisia 2014-2020, Re-lancer une nouvelle économie (Re-Né).

Conflict of Interest

The Authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Biological activity · IntegroPectin · neuroprotective · pectin · sustainable chemistry

- [1] M. B. Schulze, M. A. Martínez-González, T. T. Fung, A. H. Lichtenstein, N. G. Forouhi, *BMJ* **2018**, *361*, k2396.
- [2] K. Z. Walker, K. O'Dea, *Preventing Diabetes with Diet and Exercise In Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome*, D. Bagchi, N. Sreejayan (Ed.s), Academic Press, Cambridge (MA), **2012**, 125–134.
- [3] A. Amato, G. F. Caldara, D. Nuzzo, S. Baldassano, P. Picone, M. Rizzo, F. Mulè, M. Di Carlo, *Nutrients* **2017**, *9*, 492.
- [4] P. Picone, M. Di Carlo, D. Nuzzo, *Eur. J. Neurosci.* **2020**, *52*, 3944–3950.
- [5] Pladis Global, *Winning with Biscuits. Annual Biscuit Review 2020*, London: 2021.
- [6] L. A. Teixeira Santos, R. Cruz, S. Casal, *Food Control* **2015**, *47*, 141–146.
- [7] P. Taracón, A. Salvador, T. Sanz, S. Fiszman, A. Tárrega, *Food Res. Int.* **2015**, *69*, 91–96.
- [8] T. A. Stortz, A. K. Zetzl, S. Barbut, A. Cattaruzza, A. G. Marangoni, *Lipid Technol.* **2012**, *24*, 151–154.

- [9] P. Tarancón, S. M. Fiszman, A. Salvador, A. Tárrega, *Food Res. Int.* **2013**, *53*, 134–14.
- [10] E. I. Zoulias, V. Oreopoulou, C. Tzia, *J. Food Eng.* **2002**, *55*, 337–342.
- [11] D. Seisun, N. Zalesny, *Food Hydrocoll.* **2021**, *117*, 106575.
- [12] S. Padma Ishwarya, R. Sandhya, P. Nisha, *Crit. Rev. Food Sci. Nutr.* **2021**, DOI:10.1080/10408398.2021.1875394.
- [13] F. D. Conforti, S. A. Charles, S. E. Duncan, *J. Food Qual.* **1997**, *20*, 247–256.
- [14] B. Min, I. Young Bae, H. Gyu Lee, S.-H. Yoo, S. Lee, *Bioresour. Technol.* **2010**, *101*, 5414–5418.
- [15] D. Nuzzo, M. Contardi, D. Kossyvakis, P. Picone, L. Cristaldi, G. Galizzi, G. Bosco, S. Scoglio, A. Athanassiou, M. Di Carlo, *Oxid. Met.* **2019**, *2019*, 9481390.
- [16] D. Nuzzo, L. Cristaldi, M. Sciortino, L. Albanese, A. Scurria, F. Zabini, C. Lino, M. Pagliaro, F. Meneguzzo, M. Di Carlo, R. Ciriminna, *ChemistrySelect* **2020**, *5*, 5066–5071.
- [17] D. Nuzzo, P. Picone, C. Giardina, M. Scordino, G. Mudò, M. Pagliaro, A. Scurria, F. Meneguzzo, L. M. Ilharco, A. Fidalgo, A. Presentato, R. Alduina, R. Ciriminna, V. Di Liberto, *Antioxidants* **2021**, *10*, 669.
- [18] S. T. Minzanova, V. F. Mironov, D. M. Arkhipova, A. V. Khabibullina, L. G. Mironova, Y. M. Zakirova, V. A. Milyukov, *Polymers* **2018**, *10*, 1407.
- [19] A. Scurria, M. Sciortino, L. Albanese, D. Nuzzo, F. Zabini, F. Meneguzzo, R. V. Alduina, A. Presentato, M. Pagliaro, G. Avellone, R. Ciriminna, *ChemistryOpen* **2021**, *10*, 1055–1058.
- [20] Z. Rahmani, F. Khodaiyan, M. Kazemi, A. Sharifan, *Int. J. Biol. Macromol.* **2020**, *147*, 1107–1115.
- [21] R. Ciriminna, A. Fidalgo, R. Delisi, L. M. Ilharco, M. Pagliaro, *Agro-Food-Ind. Hi-Tech* **2016**, *27* (5), 17–20.
- [22] K. J. Palmer, M. B. Hartzog, *J. Am. Chem. Soc.* **1945**, *67*, 2122–2127.
- [23] R. M. Gohil, *J. Appl. Polym. Sci.* **2010**, *120*, 2324–2336.
- [24] U. Einhorn-Stoll, H. Kunzek, *Food Hydrocoll.* **2009**, *23*, 40–52.
- [25] A. Presentato, E. Piacenza, A. Scurria, L. Albanese, F. Zabini, F. Meneguzzo, D. Nuzzo, M. Pagliaro, D. Chillura Martino, R. Alduina, R. Ciriminna, *Antibiotics* **2020**, *9*, 586.
- [26] H.-S. Shim, M. R. Hajaligol, V. L. Baliga, *Fuel* **2004**, *83*, 1495–1503.
- [27] W. Wang, X. Ma, P. Jiang, L. Hu, Z. Zhi, J. Chen, T. Ding, X. Ye, D. Liu, *Food Hydrocoll.* **2016**, *61*, 730–739.
- [28] A. Scurria, M. Sciortino, A. Presentato, C. Lino, E. Piacenza, L. Albanese, F. Zabini, F. Meneguzzo, D. Nuzzo, M. Pagliaro, D. Francesca Chillura Martino, R. Alduina, G. Avellone, R. Ciriminna, *Molecules* **2021**, *26*, 51.
- [29] S. Zhou, Y. B. Xu, C. H. Wang, Z. F. Tian, *J. Anal. Appl. Pyrolysis* **2011**, *91*, 232–240.
- [30] D. Wu, J. Zheng, G. Mao, W. Hu, X. Ye, R. J. Linhardt, S. Chen, *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2938–2960.

Submitted: November 30, 2021

Accepted: January 14, 2022

Please note: Minor changes have been made to this manuscript since its publication in ChemistrySelect. The Editor.