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A gluten-free biscuit fortified with lemon IntegroPectin

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ABSTRACT

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 the cookie with lemon IntegroPectin, a new citrus pectin obtained from lemon processing waste via hydrodynamic cavitation showing exceptional antioxidant properties and (*in vitro*) high neuroprotective activity. The cookie's friability, palate adhesion, flavor persistency and compactness remain virtually unchanged. Only the sweetness and the smell (flavor) of the functionalized cookie are lower than those of the commercial biscuit. Production of biscuits fortified with this new pectin might result not only in gluten-free and low-calorie cookies but also in a fortified cookie capable to aid in the prevention of chronic disease such as neuronal disorders.

1. 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 unsaturated fats and calories. Furthermore, the reformulation of these products started in 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]

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

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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 today 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 nearly fifteen years later 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 gluten-free biscuit using lemon IntegroPectin, namely a new citrus pectin obtained from lemon processing waste via hydrodynamic cavitation.

Having exceptional antioxidant[16] and (*in vitro*) neuroprotective[17] activity, production of biscuits with this new pectin 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 such as neuronal disorders.

2. Materials and methods

2.1 Cookie preparation

The cookies were produced by a local company ("Le Farine dei Nostri Sacchi", Palermo, Italy) specialized in food manufacture for celiac patients using a proprietary recipe. IntegroPectin was added at 2.5 wt% at the expenses of rice flour. The reference dough is indicated as Blank dough (BD). For the cookie preparation, the doughs were kept in the oven at the temperature of 180 °C for 15 min.

2.2 Panel test

Twenty volunteer subjects were enrolled for the sensory characterization of the unmodified cookie as well as of the cookie modified with 2.5 wt% lemon IntegroPectin. The blind panel was asked to express its opinion through the evaluation of nine different descriptors. The evaluation was performed by assigning a score from 1 (very low intensity) to 5 (very high intensity) for each descriptor. The subjects were informed in advance about the evaluation conditions.

2.3 Solubilization of IntegroPectin

IntegroPectin were solubilized dissolving 1 mg of IntegroPectin powder in 10 mL of Phosphate Buffer Saline (PBS, pH = 7.4, 137 mM NaCl, 2.7 mM KCl, 8 mM Na_3PO_4). The solution was filtered using a 0.45 µm sartorius filter, aliquoted (1 mL/vial), and stored at +4 °C.

2.4 Cell cultures and treatment

Cell line of human colorectal cells (Caco-2) were cultured in T25 tissue culture flasks. Complete DMEM/F12 supplemented with 10% (v/v) FCS and antibiotics were used in a humidified atmosphere of 95% air and 5% CO_2 at 37 ◦C. All treatments were performed at least 24 h after plating in the 96-well plates. The cells were treated with dissolved IntegroPectin (10 µg/mL, 20 µg/mL and 40 µg/mL for 4 or 24 h). The control groups (Control) received an equal volume of phosphate-buffered saline (PBS) buffer solution.

2.5 Cell viability and morphology

Cells were grown at a density of 2×10^4 cell/well on the 96-well plates in a final volume of 100 µL/well. Cell viability was assessed by MTS assay, measuring the formazan absorbance at the wavelength 490 nm after 2 h incubation at 37 °C. Cell viability was expressed by normalization with the appropriate control. At the end of each experiment, the cells were washed twice with PBS and the cellular images were obtained using the Zeiss Axio Scope 2 microscope (Carl Zeiss, Oberkochen, Germany).

2.6 X-ray diffraction

The pectin samples were analyzed by a D5005 X-ray diffractometer (Bruker AXS, Karlsruhe, Germany) operating at 40 kV and 30 mA. The X-ray radiation was generated via a copper (Kα) anode and made monochromatic via the instrument's secondary monochromator. The diffraction profile of both grapefruit IntegroPectin and commercial citrus pectin (galacturonic acid ≥74.0 %, dried basis) purchased from Sigma-Aldrich (Merck Life Science, Milan, Italy) at 0.15°/min acquisition rate over the 5.0°-70.0° 2θ range.

2.7 Thermal analysis

The thermogravimetric analyses were performed on a Mettler Toledo TGA/DSC Star system heating from room temperature up to 1100 °C under a nitrogen flow (30 mL/min).

3. Results and discussion

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 adhesion, flavor persistency and compactness remained virtually unchanged.

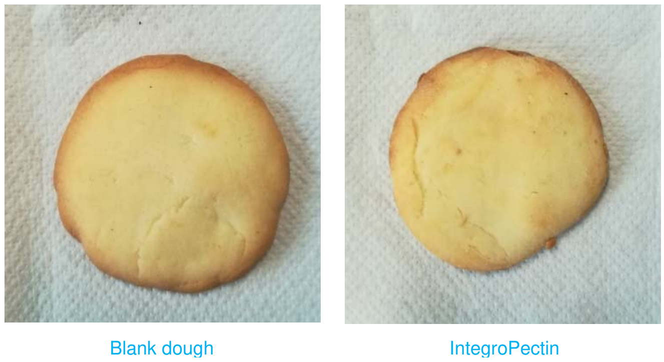

Figure 1. Unmodified rice-based cookie (*left*) and cookie modified with 2.5 wt% lemon IntegroPectin (*right*).

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.

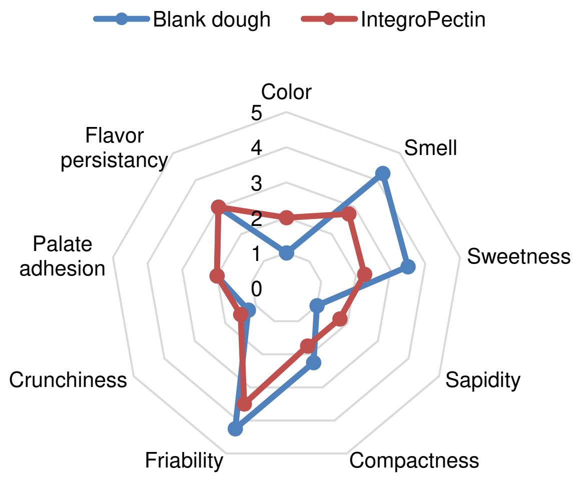

Figure 2. Sensory plot with respect to nine different descriptors of rice-based cookie (*blue line*) and cookie functionalized with 2.5 wt% lemon IntegroPectin (*red line*) evaluated by a panel of 20 subjects.

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,[17] 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).[18]

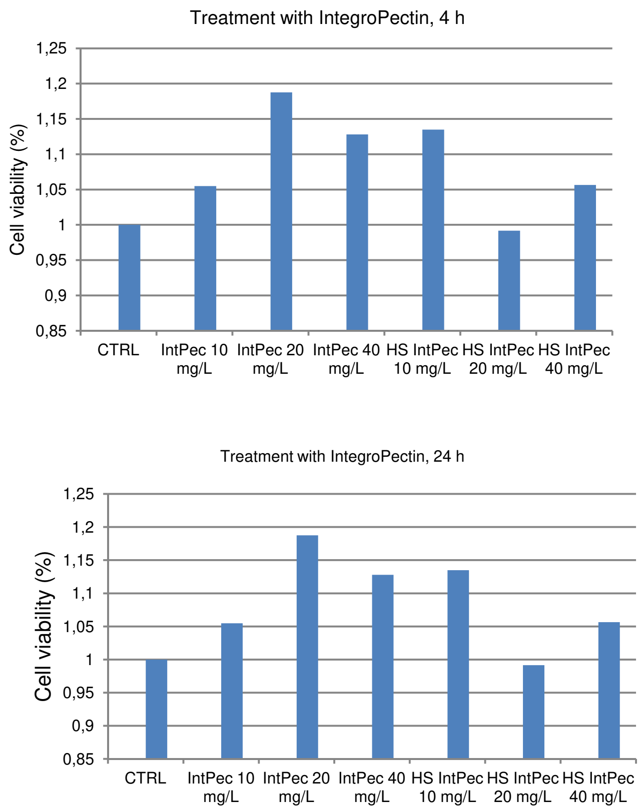

Figure 3. Viability of Caco-2 human colorectal cells cultured *in vitro* after 4 h (*top*) and 24 h (*bottom*) in the presence of increasing dosage of lemon IntegroPectin or heat-treated lemon IntegroPectin.

Figure 4 shows optical microscopy evidence that the treatment of the cells with increasing dosages of both IntegroPectin and heat-stressed IntegroPectin does 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, 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[19] disappear with the remaining peaks shifting to higher 2θ values.

This indicates that hydrodynamic cavitation 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)[20] crystallizing in hexagonal closest packing arrangement.[21] Cavitation, in other words, destroys the ‘‘fringed-micellar’’ structure of the crystalline regions of semicrystalline pectin.[22]

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 in water of the IntegroPectin at room temperature when compared to the poorly soluble commercial citrus pectin, extracted via conventional hydrolysis in hot acidic water, having DE = 70%.

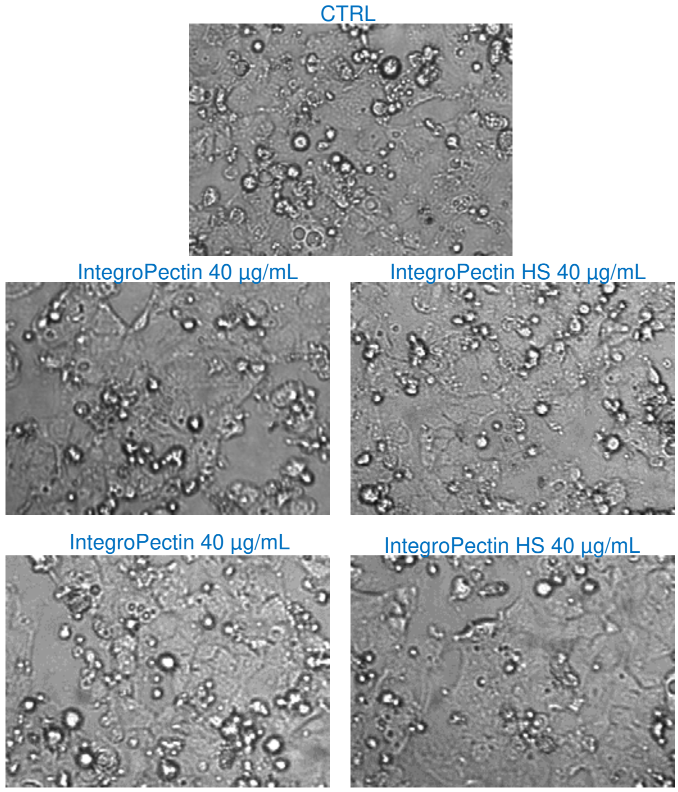

Figure 4. Optical microscopy photographs of Caco-2 human colorectal cells in the presence of increasing dosage of lemon IntegroPectin and heat-treated lemon IntegroPectin.

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.[23] Both $-COO^-$ and rhamnogalacturonan I (RG-I) lateral chains, indeed, are particularly abundant in the newly obtained lemon (and grapefruit) IntegroPectin.[24]

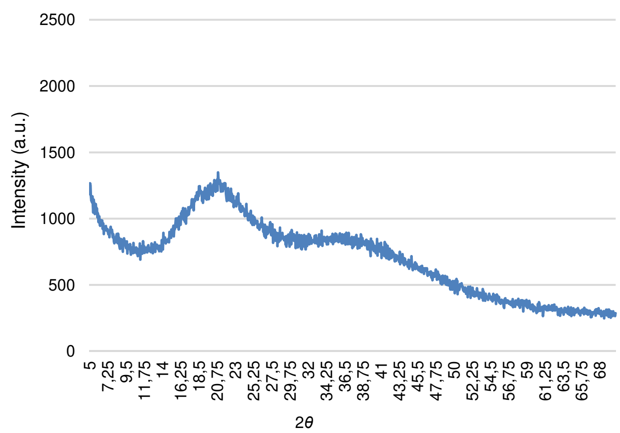

Figure 5. X-ray diffraction pattern of lemon IntegroPectin.

The thermogravimetric analysis (TGA) of the new IntegroPectin and of commercial citrus pectin provides further structural information.

In agreement to previous results,[25,26] the TGA profiles (Figure 6) are similar showing three regions at 50-200 °C, 200-400 °C and 400-600 °C. The first step corresponds to loss of water adsorbed at the surface of the hydrophilic regions of the biopolymer.

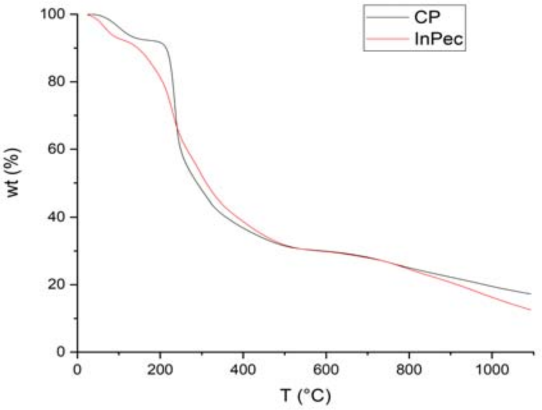

Figure 6. TGA curves of lemon IntegroPectin and commercial citrus pectin.

Likewise to what happens with grapefruit pectin obtained via acoustic cavitation,[26] the water content of the commercial citrus pectin is higher than that in lemon IntegroPectin, with a 39.10% 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 pyrolitic decomposition consisting in a primary and secondary decarboxylation (involving the acid side group and a carbon in the ring),[25] and further water loss due to cleavage of hydroxyl groups in pectin lateral chains,[28] 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 due to loss of adsorbed terpenes[27] and flavonoids[18] present in the former whole pectin, but not in commercial pectin highly purified 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.[28]

The DSC curves (not shown) have a similar shape, but whereas an exothermic transition was 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),[26] 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.

4. Conclusions

A fortified biscuit can be easily produced by replacing 2.5% of the rice-based flour with antioxidant, mitoprotective and neuroprotective lemon IntegroPectin derived from lemon processing waste via hydrodynamic cavitation (HC).

The cookie's friability, palate adhesion, flavor persistency and compactness remain virtually unchanged. Only the sweetness and the smell (flavor) of the functionalized cookie are 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. Rich in type I RG 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 (HG) portion of pectin, which partly explains the health benefits common to fruits and vegetables.[29]

The new biscuit might result in a functional cookie capable to aid in the prevention of chronic disease such as neuronal disorders. Rich in the citrus flavonoid eriocitrin,[18] the new biscuit might even result useful in the prevention of periodontitis.[30] This study shows how the high solubility of lemon, coupled to its enhanced heat resistance, make it suitable for the straightforward functionalization of bakery products. Subsequent studies are needed to investigate the clinical effects of regular assumption of this and related fortified bakery products produced using these new citrus pectins.

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Conflict of interest

The Authors declare no conflict of interest. No external funding was used for this research.

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