

Exploring the effect of utilising organic acid solutions in ultrasound-assisted extraction of pectin from apple pomace, and its potential for biomedical purposes

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Exploring the effect of utilising organic acid solutions in ultrasound-assisted extraction of pectin from apple pomace, and its potential for biomedical purposes / Girón-Hernández, Joel; Pazmino, Michelle; Barrios-Rodríguez, Yeison Fernando; Turo, Chiara Tonda; Wills, Corinne; Cucinotta, Fabio; Benlloch-Tinoco, Maria; Gentile, Piergiorgio. - In: HELIYON. - ISSN 2405-8440. - 9:7(2023), p. e17736. [10.1016/j.heliyon.2023.e17736]

*Availability:*

This version is available at: 11583/2982996 since: 2023-10-13T14:07:31Z

*Publisher:*

CELL PRESS

*Published*

DOI:10.1016/j.heliyon.2023.e17736

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24 performance were assessed biologically *in vitro* for their potential to be used in biomedical  
25 applications. Overall, the extracted pectin presented a galacturonic acid content, methoxylation and  
26 esterification degree ranged from 19.7-67%, 26.8-41.4% and 58-65.2% respectively, and were  
27 negatively charged (-24.1 to -13.2 mV). It was found that factors of time and temperature greatly  
28 influenced the response variables excepting the esterification degree, while the acid type influenced  
29 the  $\zeta$ -potential, methoxylation and esterification degrees. Additionally, it was seen that the longer  
30 extraction time (50 mins) and the higher the temperature (50 °C) exhibited the better extraction  
31 yield (~10.9%). Finally, the selected pectin showed high cytocompatibility up to 500  $\mu\text{g/mL}$  of  
32 concentration when seeded with Neonatal Normal Human Dermal Fibroblasts.

33 **Keywords:** Apple pomace, Pectin, Ultrasound-assisted extraction, Principal Component Analysis,  
34 Biomaterials.

35

## 36 1. Introduction

37 Valorisation of agro-industrial biowaste is a smart strategy that must be achieved through efficient  
38 and reproducible approaches, valuing green chemistry principles. Particularly, the extraction and  
39 purification of bioactive compounds can impact socio-environmental demands or economic  
40 challenges [1]. Although apple crop in 2022 was affected by weather conditions in Asia, around 79  
41 million tonnes of this fruit was produced worldwide [2]; in this scenario, value-added apple  
42 products such as juice, cider, jam and dried, account for 25-30% of the above volume, leading to a  
43 pomace biowaste mass that can reach up to 25% of the fresh fruit weight [3]. Particularly, apple  
44 pomace is a valuable material for extracting high attractive biomolecules like carbohydrates,  
45 polyphenols and triterpenes [4]. Pectin (PEC) is an interesting molecule present in vegetable cell  
46 walls and could be recovered from apple pomace and other vegetable biomass sources [5], it is a  
47 carbohydrate polymer with plenty of applications in the food sector. Traditionally, PEC has been  
48 used as gelling or thickening agent, this stabiliser property is complemented by the attractive  
49 utilisation of pectin as a fat replacer and health-promoting functional ingredient [6]. Alternative  
50 emerging applications include the use in the biomedical and pharmaceutical industries. Pectin, due  
51 to its simple and cytocompatible gelling mechanism, has been recently exploited for different  
52 biomedical applications including drug delivery, gene delivery, wound healing, and tissue  
53 engineering [7]. Indeed, natural biopolymers are at the centre of materials development for  
54 biomedical and biotechnological applications based on their low-toxicity, biodegradability and  
55 biofunctional key features [8].

56 Current literature reports several works focused on PEC extraction from apple pomace; on a  
57 commercial scale, diverse conditions are carried out for its purpose. However, PEC is generally  
58 extracted through water-mineral acidic solution (sulfuric, nitric, phosphoric, hydrochloric) at a pH  
59 around 1.5, where the biomass is heated at temperatures ~80 °C, followed by an ethanol  
60 precipitation at different concentrations, from 70% to absolute [9, 10]. Above-mentioned  
61 parameters can lead easily to equipment corrosion and environmental pollution derived from the

62 acidic wastewater disposal [11]. Therefore, experimental studies with apple pomace or peel, have  
63 been conducted for exploring alternatives procedures to make PEC extraction process more  
64 sustainable and to enhance its recovery. In this sense, methodologies such as: organic acid  
65 extraction, application of eutectic solvents, sequential extraction, enzymatic extraction, assisting  
66 extraction with microwaves, radio frequency, ultrasounds or the combination of this methodologies  
67 have been proposed. Indeed, Cho et al. [12] have compared different acidic extractions, by using  
68 mineral and organic acids; they found that similar amounts of pectin were extracted (~6.6%) with  
69 1M organic acids (tartaric, malic, citric) with an esterification degree ranged from 54 to 64.8%  
70 compared with conventional extraction (~6.4%) by using HCl. Furthermore, a two-step slight acidic  
71 process using H<sub>2</sub>SO<sub>4</sub> (pH 2.4) under hot stirring (100 °C) was conducted for 110 mins, leading to a  
72 PEC extraction yield of ~15%, the debris remained from the process, were used to extract cellulose-  
73 rich substances and monosaccharides, obtaining a recovery rate of 38-49% respectively [13]; this  
74 experiment represents a complete valorisation example of apple pomace; however, PEC extraction  
75 was carried out using conventional methods. Other alternative involving eutectic pre-treatments can  
76 be considered, where glycerol and lactic acid have been mixed either with choline chloride (pH 1-  
77 6.5), potassium carbonate (pH 12-14), urea or oxalic acid, leading to a final yield of extracted PEC  
78 in the range of 6-8.5% with a methoxylation degree ranged from 54 to 79%, and with an overall  
79 recovery of neutral sugars between 76-87% [14] [11]. Nevertheless, this sequential extraction lasted  
80 more than 48 h and PEC extraction yield was not significantly high compared with findings of other  
81 authors that explored different methodologies; for example, mediating the extraction process with  
82 enzymes, it was obtained ~7% of extraction yield, and the result did not present a much better  
83 performance when assisted with ultrasound (~8%). Although, in the same experiment when  
84 changing the conditions to citric acid as extractant solution at pH 2.2 and microwave assisted at pH  
85 1.8, PEC recovery was improved up to ~23% for both conditions [10]. Recently, Zheng et al [15]  
86 combined the use of citric acid solutions at a pH ranged from 1.5 to 2.5 with microwave (MWAE)  
87 and radio frequency (RFAE) assisted extractions, reaching temperatures between 80-90 °C for 20

88 minutes. Both MWAE and RFAE procedures helped to get an extraction yield of ~11%, that  
89 resulted in a higher performance compared with citric acid extraction at pH 2.2 as control (~7.5%  
90 PEC recovery). Furthermore, following RFAE method, higher content of galacturonic acid content  
91 (~63%) and esterification degree (~66%) were reported compared with MWAE and citric acid  
92 control (~41 and ~51% for the galacturonic acid, and ~54 and ~59% for the esterification degree  
93 respectively). Thus, microwaved and radiofrequency techniques can substantially reduce duration of  
94 the extraction; however, their execution could result demanding because batch processing is  
95 required [16]; additionally, microwaves generate uneven heating due to high temperature, that  
96 might cause degradation of the components in the outermost areas of the mass volume being  
97 extracted [17].

98 Finally, Dranca et al [18] proposed the use of citric acid solutions, assisted with ultrasound, up to 30  
99 minutes of extraction process. They found out that at maximum ultrasound amplitude and lower pH,  
100 PEC extraction yield and degree of esterification presented the higher values (9.1% and 88.5%  
101 respectively). Compared to the MWAE and RFAE, the ultrasound assisted procedure allows to  
102 preserve the physico-chemical structure of the extracted pectin [19].

103 Therefore, in our study we conducted a series of PEC extractions from apple pomace, ultrasound  
104 assisted, by comparing two different organic acids solutions (acetic and citric), aiming at evaluating  
105 the impact of time and temperature on PEC quality (galacturonic acid content, methoxylation and  
106 esterification degree and electrostatic charge) and extraction yield. Additionally, the obtained pectin  
107 with higher galacturonic acid content and extraction yield were assessed biologically *in vitro* by  
108 using Neonatal Normal Human Dermal Fibroblasts (NHDF) for their potential to be used in  
109 biomedical applications.

## 110 **2. Materials and methods**

### 111 **2.1 Materials and chemicals**

112 Glacial acetic acid (ACS reagent,  $\geq 99.7\%$ ), citric acid (ACS reagent,  $\geq 99.5\%$ ), hydrochloric acid  
113 (ACS reagent, 37%), ethanol 96%, sodium chloride (ACS reagent,  $\geq 99.0\%$ ), phenol red (indicator  
114 ACS), sodium hydroxide (reagent grade,  $\geq 98\%$ ), m-hydroxydiphenyl, D-(+)-Galacturonic acid  
115 monohydrate (analytical standard), sodium tetraborate, sulphuric acid (ACS reagent, 95.0-98.0%)  
116 and all other chemicals were purchased from Sigma-Aldrich, UK. Deionised water was obtained  
117 throughout Milli-Q® Water Purification System (IQ 7005, Merk, UK).

### 118 **2.2 Apple biowaste processing and preparation**

119 Apples (*Malus domestica* Bork) var. Royal Gala, from different origins (France, UK, South Africa,  
120 Chile), were purchased in a local supermarket. Subsequently, samples were visually verified to  
121 remove any damaged areas and hand-washed with tap water. Then, they were cut and ground using  
122 a fruit juicer (Cookworks, Argos, UK). The resulting pulp was passed through the juicer 3 more  
123 times to maximise the water removal and get smaller solid particles. Apple pomace yield in relation  
124 to whole apple and moisture content of apple pomace were determined by using the AOAC method  
125 [20], while the soluble solids from the extracted juice were measured by using a digital  
126 refractometer (RS PRO, UK).

127 Wet apple pomace was dried at 68°C in a vacuum oven (SVAC1-2, SHEL LAB, UK) for 24 h  
128 before milling with an electric grinder (Blender LB20E, Waring Commercial, US) into powder and  
129 then, stored in grip seal bags in desiccator until further use.

### 130 **2.3 Experimental design of the pectin extraction from the apple pomace**

131 Extraction of pectin from apple pomace was carried out using a combination of variables including  
132 acidic solution from acetic acid (AA) or citric acid (CA), sonication time (25 or 50 min) by using an  
133 ultrasound water bath and temperature at 40 and 80 °C. The processing parameters were selected

134 based on the most reported values in literature for successfully extracting pectin from other food  
135 waste biomasses [21-23].  
136 Ultrasound assisted extraction was performed by mixing 15 g of apple pomace powder with 300 mL  
137 (to reach a ratio of 1g /20 mL) of distilled water in which citric acid or acetic acid was added to  
138 reach a pH value of 1.5 by titration with 1M HCl. The ultrasound water bath (USC 300T, VWR,  
139 UK) was set at 45 kHz, 80 W, and 100% amplitude. After sonication the mixture was centrifuged at  
140 4400 rpm for 20 mins (SORVALL ST 8R, Thermo-Fisher, UK), and the supernatant was collected,  
141 filtered using a nylon mesh, and transferred to standard glass flasks. Equal amount of ethanol was  
142 added to the supernatant and the resulting solution was kept for 24 h at 4-6 °C. Then, the  
143 precipitated pectin was centrifuged at 4400 rpm for 10 min and consecutively washed with ethanol  
144 while filtering through nylon mesh. The resulting pectin was dried at 45 °C on a heated incubator  
145 (MIR-162, Panasonic, Japan) until constant weight and kept and stored in grip seal bags in  
146 desiccator until further use.

147 The yield of the extracted pectin was calculated with the following formula (Eq.1):

$$148 \text{ Pectin yield (\%)} = \frac{\text{dried pectin weight}}{\text{dried apple pomace weight}} \times 100 \quad \text{Eq.1}$$

## 149 **2.4 Characterisation of the extracted pectin**

### 150 **2.4.1 Determination of the anhydrouronic acid contents and the degree of methoxylation and** 151 **esterification**

152 The degree of methoxylation (DM) and anhydrouronic acid (AUA) contents and degree of  
153 esterification (DE) in pectin samples were analysed by conventional methods [21, 24]. To 50 mg of  
154 pectin, 500 µL of ethanol, 10 mL of distilled water, 0.10 g NaCl and one drop of phenol red  
155 indicator were added. The solution was stirred for 15 min to dissolve all of the components, and  
156 then titrated with 0.1 M NaOH until the colour changed (Titration A). Subsequently, 2.5 mL of 0.25  
157 M NaOH was added to the mixture and allowed to stand for 30 mins at room temperature. Finally,  
158 2.5 mL of 0.25 M HCl was added, and the mixture was titrated again with 0.1 M NaOH until the



159 colour turned red (Titration B). The degree of methoxylation was calculated by using the following  
160 equation (Eq.2):

$$161 \quad DM(\%) = \frac{\text{meq Titration B} \times 31 \times 100}{\text{weight of sample (mg)}} \quad \text{Eq.2}$$

162 Where meq Titration B are the milliequivalents of NaOH used for the Titration B, and 31 is the  
163 molecular weight of the methoxyl group.

164 The anhydrouronic acid content was calculated according to the equation 3 (Eq.3):

$$165 \quad AUA(\%) = \frac{176 \times 100}{z} \quad \text{Eq.3}$$

166 Where 176 is the molecular weight of AUA and

$$167 \quad z = \frac{\text{weight of sample (mg)}}{\text{meq Titration A} + \text{meq Titration B}} \quad \text{Eq.4}$$

168 Finally, the degree of esterification of the extracted pectin was calculated by:

$$169 \quad DE(\%) = \frac{176 \times DM\% \times 100}{31 \times AUA\%} \quad \text{Eq.5}$$

#### 170 **2.4.2 Galacturonic acid content analysis**

171 A colorimetric method based on the m-hydroxydiphenyl reagent was used to measure the total  
172 galacturonic acid (GA) content of the extracted pectin following the protocol proposed by  
173 Gharibzahedi et al. [25]. Briefly, 500  $\mu\text{L}$  of pectin solution (concentration of 200  $\mu\text{g}/\text{mL}$ ) was  
174 poured into a glass tube vial, and then 3 mL of sulfuric acid/sodium tetraborate was added and  
175 immediately cooled in a bath containing cold water. A continuous operation including shaking the  
176 tubes for 30 s with a vortex mixer (VORTEX 3, IKA, Germany), heating in a water bath (GLS  
177 Aqua 12 Plus, Grant, UK) at 100  $^{\circ}\text{C}$  for 5 mins and cooling in ice water was performed. Then, 100  
178  $\mu\text{L}$  of m-hydroxydiphenyl (0.15% in 0.5% NaOH) were added to the vial and kept under shaking  
179 for 5 minutes (SSM1, Stuart, UK). Finally, the absorbance of the resulting solutions was read at  
180 525 nm using a multiplate reader (FLUOstar Omega, BMG Labtech, Germany). For the preparation  
181 of the calibration curve, solutions of galacturonic acid (between 1-200  $\text{mg} \cdot \text{mL}^{-1}$ ) were used.

#### 182 **2.4.3 NMR measurement**

183 The extracted pectin samples were analysed by NMR spectroscopy. Saturated samples were  
184 prepared in 0.7 mL D<sub>2</sub>O with TMSP-d<sub>4</sub> [3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid]  
185 (Sigma-Aldrich, UK) added as an internal reference (0.0 ppm). The <sup>1</sup>H NMR spectra were obtained  
186 at 80 °C on a Bruker Avance III HD 700 MHz NMR spectrometer using a Prodigy TCI cryoprobe.  
187 Each spectrum was acquired with 16 scans and 32 K datapoints (transformed to 128 K). Baseline  
188 corrections were applied before integration.

#### 189 **2.4.4 Molecular weight determination**

190 The molecular weight of the extracted pectin was assessed by size-exclusion chromatography (SEC;  
191 1260 Infinity GPC/SEC System, Agilent), equipped with a PL aquagel-OH MIXED-H 8 µm  
192 column. The samples were dissolved overnight at 2 mg/mL concentration in a recommended buffer  
193 (0.2 M NaNO<sub>3</sub> + 0.01 M NaH<sub>2</sub>PO<sub>4</sub> at pH 7), and, then, filtered through a 0.45 µm membrane (Titan  
194 3, PTFE, ThermoScientific, UK) prior to injection (20 µl). The column set was calibrated with  
195 narrow pullulan standards and, thus, all molecular weight values were determined.

#### 196 **2.4.5 Fourier transform infrared spectroscopy (FTIR-ATR)**

197 FTIR-ATR spectroscopy analysis was performed on the extracted pectin. The infrared spectra were  
198 obtained with a spectrophotometer Spectrum one equipped with UATR accessory. The readings  
199 were taken in the wavelength range of 4000–550 cm<sup>-1</sup>, for each of the eight independent samples of  
200 each combination: acidic solution x sonication time x temperature, at least five consecutive readings  
201 were taken from pectin flakes. The average value was considered as representative for each sample.

#### 202 **2.4.6 ζ -potential measurement**

203 The ζ-potentials of pectin solutions (1:1 mg mL<sup>-1</sup>) were measured by laser Doppler electrophoresis  
204 (Zetasizer Nano, Malvern instrument, US). Three sets of at least 10 measurements were averaged to  
205 get the final ζ-potential value for each PEC solutions.

#### 206 **2.4.7 Rheological analysis**

207 PEC solutions were solubilised in deionized water at 2% (w/w) under stirring at 25 °C for 16 h, then  
208 solutions were allowed to rest overnight at 4°C prior to the rheological experiments. The tests were  
209 performed by using a stress-controlled rheometer (MCR302, AntonPaar GmbH, Graz, Austria)  
210 equipped with 25 mm parallel plate geometry. For each test, each pectin solution was poured on  
211 lower plate at 25°C. De-hydration was prevented by a water trap while temperature control was  
212 guaranteed with a Peltier system. The shear strain amplitude on each pectin solution was measured  
213 by the shear strain test at 25°C (rotational oscillation 1 Hz, strain from 0.01% to 500%), while the  
214 frequency sweep test was performed using angular frequencies ( $\omega$ ) from 100 to 0.1 rad/s and a  
215 strain value within the linear viscoelastic region of 1%. Furthermore, the solution viscosity was  
216 determined using a shear rate from 0.1 to 100 1/s and a strain of 1%. Rheological tests were  
217 performed in triplicate.

#### 218 **2.4.8 Pectin as biomaterials: *in vitro* cell tests**

##### 219 *2.4.8.1 Cell culture and seeding*

220 Neonatal Normal Human Dermal Fibroblasts (NHDF) were purchased from Lonza Biosciences  
221 (Switzerland) and cultured as recommended by the seller. Briefly, fibroblasts were grown at 37 °C,  
222 5% CO<sub>2</sub>, in Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% fetal  
223 bovine serum (FBS), 2 mM L-glutamine and a 1% antibiotic mixture containing penicillin and  
224 streptomycin (100 U mL<sup>-1</sup>). To perform biocompatibility assays, PEC solutions at different  
225 concentrations (10, 25, 50, 100, 250, 500 and 1000 µg/mL) were prepared by dissolving the pectin  
226 powders in DMEM and then sterilised by filtration through a 0.22mm Millex GP PES membrane  
227 syringe-driven filter unit (Millipore, SLS, UK) using 5 ml plastic syringes. Suspensions of 8 x 10<sup>4</sup>  
228 cells and 10 x 10<sup>4</sup> cells in DMEM were seeded on each well of a 96 and 48-multiwell plates  
229 respectively, with the different diluted PEC solutions, and then incubated with at 37 °C, 5% CO<sub>2</sub> for  
230 the necessary biological tests.

##### 231 *2.4.8.2 Cytocompatibility studies*

232 Cell viability was assessed with the live/dead staining (LIVE/DEAD® Cell Imaging Kit, Life  
233 Technologies, Thermo Scientific, US) at 24 h in 48-multiwell plates. According to the  
234 manufacturer's protocol, membranes were washed with phosphate buffered saline (PBS, Sigma-  
235 Aldrich, UK) and stained with 150 µl solution of 4 µM Ethidium homodimer-1 and 2 µM calcein in  
236 PBS. After 30 min of incubation at room temperature, cells were imaged with a EVOS M5000  
237 fluorescence microscope to detect calcein (ex/em 488 nm/515 nm) and Ethidium homodimer-1  
238 (ex/em 570 nm/602 nm), respectively.

239 Furthermore, at the same time point, Presto Blue assay was exploited to test the metabolic activity  
240 of cells seeded with the different diluted PEC solutions in 96-multiwell plates. A Filter-based  
241 FLUOstar® Omega multi-mode reader (FLUOstar® Omega, Germany) was used to measure the  
242 fluorescence (560 nm excitation and 590 nm emission) after 1.30 h of incubation with a 10%  
243 aliquot of Presto Blue (Thermo Scientific, USA). Results were expressed as mean ± standard  
244 deviation.

245 Finally, the cell morphology was observed by nucleus and cytoskeleton staining after 48 hours of  
246 cell seeding. Briefly, cells were fixed with 4% paraformaldehyde solution for 15 min, followed by  
247 three washing steps with PBS. Cells were then permeabilised using 0.1% v/v Tween20® in PBS for  
248 5 min. Rhodamine-phalloidin was prepared using 1:100 dilution of phalloidin-  
249 tetramethylrhodamine B isothiocyanate (Sigma Aldrich, P1951) in 1% v/v Tween20® in PBS for  
250 30 min, and then washed three times with PBS. One drop of DAPI (VECTASHIELD®) antifade  
251 mounting media was added to each sample, then covered with a glass slide and imaged using a  
252 EVOS M5000 fluorescence microscope.

#### 253 **2.4.9 Statistical analysis**

254 The analytical determination results were processed by one-way ANOVA, with mean separation by  
255 Tukey's test at 95% confidence level. A multifactor ANOVA was performed on the extraction  
256 parameters: Acid type (A), Extraction time (Et), Temperature (Tp) and their interactions, to evaluate  
257 their effects on the analytical determinations performed on the extracted pectin. The infrared

258 information was analysed by Principal Component Analysis (PCA) to group the different  
259 extractions. The spectra were pre-processed to compensate and remove the bias linked to the  
260 experimental assessment by baseline correction (MicroLab Expert, FTIR Software, Agilent, US).  
261 Subsequently, different methods such as standard normal variance (SNV), multiplicative dispersion  
262 correction (MSC), and first and second derivatives were evaluated on the range of 1800 - 650  $\text{cm}^{-1}$   
263 of the spectra (known as fingerprint) that provided key information to differentiate samples from  
264 different treatments [26]. Data processing was performed using Statgraphics Centurion 19  
265 (Statpoint Technologies, Inc., USA) and R statistical software (version 3.6.3, R statistics, US).

## 266 3. Results

### 267 3.1 Analytical determination

268 In this work, Royal gala apples have been bought from a local store and they were characterised by  
269 soluble solids and moisture content, presenting  $12.41 \pm 0.62^\circ$  Brix, and after removing the water  
270 from the pomace, dry matter represented  $19.63 \pm 0.43\%$  apple pomace (dry base).

271 The effect of ultrasound-assisted extraction with the combination of different processing parameters  
272 (acid type, temperature, and time of extraction) on the analytical properties of the extracted pectin  
273 have been investigated in this work. These results are summarised in **Table 1** and elaborated by the  
274 multifactor ANOVA to investigate if these variables were statistically significant or not (**Table 2**).

275 The yield of the pectin obtained from the different extractions ranged from 1 to 12%, depending on  
276 the type of acid, time, and temperature of extraction. Particularly, it can be observed that the yield  
277 increased with increasing time and temperature. As example, for the citric acid, the yield increased  
278 from  $3.1 \pm 0.7\%$  at  $40^\circ\text{C}$  for 25 min to  $11.8 \pm 1.5\%$  at  $80^\circ\text{C}$  for 50 min of extraction. According to  
279 the F-ratio reported in **Table 2**, the temperature of extraction ( $T_p$ ) presented the highest influence  
280 ( $47.33^{***}$ ) followed by the extraction time ( $E_t$ ,  $17.18^{**}$ ) while the acid type ( $A$ ) presented a  
281 statistical effect only in interaction with the other 2 processing factors ( $A \times E_t \times T_p$ ,  $11.49^{**}$ ). Thus,  
282 temperature was a crucial parameter, because its increase allowed the increase of pectin solubility,  
283 resulting in a higher yield. The behavior was described in literature in different works reporting  
284 extraction of pectin from different biomass [27, 28].

285 The same substantial influence of the time and temperature of extraction was observed for the  
286 content of galacturonic acid ( $E_t$ ,  $239.36^{***}$  and  $T_p$ ,  $792.12^{***}$ ). GalA is the most prevailing  
287 building block of pectin, which makes its determination a very important step in the analysis of  
288 pectin's chemical structure [29]. The range of the analysed galacturonic acid was between  $\sim 20\text{-}50\%$   
289 with the highest content found in the pectin extracted with acetic acid at  $80^\circ\text{C}$  for 50 minutes.

290 Commercial apple pectin purchased from Sigma-Aldrich was used as control and it was found to be  
291 characterised by 67% GalA within the range reported into the specification sheet of the supplier.

292 Furthermore, the degree of esterification (DE) is another parameter that affects pectin quality and  
293 applications. Indeed, according to the extraction conditions, different proportions of the acid groups  
294 of the GalA units are esterified and this is known as DE [30]. Moreover, GalA units can be partly  
295 methoxylated, where the backbone presents methyl ester forms (-COOCH<sub>3</sub>), and this can be  
296 calculated as degree of methoxylation (DM) [31].

297 In our work, all the extraction conditions led to a DE ranging from 58 to 65%. In contrast with the  
298 other analytical determinations, the use of the different acid type influenced the DE (5.46 \*) where  
299 the citric acid extractions provided the highest values (at 80 °C for 25 minutes, 63.0±5.6% for CA  
300 respect with 58.0±0.3% for AA). As shown in **Table 1**, both acids presented a similar DE to that of  
301 commercial SIG-APP (58.9±2.4%). Numerous researchers described that the pectin solubilisation  
302 into the solvent happened due to the breakage of the plant cell wall under the influence of the  
303 ultrasound [32, 33]. Particularly, ultrasound is a green method that rises the selectivity, decreases  
304 reaction time, and encourages macro- and micro- mixing via acoustic cavitation, creating  
305 cavities/bubbles. After collapse, these can release huge amounts of energy that is made available to  
306 break the structure where pectin is contained [34]. As demonstrated by Zhang et al. [35] high  
307 intensity ultrasound (up to 300 W cm<sup>-2</sup>) can increase the DE >70%. They reported a similar value of  
308 DE close to 60% when using a lower ultrasound power (~60W cm<sup>-2</sup>) at 20 °C for 30 minutes.  
309 Then, according to the DM, pectin can be categorised as high methoxy pectin (DM > 50 %) and low  
310 methoxy (DM < 50 %) [36]. **Table 1** shows that the DM values of all the apple pectin were in the  
311 ranges of ~27-41%; thus, our pectin can be classified as low methoxyl. Moreover, the pectin with  
312 the highest DM was obtained with the citric acid by comparing the same extraction conditions (time  
313 and temperature) of the acetic acid. This trend was confirmed by the F-ratio (21.5\*\*). These  
314 considerations on the DM are important for selecting the use of the pectin in biomedical application  
315 as bioink and hydrogel for tissue engineering and regenerative medicine. Particularly, low or high  
316 DM require different conditions for crosslinking pectin. Pectin with low DM is characterised by  
317 high number of free carboxyl groups with high cation-binding ability. The binding of divalent

318 cations e.g.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  produces junction zones between two polyguluronate chain dimers. These  
 319 segments present an “egg-box” structure, where the binding of the cation to the carboxyl groups of  
 320 two opposite pectin chains was stabilised by van der Waals interactions and hydrogen bonds [37].  
 321 Thus, our extracted pectin in all the conditions can be suitable to manufacture bioprinted constructs  
 322 or *in situ* gelling systems. Indeed, the  $\zeta$ -potential values ranging from -13 to -24 mV confirmed the  
 323 presence of a high number of free COOH groups, fundamental for the further ionotropic gelation  
 324 with divalent ions. Furthermore, this negative charge of the extracted pectin can allow to use it as  
 325 polyelectrolyte (specifically as polyanion) for the surface functionalisation of medical devices by  
 326 technique of Layer-by-Layer (LbL) assembly. LbL is an environmental-friendly technique that  
 327 allows to create a multilayered coating at the nanoscale, exploiting the electrostatic interaction of  
 328 polyelectrolytes, for modifying the surface topography and/or entrapping biomolecules/drugs to  
 329 impart specific biological activities [38, 39].

330 **Table 1.** Yield, galacturonic acid content, methoxylation and esterification degree, and  $\zeta$ -potential  
 331 of the extracted pectin samples from apple pomace obtained by conventional acidic extraction at  
 332 pH= 1.5 with different temperatures and times. The values are shown as average  $\pm$  SD.

Code	Acid	Temp. (°)	Time (min)	Yield (%)	GalA (%)	DM (%)	DE (%)	$\zeta$ -potential (mV)
CA40-25	CA	40	25	3.1 $\pm$ 0.7	27.1 $\pm$ 4.8	41.4 $\pm$ 2.0	59.1 $\pm$ 1.3	-22.9 $\pm$ 1.1
CA80-25	CA	80	25	7.1 $\pm$ 1.9	31.0 $\pm$ 3.9	37.5 $\pm$ 2.1	63.0 $\pm$ 5.6	-13.2 $\pm$ 0.7
AA40-25	AA	40	25	1.2 $\pm$ 0.3	19.7 $\pm$ 0.5	33.8 $\pm$ 3.2	58.1 $\pm$ 1.9	-22.7 $\pm$ 2.8
AA80-25	AA	80	25	10.8 $\pm$ 2.9	43.9 $\pm$ 0.7	27.5 $\pm$ 0.8	58.0 $\pm$ 0.3	-16.1 $\pm$ 0.4
CA40-50	CA	40	50	5.0 $\pm$ 0.3	36.9 $\pm$ 2.0	36.6 $\pm$ 3.1	65.2 $\pm$ 4.4	-13.5 $\pm$ 1.2
CA80-50	CA	80	50	11.8 $\pm$ 1.5	43.7 $\pm$ 2.6	33.2 $\pm$ 1.4	61.4 $\pm$ 1.4	-15.8 $\pm$ 0.2
AA40-50	AA	40	50	8.6 $\pm$ 2.3	24.4 $\pm$ 1.4	32.0 $\pm$ 4.4	61.2 $\pm$ 5.2	-20.9 $\pm$ 0.8
AA80-50	AA	80	50	10.1 $\pm$ 2.4	49.2 $\pm$ 2.4	26.8 $\pm$ 1.7	58.2 $\pm$ 3.4	-18.6 $\pm$ 0.5
SIG-APP	-	-	-	-	67.0 $\pm$ 2.6	31.9 $\pm$ 1.3	58.9 $\pm$ 2.4	-24.1 $\pm$ 1.1

333 **Table 2.** F-ratio values and significance levels obtained in multifactor ANOVA for the physico-  
 334 chemical parameters according to the factors: Acid type (A), Extraction time ( $E_t$ ), Temperature ( $T_p$ )  
 335 and their interactions.

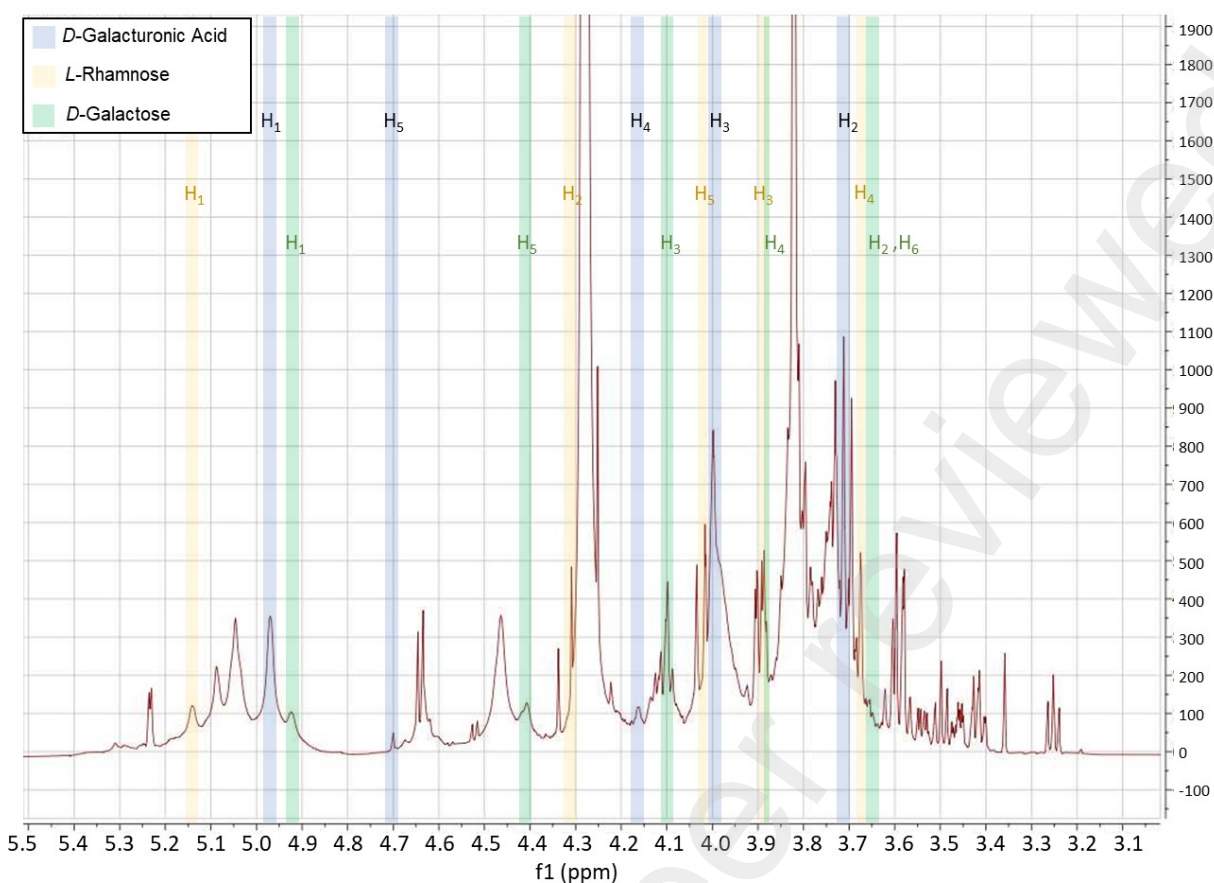
A	$E_t$	$T_p$	A x $E_t$	A x $T_p$	$E_t$ x $T_p$	A x $E_t$ x $T_p$
---	-------	-------	-----------	-----------	---------------	-------------------



<b>Yield (%)</b>	1.37 <sup>NS</sup>	17.18 <sup>**</sup>	47.33 <sup>***</sup>	0.0 <sup>NS</sup>	0.01 <sup>NS</sup>	2.72 <sup>NS</sup>	11.49 <sup>**</sup>
<b>GalA (%)</b>	0.72 <sup>NS</sup>	239.36 <sup>***</sup>	792.12 <sup>***</sup>	35.67 <sup>***</sup>	320.16 <sup>***</sup>	2.95 <sup>NS</sup>	1.44 <sup>NS</sup>
<b>DM (%)</b>	21.5 <sup>**</sup>	4.57 <sup>*</sup>	10.11 <sup>*</sup>	1.21 <sup>NS</sup>	0.73 <sup>NS</sup>	0.24 <sup>NS</sup>	0.01 <sup>NS</sup>
<b>DE (%)</b>	5.46 <sup>*</sup>	1.72 <sup>NS</sup>	0.19 <sup>NS</sup>	0.06 <sup>NS</sup>	0.24 <sup>NS</sup>	3.18 <sup>NS</sup>	0.74 <sup>NS</sup>
<b>ζ-potential (mV)</b>	57.23 <sup>***</sup>	12.49 <sup>**</sup>	91.87 <sup>***</sup>	18.94 <sup>**</sup>	0.72 <sup>NS</sup>	88.63 <sup>***</sup>	19.79 <sup>**</sup>

336 <sup>NS</sup>, not significant. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

337 <sup>1</sup>H NMR spectra of the extracted and commercial apple pectin were compared. All the spectra were  
338 characterised by a broad signal chain (i.e. CH<sub>3</sub> and CH<sub>2</sub> groups) ranging from 0 to 2.5 ppm [40]  
339 (**Figure S1**). Particularly, signals at 2.11 and 1.91 ppm are from the -COCH<sub>3</sub> groups located at 3-*O*-  
340 and 2-*O*-galacturonic acid. Then, signals at 1.30 ppm and 1.27 ppm are from the CH<sub>3</sub> group of *L*-  
341 rhamnose. The peak at 3.92 ppm is derived from the CH<sub>3</sub> group that is associated with the  
342 carboxyl groups of GalA. The remaining pectin signals are assigned to the 5 protons found in GalA  
343 (H<sub>1</sub>, 4.97 ppm; H<sub>2</sub>, 3.73 ppm; H<sub>3</sub>, 3.97 ppm; H<sub>4</sub>, 4.16 ppm, and H<sub>5</sub>, 4.70 ppm) (labelled in blue in  
344 **Figure 1** and reported in **Table 3**). Furthermore, signals at 5.13 ppm and 4.92 ppm located in the  
345 anomeric region are assigned to H<sub>1</sub> Rha and H<sub>1</sub> Gal, respectively. Furthermore, the extracted pectin  
346 showed differences compared with the control SIG-APP. Indeed, the acetyl groups of GalA acid  
347 and methyl groups of Rha were not visible in the <sup>1</sup>H NMR spectrum at range 2.5-1 ppm (**Figure**  
348 **S1**). Also, the extracted pectin showed a visible increase in the intensities of the peaks at 4.92 ppm  
349 of the H<sub>1</sub> Gal, that could overlap the peak at 4.97 ppm of H<sub>1</sub> GalA, and at 4.70 ppm of the H<sub>5</sub> GalA.  
350 However, all the other protons, characterising the GalA [41], were less intense or not detected.



351  
352 **Figure 1.**  $^1\text{H}$  NMR spectrum of pectin from apple pomace extracted by using acetic acid at  $80^\circ\text{C}$  for  
353 25 minutes ultrasound-assisted.

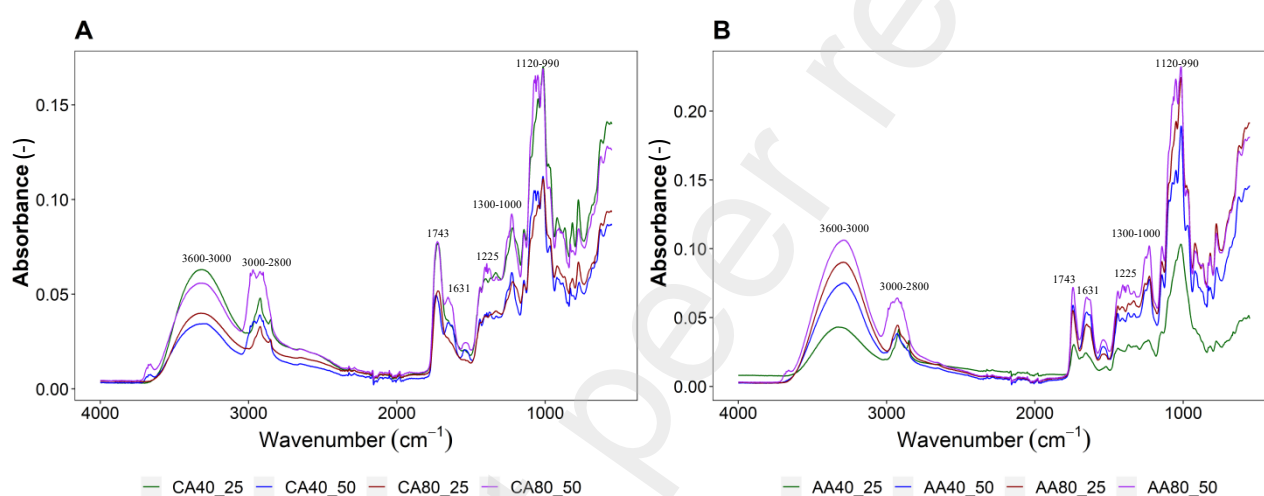
354 **Table 3.**  $^1\text{H}$  NMR chemical shifts of pectin from apple pomace extracted by using acetic acid at  
355  $80^\circ\text{C}$  for 25 minutes ultrasound-assisted.

	<b>H<sub>1</sub></b>	<b>H<sub>2</sub></b>	<b>H<sub>3</sub></b>	<b>H<sub>4</sub></b>	<b>H<sub>5</sub></b>	<b>H<sub>6</sub></b>
<b><i>D</i>-galacturonic acid</b>	4.97	3.73	3.97	4.16	4.70	n.d.
<b><i>L</i>-rhamnose</b>	5.13	4.31	3.88	3.67	4.02	n.d.
<b><i>D</i>-galactose</b>	4.92	3.64	4.10	3.87	4.41	3.66

356 n.d.= not detected.

357 FTIR-ATR pectin spectra obtained after acidic extraction for all the different treatments are  
358 illustrated in **Figure 2**. The main absorption peaks recorded around  $3600\text{-}3000\text{ cm}^{-1}$  were caused by  
359 O-H stretching, while characteristic absorption peak of pectin-reproduced polysaccharides due to C-  
360 H stretching of  $\text{CH}_2$  groups was observed between  $3000\text{-}2800\text{ cm}^{-1}$  [18, 24]. Stretching vibration  
361 (C=O) of methyl-esterified and carboxylate ions (free carboxyl groups) of pectin resulted in the  
362 bands at  $1743\text{ cm}^{-1}$  and  $1631\text{ cm}^{-1}$ , respectively [42]. The tendency of increasing intensities and

363 band area of esterified carboxyl groups may indicate an increase in degree of esterification [43].  
364 Certainly, esterified carboxyl groups exhibit an increasing trend in their intensities and band areas,  
365 as esterification degree value increases [44]. Also, the higher absorbance for esterified carboxylic  
366 groups, compared to free carboxylic groups, would indicate a higher degree of esterification [45].  
367 Bands related to the stretching of the C-O bond were observed between 1300 and 1000  $\text{cm}^{-1}$  [24],  
368 while the absorption band at 1225  $\text{cm}^{-1}$  was due to the cyclic C-C bond in the ring structure of  
369 pectin. Finally, the region between 1120-990  $\text{cm}^{-1}$  has been reported for the spectral identification  
370 of galacturonic acid in peptide polysaccharides [46].



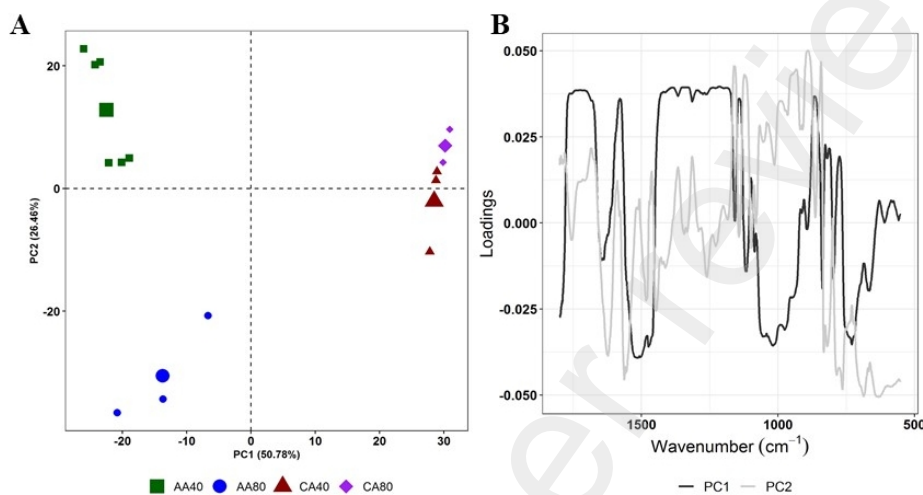
371

372 **Figure 2.** ATR-FTIR spectra with baseline correction of the apple pectin samples obtained in the  
373 mid-infrared 4000-650  $\text{cm}^{-1}$  range after acidic extraction with citric (A) and acetic acid (B).

374 Furthermore, some more considerations can be done on mid-infrared (MIR) spectra where the  
375 wavenumber range of 4000-650  $\text{cm}^{-1}$  can be classified into two different regions: functional group  
376 (4000-1500  $\text{cm}^{-1}$ ) and fingerprint region (1500-650  $\text{cm}^{-1}$ ). In both regions, changes in absorbance  
377 values are observed due to the different treatments (Figure 2). However, differences in the  
378 fingerprint region are more evident in peaks of interest such as those associated with the degree of  
379 esterification (743  $\text{cm}^{-1}$  and 1631  $\text{cm}^{-1}$ ), gallic acid (1120-990  $\text{cm}^{-1}$ ) and pectin structure cycle  
380 (1300-1000  $\text{cm}^{-1}$ ). Therefore, the chemometric analysis of the spectrum was performed within  
381 information in the region of 1800-650  $\text{cm}^{-1}$  wavenumbers.

382 Following the baseline correction, an exploratory PCA analysis was performed with the information  
383 of the region between 1800-650  $\text{cm}^{-1}$  for 25-minute treatments, using different processing

384 techniques such as SNV, MSC, and first and second derivatives. The best clustering results were  
385 evidenced with MSC, which are illustrated in **Figure 3**. As can be seen, the first two components  
386 explain almost all the variability of the MIR information (77.24%) (**Figure 3A**). The first principal  
387 component (PC1) provides the main contribution (50.78%), while the second (PC2) explains  
388 26.46%.



389

390 **Figure 3.** (A) PCA of the processed infrared signal spectra of extracted pectin (25 min) with  
391 baseline correction + MSC normalisation; (B) Pectin apple spectrum and loadings for PC1 and PC2.

392 The scatter plot shows three different groups according to the treatment applied: i) AA40, ii) AA80

393 and iii) CA40 and CA80. This shows a clear effect on pectin composition when temperature is

394 varied from 40 to 80 °C in the acetic acid extraction, while this effect is not observed with citric

395 acid. Thus, FTIR analyses confirmed the influence of temperature during the extraction on the

396 pectin structure and the content of GAs, which were lower in AA40 than those obtained in AA80

397 (**Table 1**), which could explain the differences evidenced by the analysis of the IR spectra.

398 The loadings plot for the first two components indicates that the region between 1800 to 1700 cm<sup>-1</sup>

399 and 1420 to 1180 cm<sup>-1</sup> are strongly associated with the samples in grouped PC1 positive region,

400 where CA40 and CA80 samples were located. As discussed above, these regions are associated with

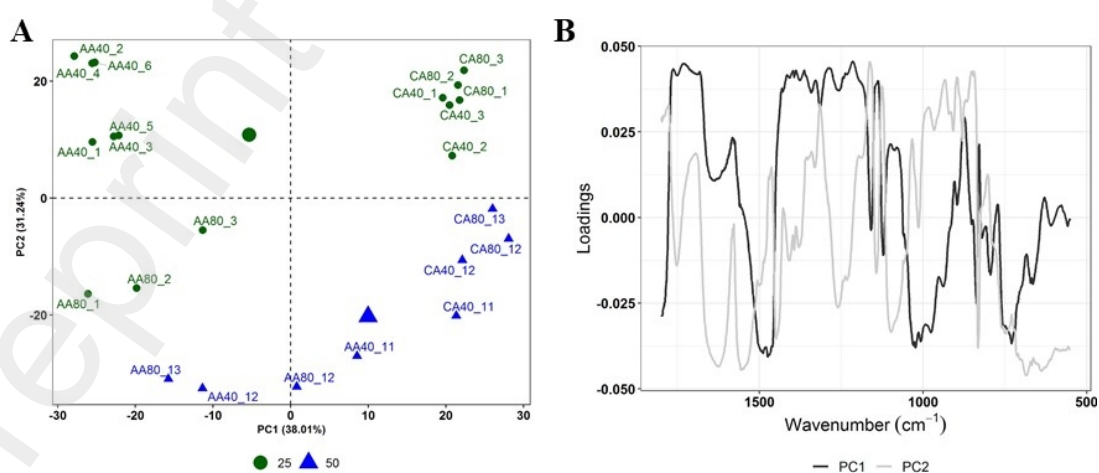
401 the degree of esterification and C-O stretching, respectively. This is consistent with the significant

402 higher degree of esterification for the samples extracted with citric acid (**Table 1 and 2**) An

403 important contribution from the region between 1200-900 cm<sup>-1</sup>, it is also evident in the negative part

404 of PC1 where most of the AA40 samples were observed. This zone could be influenced by the

405 presence of galacturonic acid in the pectin, and as shown in **Table 1**, where these samples presented  
 406 the lowest concentrations of galacturonic acid. Finally, AA80 samples were grouped in PC2  
 407 negative region, loadings plot evidence a considerable contribution of the band 1631 and 1565  $\text{cm}^{-1}$ .  
 408 The peak at 1636-1606  $\text{cm}^{-1}$  indicated (C=O) stretching vibration of carboxylate ion. The ratio of  
 409 the area of the peak at 1743  $\text{cm}^{-1}$  (COO-R) to the sum of the areas of the peaks at 1743  $\text{cm}^{-1}$  and  
 410 1636  $\text{cm}^{-1}$  (COO<sup>-</sup>) can be used to quantify the degree of esterification [18].  
 411 Furthermore, when comparing data from pectin spectra extracted at 25 and 50 mins, PCA analysis  
 412 evidenced the effect of the extraction time on the chemical characteristics of the pectin, which  
 413 allows their aggrupation (**Figure 4**). Samples obtained at 50 minutes (quadrant IV, samples CA80)  
 414 and pectin obtained at 25 minutes (quadrant I, samples CA40 and CA80) were grouped in the  
 415 positive region of PC1. The wavenumber between 1800-1650  $\text{cm}^{-1}$  (associated with the  
 416 esterification degree) and 1400-1100  $\text{cm}^{-1}$  shows an important contribution for the separation of this  
 417 type of samples (**Figure 4B**), which is consistent with the higher degrees of esterification of the  
 418 samples extracted with CA at 25 and 50 min (**Table 1**). Samples obtained at 25 mins on the  
 419 negative region of PC1 (quadrant II pectin AA40; quadrant III pectin AA80), are mostly associated  
 420 with the 1100 -900  $\text{cm}^{-1}$  wave numbers, influenced by galacturonic acid. Finally, pectin obtained at  
 421 50 mins distributed over the negative region of PC2 were associated with bands at 1631  $\text{cm}^{-1}$ , 1550  
 422  $\text{cm}^{-1}$ , 1450  $\text{cm}^{-1}$ , 1250  $\text{cm}^{-1}$ , and 1100  $\text{cm}^{-1}$ .



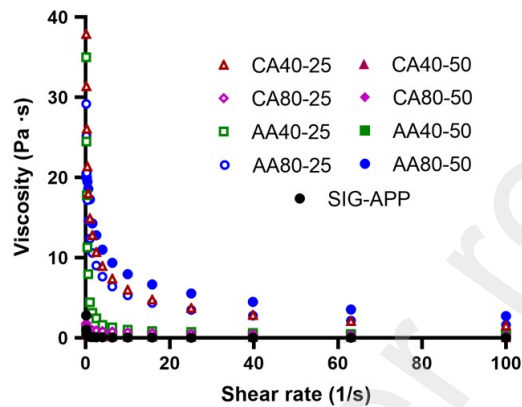
423  
 424 **Figure 4.** (A) PCA of all processed infrared signal spectra of extracted pectin (25 and 50 min) with  
 425 baseline correction + MSC normalisation; (B) Pectin apple spectrum and loadings for PC1 and PC2.

426 Finally, the molecular weight is a key-parameter for evaluating the relationship between  
427 polysaccharide structure and function [47], where its value is associated with the pectin gelling  
428 properties, fundamental for being considered suitable for the manufacturing of hydrogels in tissue  
429 engineering [48] The  $M_w$  of the extracted pectin samples ranged from 1.11 to  $1.15 \times 10^5$  Da. The  
430 commercial pectin had similar value ( $1.13 \times 10^5$  Da) in accordance with the literature [49].  
431 Therefore, no differences have been noticed among all the extracted pectin samples.

### 432 **3.2 Rheological analysis**

433 Rheological analysis measurements reported a different behaviour for extracted pectin solutions  
434 from the apple pomace compared to commercial pectin from Sigma-Aldrich (SIG-APP). Flow  
435 curves revealed a lower viscosity for SIG-APP compared to extracted pectin solutions (**Figure 5**)  
436 while the frequency sweep tests at 25°C showed an opposite trend of  $G'$  and  $G''$  (**Figure 6A**)  
437 having a SOL state ( $G' < G''$ ) for extracted pectin solutions and a GEL state for SIG-APP one ( $G' > G''$ ).  
438 Strain sweep tests allowed to identify the linear viscoelastic region (LVE) which indicates  
439 the range in which the test can be carried out without destroying the structure of the sample. LVE is  
440 visible in all the extracted pectin (except for CA 40-50) reaching a yield point for strain up to 50%  
441 (**Figure 6B**). On the other hand, the SIG-APP solution shows a narrow LVE with a yield point at  
442 5% strain. Furthermore, rheological measurements highlighted the effect of extraction process on  
443 the mechanical behaviour of pectin solutions. Indeed, the use of AA or CA strongly influenced the  
444 properties of the final solutions, higher viscosity was obtained when the extraction process was  
445 performed using AA at 80°C (AA80-25 and AA80-50) while for CA a reduction of the viscosity  
446 was observed increasing the temperature and the time (**Figure 5**). All the tested conditions  
447 maintained a SOL state at 25°C however differences in the frequency and strain sweep test plots  
448 were observed ascribed to the acidic conditions (CA or AA) used within the extraction process  
449 (**Figure 6**). When CA was used,  $G'$  and  $G''$  decreased for the higher temperature while the longer  
450 time reduced the stability of the solutions to strain exhibiting a lower yield point. On the contrary,  
451 for AA the process at 80°C guaranteed higher  $G'$  and  $G''$  values compared to 40°C, however the

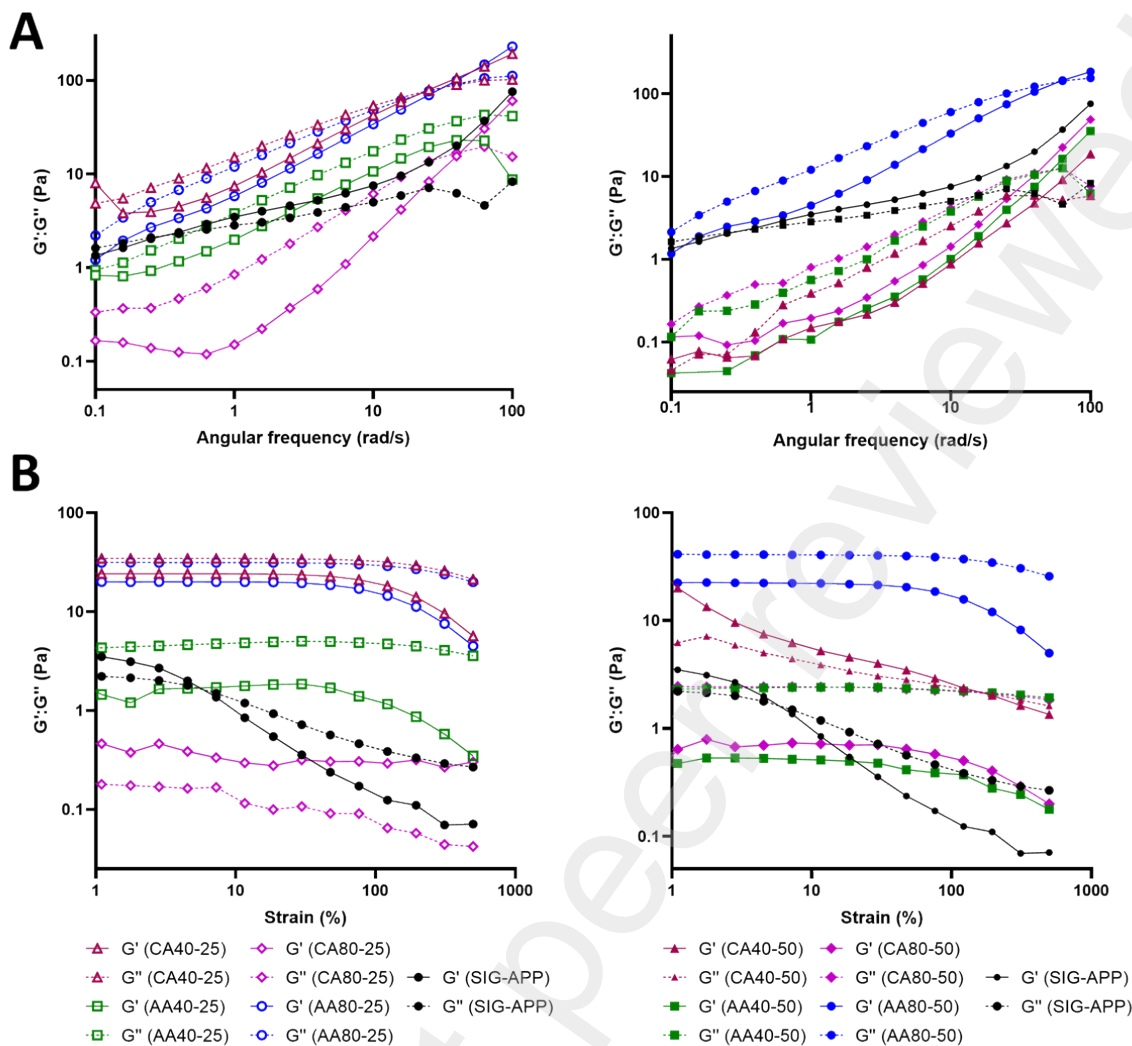
452 extraction time did not affect the mechanical properties of the solutions tested, indeed only a slight  
453 reduction of  $G'$  and  $G''$  values was observed for AA40-50 compared to AA40-25.  
454 The tested pectin solutions show  $G'$  and  $G''$  values of few Pa, highlighting the potential of this  
455 material to be applied in the field of soft tissue engineering and regenerative medicine as the  
456 mechanical properties of several human tissues are in the range from few Pa to kPa [50].



457

458 **Figure 5.** Flow-curves of the extracted pectin solutions from different acidic conditions. Apple  
459 pectin from Sigma-Aldrich (SIG-APP) has been used as control.





460

461 **Figure 6.** Rheological properties of pectin solutions obtained from (A) frequency and (B) strain  
 462 sweep tests after 25 (left) and 50 (right) mins of extraction. Apple pectin from Sigma-Aldrich (SIG-  
 463 APP) has been used as control.

464

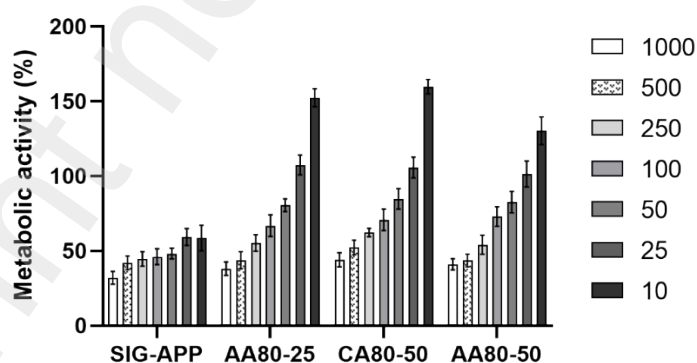
### 465 3.3 *In vitro* cell tests

466 Neonatal Normal Human Dermal Fibroblasts were seeded on the tissue culture plates with different  
 467 concentrations of the extracted and commercial pectin to assess their cytocompatibility for  
 468 biomedical applications, particularly for tissue engineering and regenerative medicine.

469 The NHDF metabolic activity was assessed by using Presto Blue assay (**Figure 7**) after 48 hours,  
 470 showing a significant increase when the concentration of the dissolved pectin is below 250  $\mu\text{g/mL}$ ,  
 471 confirming the results observed by the live/dead staining assay (**Figure 8**). Interestingly, the AA80-  
 472 25 and CA80-50 exhibited the highest metabolic activity of the NHDF when compared to the

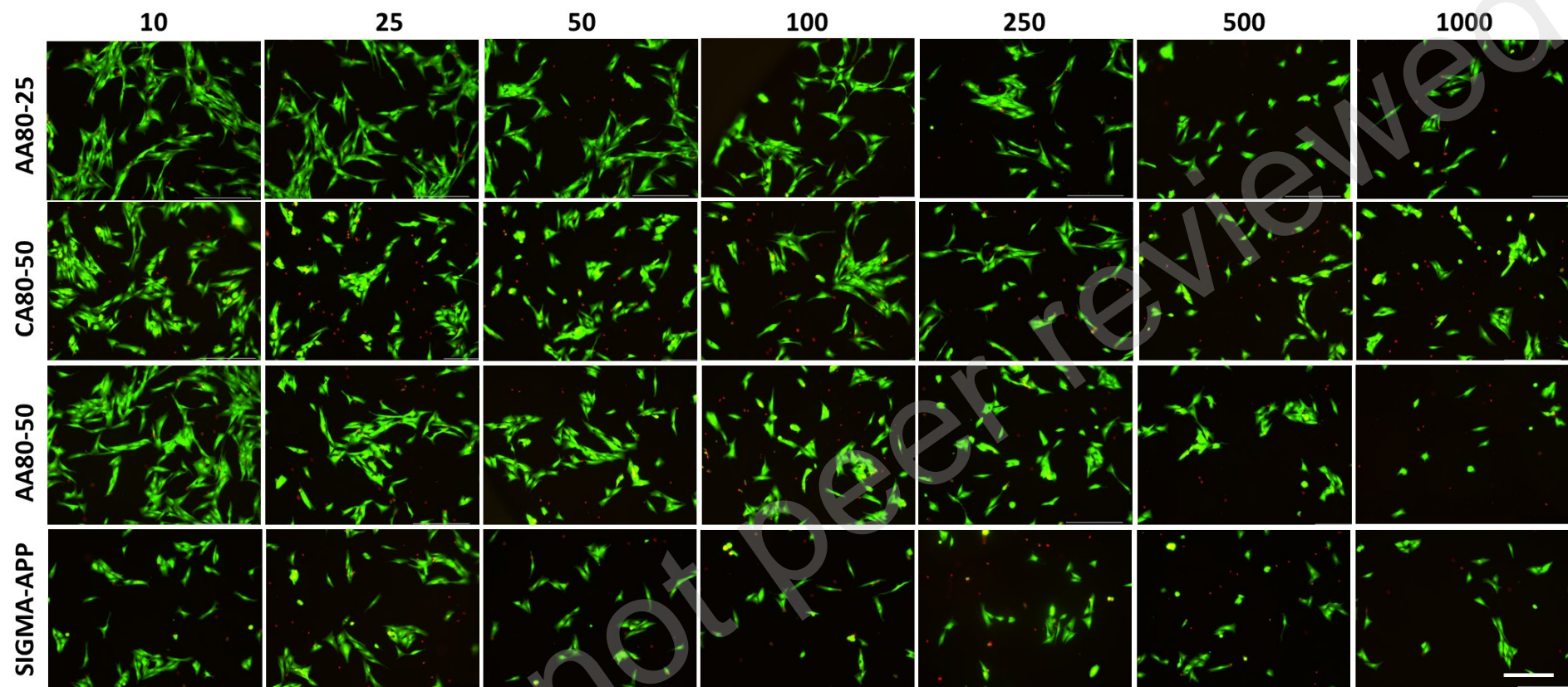


473 remaining sample AA80-50 (at 1000  $\mu\text{g/mL}$   $p < 0.01$ ). However, all the samples containing the  
 474 extracted pectin encouraged the growth and a quicker spreading of the cells. In contrast, a  
 475 significant viability reduction was observed on the cells seeded with the commercial pectin. After  
 476 48 hours, a reduction of more than 50% compared to the other samples was detected at  
 477 concentrations in the range of 10-50  $\mu\text{g/mL}$ . Furthermore, the viability of the NHDF was assessed  
 478 by live/dead staining assay after 48 h of seeding, as shown in **Figure 8**. Lower concentrations  
 479 showed a high cell viability and ability to promote cell attachment. NHDF showed the typical  
 480 elongated and flattened morphology on all the extracted pectin samples and spreading  
 481 homogeneously along the TCP surface. On the other hand, highest concentrations seemed to have  
 482 affected the cell behavior. Particularly, from the concentration of 500  $\mu\text{g/mL}$ , it was noticed  
 483 different dead cells (labelled in red) mainly for the samples AA80-50 and SIG-APP.  
 484 Immunostaining assays confirmed the previous results with the cell maintained spindle-shape in the  
 485 presence of low concentrations of the extracted pectin, while cells at higher concentrations  
 486 evidenced a rounded shape and cellular contraction with smaller nucleus (**Figure 9**). This can be  
 487 related with the cytotoxic effect of pectin confirmed by low metabolic activity detected by Presto  
 488 Blue assay and Live/Dead staining.

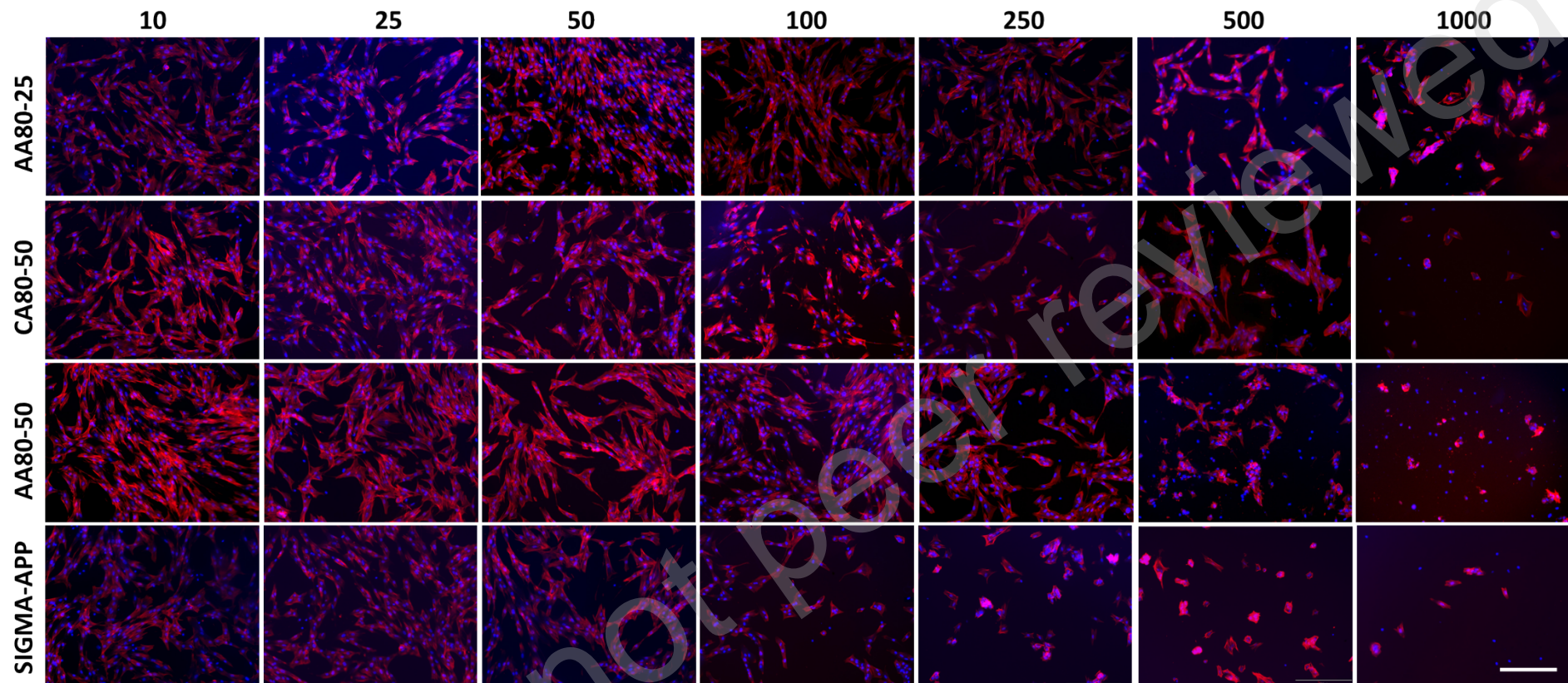


489

490 **Figure 7.** Metabolic activity of Neo-dermal fibroblast cells after 48 hours of seeding in presence of  
 491 different concentration (from 1000 to 10  $\mu\text{g/mL}$ ) of the extracted pectin. Apple pectin from Sigma-  
 492 Aldrich (SIG-APP) has been used as control. The results are shown as average  $\pm$  SD after  
 493 normalisation to the control of cells seeded on TCPs.



**Figure 8.** Live/dead images of Neo-dermal fibroblast cells after 24 hours of seeding in presence of different concentration (from 1000 to 10  $\mu\text{g/mL}$ ) of the extracted pectin. Commercial apple pectin purchased from Sigma-Aldrich (SIG-APP) has been used as control. Scale bar= 300  $\mu\text{m}$ .



**Figure 9.** Immuno-staining images of Neo-dermal fibroblast cells after 48 hours of seeding in presence of different concentration (from 1000 to 10  $\mu\text{g/mL}$ ) of the extracted pectin. Commercial apple pectin purchased from Sigma-Aldrich (SIG-APP) has been used as control. Scale bar= 300  $\mu\text{m}$ .

#### 4. Conclusion

A comprehensive comparison between different processing factors of a combined organic acidic and ultrasound-assisted extraction applied to obtain pectin from apple biowaste was made to evaluate the procedure performance, including yield and physico-chemical properties, to propose an alternative methodology to the mineral acidic extraction. We found in this work that temperature and time mainly influenced the properties of the extracted pectin in terms of extraction yield, GalA content and methoxylation degree, where temperature presented the highest influence on the process. Moreover, we observed that the acid type only showed effect on the  $\zeta$ -potential of the extracted materials. Considering the highest cytocompatibility of the extracted pectin compared with the commercial one, the evaluated procedure allows to obtain materials that can be proposed for different biomedical applications, including as hydrogels for soft tissue engineering and regenerative medicine, thanks to the low moduli measured through rheology, and as polyelectrolyte for the development of multilayered coating to modify the surface of medical devices and/or to allow the controlled release of biological molecules and drugs.

**Conflicts of interest**

The authors that they have no conflict of interest to declare for this publication.

**Acknowledgement**

This work was supported from the ONE Planet studentship that funded the experimental activities of Michelle Pazmino at Newcastle and Northumbria Universities.



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