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Pea cell wall polysaccharides and their structural integrity influence protein bioaccessibility and hydrolysis

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ABSTRACT

Pea cell walls have been shown to encapsulate nutrients inside cells, thereby limiting their hydrolysis by digestive enzymes. However, it is unknown how the cell wall performs this barrier function. In particular, this could be due to the presence of specific polysaccharides or, most probably, the organisation of the components within the cell wall. This study aimed to investigate how cell walls prevented protein hydrolysis. To address this objective, isolated cells were obtained using different treatments thought to affect cell walls differently (incubations in water, salt or EDTA solutions) and digested *in vitro* using a three-phase (oral, gastric and intestinal) model. Purified polysaccharides (cellulose, rhamnogalacturonan I and xyloglucan) and solutions obtained from the incubation of pea fibres and flour were also used in our digestion experiments. We found that protein bioaccessibility (here defined as the amount of protein released from the pea cells) and hydrolysis was lower after the gastric phase for the isolated cells prepared with the salt solution compared to the other treatments. Regardless of the treatment, between 47% and 93% of proteins were released from the cells (bioaccessible) and hydrolysed, respectively. Therefore, after prolonged incubation time proteases seem to be able to penetrate the cells during digestion. In terms of purified polysaccharides, rhamnogalacturonan I had the greatest effect on protein hydrolysis. Incubation solutions made from the pea fibres reduced proteolysis to a greater extent than the pea flour. The present study showed that pea cell walls delay protein digestion mainly via its structural organisation within the cell wall, with purified polysaccharides having a more limited effect.

Keywords

Cell wall; Protein digestion, Polysaccharides; Mechanism; Structure.

1 **1. Introduction**

2 Pulses such as pea are a potential source of nutrients, such as protein, for both humans and
3 animals. However, previous research has shown that pea cell walls limited the digestibility of
4 nutrients, particularly protein and starch (Grundy, Labarre, Mayeur-Nickel, van Milgen, &
5 Renaudeau, 2023; Junejo et al., 2021). Indeed, the cell wall can influence macronutrient
6 hydrolysis and absorption via a range of mechanisms including encapsulation (Grundy,
7 Edwards, et al., 2016). However, it is still unknown how this effect is achieved and which
8 component(s) of the cell wall is (are) responsible. One hypothesis is that the presence of
9 specific polysaccharides within the cell wall and the cross-links between them and other
10 molecules (proteins and phenolic compounds), and/or their organisation within the cell wall
11 which controls porosity. In order to predict the impact that pulses such as peas can have on
12 health, including digestive functions, it is essential to understand how cell walls modulate
13 nutrient bioaccessibility (i.e., release of a nutrient from the food matrix) and hydrolysis.

14 Isolated pea cells are an interesting model that can be used to understand the effect of cell walls
15 on nutrient digestion, which is why their study has recently become popular (Bhattarai, Dhital,
16 Mense, Gidley, & Shi, 2018; Edwards et al., 2020; Grundy, Carriere, et al., 2016; Junejo et al.,
17 2021; Liu, Hao, Chen, & Zhu, 2020; Pallares Pallares et al., 2018; Zahir, Fogliano, & Capuano,
18 2020). Different methods have been used to obtain these isolated cells and even though it is
19 assumed that the cell wall is intact, the impact of the treatment on the polysaccharides within
20 the cell wall remain unclear. Chelating agents, such as cyclohexanediamine tetraacetic acid
21 (CDTA) and ethylenediaminetetraacetic acid (EDTA), can be used to remove the calcium
22 crosslinking the pectic polysaccharides of adjacent cells (located in the middle lamella)
23 together and make cell separation possible, particularly for hard plant tissue (Jarvis, 1982). For
24 legumes, cell separation can be achieved with thermal treatment, but this can also result in cell
25 wall swelling (Edwards et al., 2020; Waldron, Parker, & Smith, 2003). Finally, soaking in salt

26 solution, often of bicarbonate, combined with thermal treatment, can be used to accelerate the
27 softening of the plant tissue and thereby facilitate cell separation (Schoeninger, Coelho, Christ,
28 & Sampaio, 2014).

29 Cell walls of pea has been previously studied in term of composition, physical properties and
30 functionalities (Dopico, Labrador, & Nicolas, 1986; Grundy et al., 2023; Guevara-Zambrano
31 et al., 2023; Jebalia, Della Valle, Guessasma, & Kristiawan, 2022; Junejo et al., 2021; Noguchi
32 et al., 2020; Talbott & Ray, 1992). Depending on the variety, pea dietary fibre content ranges
33 from 13 to 31%, with most of it being classified as insoluble fibre (Brummer, Kaviani, & Tosh,
34 2015; Grundy et al., 2023; Kan et al., 2018). Pea cell walls are made predominantly of pectin
35 (mainly rhamnogalacturonan I) and hemicellulose (mainly xyloglucan) (Brillouet & Carré,
36 1983; Dopico et al., 1986; Noguchi et al., 2020).

37 One of the difficulties faced when studying cell walls is to obtain precise, reliable values on
38 their composition and structure. Given that cell walls have to be disrupted to extract the
39 polysaccharides they contain, information about their organisation, spatial structure, and
40 certain cross-links is lost. Other, indirect means ought to be employed to compensate for this,
41 for instance particle size measurements and microscopy observations can provide information
42 about the structure and integrity of the cell wall (Holland, Ryden, Edwards, & Grundy, 2020).
43 However, these approaches still do not permit to identify the cross-links between the
44 polysaccharides within the cell wall. Using both intact cell walls, individual (extracted) and
45 solubilised polysaccharides can overcome this missing information. Therefore, this study
46 aimed to shed light on the cell wall composition and structure responsible for the observed
47 decrease in protein digestibility. To do so, cells from pea seeds were isolated using different
48 treatments. In parallel, individual polysaccharides and solutions obtained following incubation
49 of pea flour or fibres were added to pea protein to investigate their potential interaction and
50 impact on proteolysis.

51 2. Materials and Methods

52 2.1. Materials

53 Pea (*Pisum sativum L.*) seeds, protein, and fibres were provided by Roquette (Vic-sur-Aisne,
54 France). As indicated by the supplier, pea proteins were isolated through wet extraction of
55 yellow peas, separated by flocculation and spray-dried. Pea fibres were obtained from the wet
56 extraction of yellow peas, and were composed mainly of internal cell walls from the
57 cotyledons, (the hull having been removed) and residual starch (see Table 1 for composition).
58 Those fibres were separated by centrifugation and decantation, and then dried. Pea cells were
59 isolated using four different methods based on previous work (Bhattarai, Dhital, Wu, Chen, &
60 Gidley, 2017; Grundy, Wilde, Butterworth, Gray, & Ellis, 2015; Guevara-Zambrano et al.,
61 2023; Junejo et al., 2021): i) incubation of pea seeds in water for 16 h, cooking at 60°C for 1 h
62 and separation with a high shear mixer (IKA® T25 ultra-turrax, treatment 1, Mixer water
63 60°C), ii) incubation in water for 16 h at room temperature, cooking at 60°C for 1 h and
64 separation gently crushing the peas with a mortar and pestle (treatment 2, Water 60°C), iii)
65 incubation in NaHCO₃ (1.5%, w/v) and Na₂CO₃ (0.5%, w/v) for 16 h at room temperature (pH
66 9.5), cooking at 60°C for 1 h and separation by gently crushing the peas with a mortar and
67 pestle (treatment 3, Salt 60°C), and iv) incubation in EDTA (50 mM) for 16 h at room
68 temperature (pH 7) without a cooking step, and separation gently crushing the peas with a
69 mortar and pestle (treatment 4, EDTA). Each cell preparation was then loaded on a stack of
70 sieves of 1 000, 150 and 71 µm aperture as well as a sieve base to collect the liquid. After
71 washing the preparation with water to separate the cells from other particles and cell fragments,
72 the cells were collected on the 71 µm sieve. For treatment 4, a dialysis step using a dialysing
73 membrane (Float-ALyzer G2 10 mL, 3.5–5 kDa, Merck) was added to remove the EDTA as
74 the latter can affect enzymes activity. Pea flour was obtained by grinding pea seeds using a
75 hammer mill (40 HP, capacity 3T/H; Stolz, Pontivy, France) fitted with a 2.5 mm grid.

76 Pea flour and fibres were incubated in deionised water at 4°C on a rotator for periods of 6, 24
77 and 72 h to obtain solubilised pea fibres. The end point of 72 h was chosen to represent the
78 maximum time that the pea material (flour or extracted fibres) could reside in the
79 gastrointestinal tract before being fermented by colonic microorganisms. This complemented
80 the experiments performed on pure polysaccharides, cellulose, rhamnogalacturonan and
81 xyloglucan, extracted from different plant sources (see below for references).

82 Pepsin from porcine gastric mucosa (#P6887, 2145 U/mg of solid), bovine bile extract
83 (#B3883), pancreatin from porcine pancreas (#P7545, 3.4 U/mg of solid based on trypsin
84 activity) and cellulose (#310697) were purchased from Merck (Saint Quentin Fallavier,
85 France). Rhamnogalacturonan I (from soybean, #P-RHAGN) and xyloglucan (from tamarind,
86 #P-XYGLN) were purchased from Megazyme (Libios, Vindry-sur-Turdiner, France). All other
87 chemicals, solvents and reagents were purchased from Merck (Saint Quentin Fallavier, France)
88 or Thermo Fisher Scientific (Illkirch-Graffenstaden, France).

89

90 *2.2. Physical and chemical characterisation of materials*

91 Each pea cell preparation and the polysaccharides (cellulose, rhamnogalacturonan and
92 xyloglucan) were analysed for moisture (oven-dried at 102°C), protein (Dumas method with a
93 nitrogen conversion factor of 5.4, LECO, FP828 Carbon/Nitrogen/Protein Determinator,
94 Villepinte, France, AOAC 968.06), and total dietary fibre (method AOAC 991.43) content.
95 The pea flour and fibres were analysed as described in Grundy et al. (2023) and presented in
96 Table 1. The average particle size of the cells obtained via the different treatments was
97 measured using Malvern laser diffraction particle sizer 3000 equipped with a dispersant unit
98 (Hydro 2000G) filled with water (Malvern Instruments Ltd.). Triplicate measurements were
99 made for each analysis.

100

101 Table 1 Composition of the pea flour and fibres (% on a dry weight basis).

	Pea flour	Pea fibre
DM (%)	87.68	95.65
Protein (%)	20.79	6.75
Starch (%)	49.04	36.14
TDF (%)	19.58	52.13
IDF (%)	13.75	47.99
SDF(%)	5.83	4.14
Ash (%)	3.71	3.06

102

103
104 *2.3. In vitro digestion*

105 The cells isolated with treatments 2, 3 and 4 were digested using the three phase Infogest
106 standardised protocol (Brodkorb et al., 2019; Grundy, Tang, van Milgen, & Renaudeau, 2022).

107 The cells isolated with treatment 1 were not digested *in vitro* as their cell walls were ruptured
108 (protein 100% bioaccessible and highly hydrolysable).

109 Pea protein was mixed with the different polysaccharides (either purified or solubilised
110 following incubation) to identify their effect on protein hydrolysis. the extent of protein
111 hydrolysis was assessed after the intestinal phase.

112 The different materials (cells, pea protein plus polysaccharides, and pea protein plus incubation
113 solutions) were added to simulated salivary fluid so the quantity of protein and dietary fibre (or
114 purified polysaccharides) was equal to 50 mg and 100 mg (dry weight basis), respectively. The
115 composition of the simulated fluids used for each digestion phase can be found elsewhere
116 (Brodkorb et al., 2019). After about 6 h of incubation (2 min of oral phase pH 7, 2 h of gastric
117 phase at pH 3, and 4 h of intestinal phase at pH 7), the enzymatic reactions were stopped by
118 increasing the pH to 9 with 5 N NaOH at the end of the intestinal phase.

119 Each digestion was performed in triplicate, without (blank sample, to determine protein
120 bioaccessibility) and with (digested sample, determination of protein hydrolysis) enzymes.

121

122 2.4. Protein and proteolysis products assessment

123 For the isolated cells, the extent of proteolysis was estimated from the crude protein
124 measurement of the recovered *in vitro* digesta by the Dumas method (see section 2.2). Briefly,
125 following centrifugation at 2,500 g at 4 °C for 10 min, the pellet containing the undigested
126 material was washed, filtered using a cell-strainer (Falcon®, 40 µm aperture) and dried at 80°C
127 overnight as previously described (Grundy et al., 2022).

128 For the other samples (digestions with the purified polysaccharides and the incubation
129 solutions), the OPA method was used to quantify the amino groups. Thus, after centrifugation,
130 the supernatant was collected, proteins precipitated in methanol and the small peptides
131 hydrolysed with sulfuric acid as described in Sousa et al. (2023).

132

133 2.5. Microstructural characterisation

134 Samples were mounted on microscopy slides, and then visualised with an Apotome microscope
135 and Zen software (Apotome, Zeiss, France). Some of the cells were also dyed with calcofluor
136 white and fast green FCF to identify cell wall and protein components, respectively. Images
137 were captured using 10x and 20x objective lenses.

138

139 2.6. Statistical analysis

140 The data were analysed using R studio version 4.1.2. For all tests, the significance level was
141 set at $P < 0.05$ (2 tailed) and all data were expressed as means of triplicates. The differences in
142 protein bioaccessibility and digestibility between the pea materials were assessed by one-way
143 analysis of variance (ANOVA) followed by Tukey's post-hoc test.

144

145

146

147 **3. Results and discussion**

148 This study was conducted to better understand how pea cell walls influenced protein digestion,
149 either via its structural organisation (physical barrier) and/or via the properties of its
150 constitutive polysaccharides (e.g., “binding” with proteases, or increase in the viscosity of the
151 gastrointestinal content). To achieve this, we employed an approach similar to our previous
152 work (Grundy et al., 2017) where dietary fibres with different levels of complexity were used:
153 i) isolated cells obtained with different treatments (it is assumed that the cell walls are intact
154 but each treatment is likely to have a different impact of the cell wall integrity and structure),
155 ii) pea polysaccharides solubilised following the incubation of either flour or fibres extracted
156 from pea, and iii) purified polysaccharides chemically similar to the ones found in pea cell
157 walls but extracted from other plant sources (commercially available). A set of analyses were
158 then carried out to characterise the pea cells (nutritional composition, particle size, and
159 microscopy) before and after simulated digestion, and the extent of proteolysis estimated from
160 the material recovered after digestion (either the cells or products of proteolysis).

161

162 *3.1. Characterisation of the studied materials*

163 Differences in the content of protein and dietary fibre were observed between the four cells
164 preparations (Table 2). Some protein was lost during the isolation of the cells, notably for
165 EDTA (14.2%) compared to Salt 60°C (18.6%, $P < 0.05$). However, the dietary fibre content
166 was higher for the EDTA compared to the other treatments (TDF value of 56% for EDTA,
167 between 6.1 and 23.7% for the other cell samples, $P < 0.05$), in particular the insoluble fraction
168 seemed to have been more impacted by the preparation. It is likely that this fraction
169 corresponded to resistant starches that were not gelatinised due to the absence of cooking. Even
170 though a thermal treatment is included in the TDF analysis (100°C for 4 h in presence of
171 pancreatic α -amylase and amyloglucosidase), it is possible that either the enzymes could not

172 penetrate the cell wall or the starch was not fully gelatinised and/or hydrolysed (Dhital,
173 Bhattarai, Gorham, & Gidley, 2016). Therefore, the analysis did not discriminate between cell
174 wall components and resistant starches.

175 As expected, the incubations in water did not sufficiently weaken the middle lamella, which
176 resulted in cell wall rupture (Fig. 1.1 and 1.2), losses in dietary fibre (6.08 and 13.83% for
177 Mixer water 60°C and Water 60°C, respectively) and protein (8.6% compared to 18.6 and
178 14.6% for Salt 60°C and EDTA, respectively). Therefore, differences in the integrity of the
179 cells were visible in the microscopy images (Figure 1). The cells isolated using water and the
180 mortar appeared less damaged than those obtained after mixing. However, a limited number of
181 cells seemed to have intact cell walls with starch (gelatinised) “holding” the cell shape together,
182 some cells also appeared emptied of their content (Fig.1.2A and 1.2B). Because of the absence
183 of heat treatment, the starch for the EDTA cells were not gelatinised (starch granules appear
184 intact). The cell wall of the cells isolated with Salt 60°C and EDTA seemed to be undamaged
185 as demonstrated by the blue “layer” (cell wall stained with calcofluor White) delimiting the
186 cells (Fig. 1.3a, 3b, 4a and 4b). In addition, for those treatments the protein was only observed
187 within the cells (stained in green) unlike the treatments with water where some of the nutrients
188 (starch and proteins) were “solubilised” with empty cells and cell wall fragments present in the
189 preparation (Fig. 1.1 and 1.2).

190

191 **Table 2** Protein and dietary fibre composition of the cells obtained from the four treatments

	Mixer water 60°C	Water 60°C	Salt 60°C	EDTA
DM (%)	24.75	21.07	26.05	24.03
Protein (%) ^a	5.09	8.57	18.55	14.20
TDF (%) ^a	6.73	13.83	23.66	55.84
IDF (%)	5.43	13.22	21.97	52.58
SDF(%)	1.30	1.24	3.39	4.89

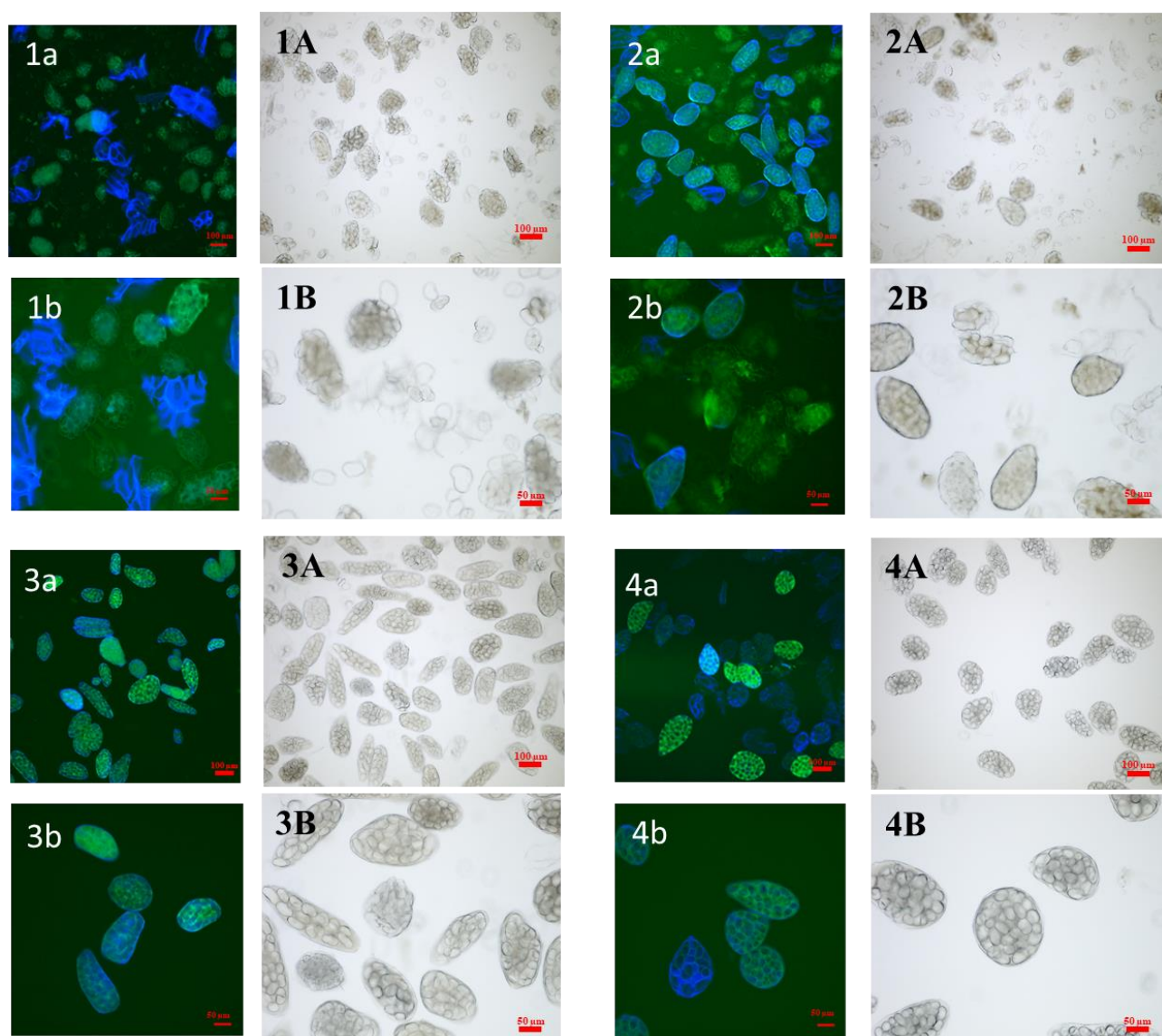
192

193 ^a Data expressed on a dry weight basis.

194 Values are presented as means of triplicates.

195

196



197

198 **Fig. 1.** Microscopy images of the cells at baseline for different preparations: treatment 1 (Mixer

199 water 60°C, 1), treatment 2 (Water 60°C, 2), treatment 3 (Salt 60°C, 3) and treatment 4 (EDTA,

200 4). For images a and b, cell walls are stained in blue with calcofluor White, and proteins in

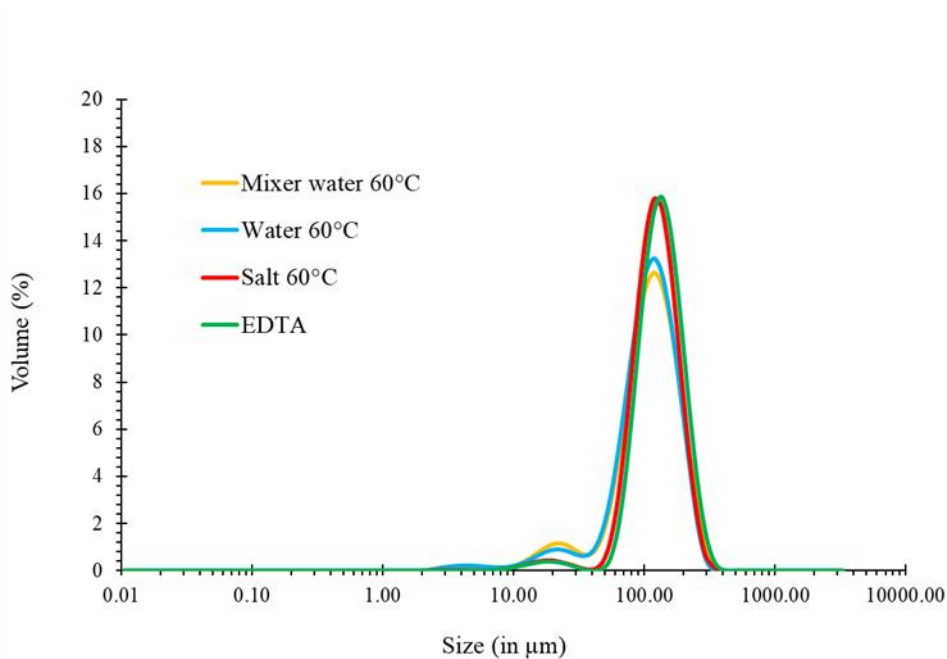
201 green with Fast Green. Scale bars are 100 μm for a and A; and 50 μm for b and B.

202

203 These observations were confirmed with the particle size distributions (Fig. 2) that showed a

204 mean cell size of 135 μm for the Salt 60°C and EDTA treatments. This value is slightly smaller

205 than those reported in other studies, that is around 150 μm (Edwards et al., 2020; Junejo et al.,
 206 2021), which could be due to differences in the variety of pea used. A second peak at around
 207 20 μm can be identified, especially for the two water treatments, which is likely to correspond
 208 to starch granules, protein aggregates, and other cell fragments solubilised during the analysis
 209 in the dispersant unit (as distinguishable in Fig1.1B and 1.2B).



210

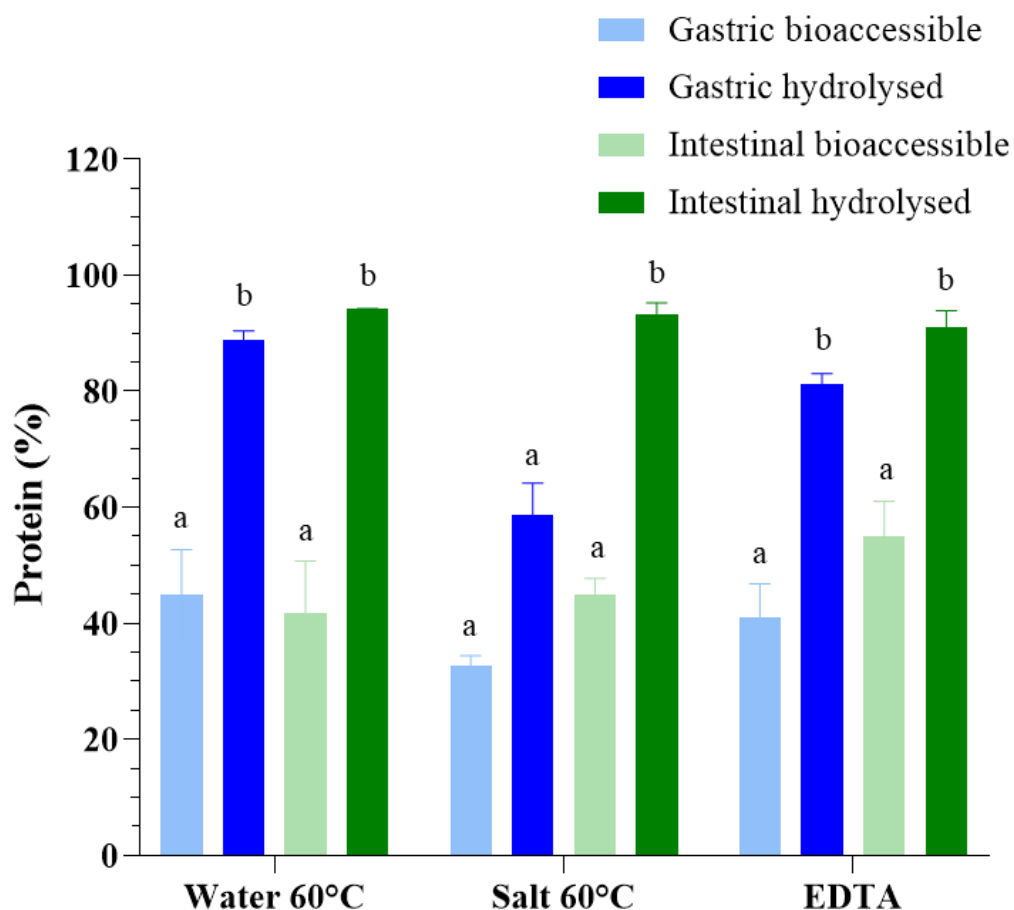
211 **Fig. 2.** Particle size distribution of the isolated cells obtained with the four treatments.

212

213 3.2. Impact of cell isolation treatment on protein digestion

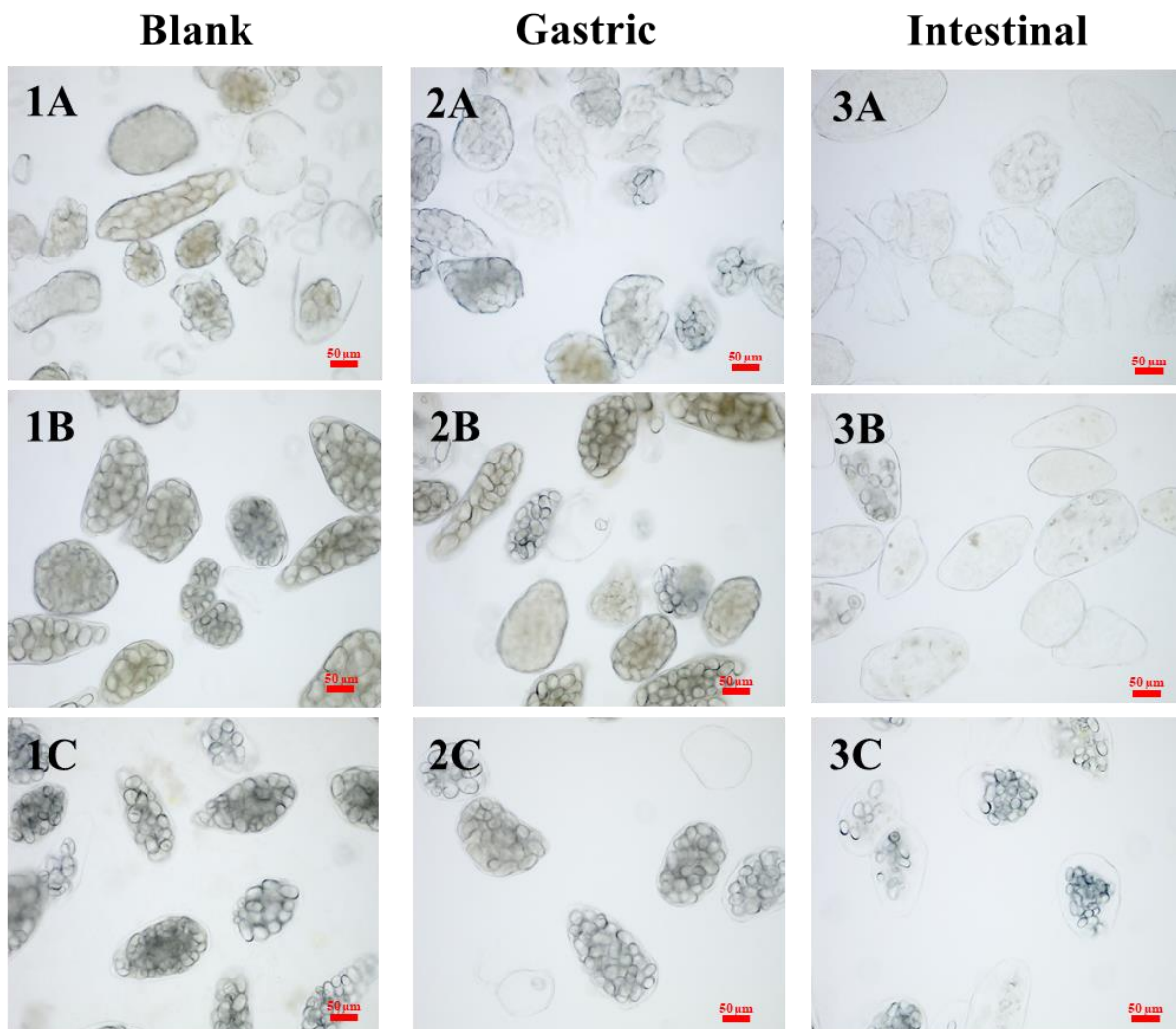
214 Figure 3 reveals that more than half of the protein remained inside the cells during the
 215 incubation (on average ~52% of protein remained encapsulated), although the amount for the
 216 cells obtained with treatment 2 (water 60°C) was actually lower given that some protein was
 217 lost during the preparation process (only 8.6 % of protein remained in the sample, see Table
 218 2). This is visible in Fig. 4.1 where the cells appear more damaged for the water treatment than
 219 for Salt 60°C and EDTA. The protein hydrolysis after the gastric phase was lower ($P < 0.05$)
 220 for the cells prepared with the treatment 3 (Salt 60°C) compared to the other treatments.
 221 However, the digestions at the end of the intestinal phase were similar for the three types of

222 cells (~93%). The extent of protein hydrolysis in pea cells reported in the literature is lower
 223 than that of the present work (Bhattarai et al., 2017; Junejo et al., 2021). This could be due to
 224 the incubation time that was longer (6 h instead of 2 or 4 h in other studies) and to the variety
 225 and growing conditions of the peas that may also have been different. Indeed, it is known that
 226 the variety and the conditions in which a plant grows can influence its composition, including
 227 dietary fibre, and thereby the nutritional value and digestibility of the food products derived
 228 from it (Givens, Davies, & Laverick, 2004; Grundy et al., 2020; Hall, Hillen, & Robinson,
 229 2017).



230

231 **Fig. 3.** Amount of protein released from the cells (bioaccessible) and hydrolysed following
 232 gastric and intestinal incubation. Different letters indicate significant differences as determined
 233 by one-way ANOVA and Tukey's post-hoc test ($P < 0.05$).



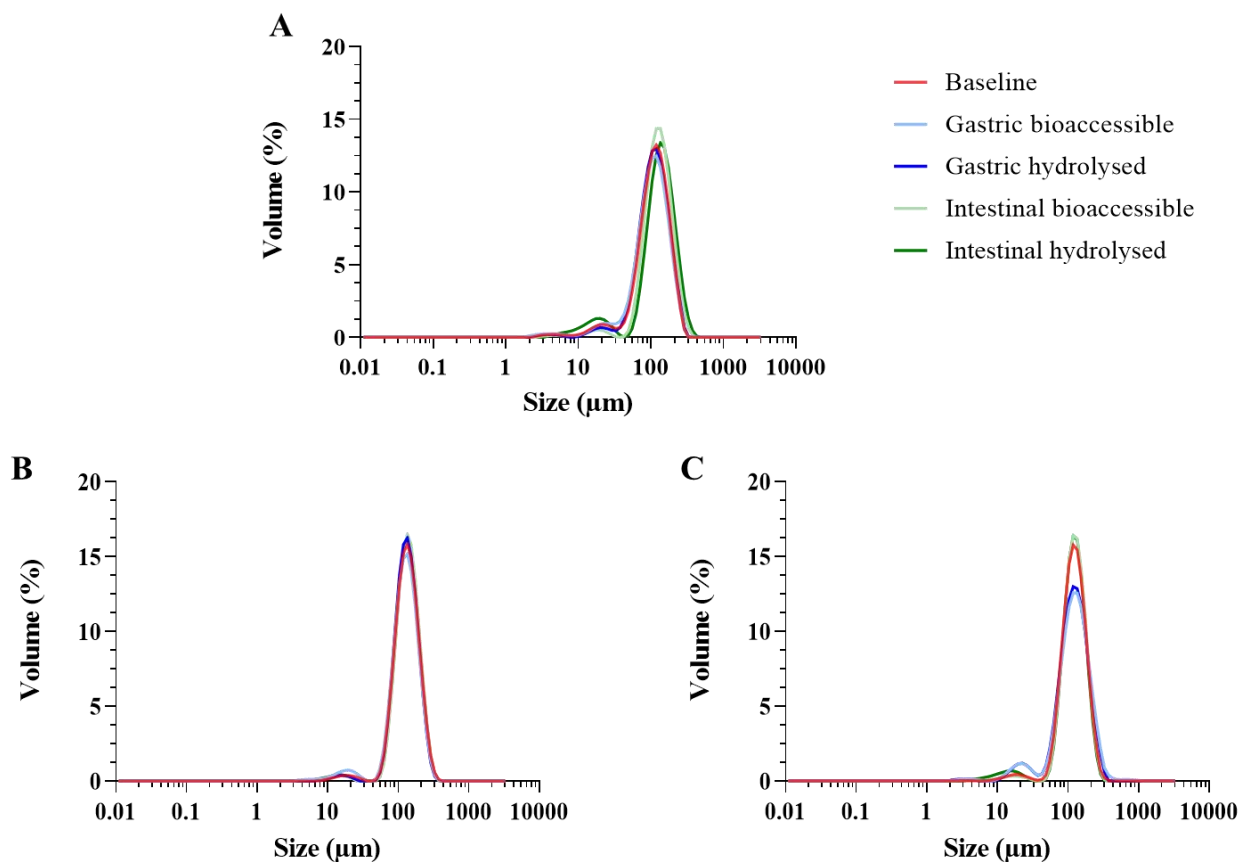
234

235 **Fig. 4.** Microscopy images of pea cells recovered following *in vitro* digestion for the blank (1,
 236 incubation for 6 h without enzyme), gastric (2) and intestinal (3) samples for Water 60°C (A),
 237 Salt 60°C (B) and EDTA (C) treatments.

238

239 Particle size analysis of the recovered cells at different time points of digestion shows an overall
 240 increase in the mean volume of particles with a 135 μm diameter (main peak) demonstrating
 241 the disappearance of most of the solubilised material (loss of the smaller, soluble and
 242 hydrolysable particles such as protein aggregates) (Fig. 5). This is particularly true for the salt
 243 treatment, whereas little changes in cell size were observed for the EDTA treatment. Even

244 though the size of the cells was unchanged, their content was hydrolysed as illustrated in Fig 4
 245 (3A and 3B) where “ghost” cells can be seen.



246
 247 **Fig. 5.** Particle size distribution of the collected samples after gastric and intestinal incubation
 248 for water (A), salt (B) and EDTA (C) cell preparations.

249
 250 Starch, due to the size of the granules, remained entrapped within the cell wall matrix whereas
 251 protein may have leaked out depending on the treatment employed to generate the cells (Fig
 252 4.3A, 3B and 3C). Starch appeared to be constrained within the cell wall of cell treated with
 253 EDTA, which hindered their hydrolysis. More importantly, as mentioned above, having not
 254 been hydrothermally treated, the starch was not gelatinised, the compact/semi-crystalline
 255 structure thus preventing amylase access to the glycosidic bonds (Fig. 4.2B, 2C and 3C).
 256 Regardless of the treatment, a significant amount of starch remained inside the cells after the
 257 gastric phase. The protein however was highly hydrolysed indicating that proteases had access

258 to their substrate. Therefore, while the extra cellular proteins present in the simulated digestive
259 fluids were readily “available” for protease hydrolysis, it is also likely that the enzymes
260 penetrated the cell wall (and hydrolyse the proteins inside the cells) as shown with the diffusion
261 of Fluorescein isothiocyanate (FITC)-labelled dextrans of 10 and 40 kDa in some of the cells
262 (Figure 2S). This size range includes the molecular weight of pepsin (35 kDa) and trypsin (24
263 kDa). In another study, it was reported that amylase (50 kDa) was unable to penetrate the cell
264 wall of pea (Bhattarai et al., 2017). Here, some amylase must have entered the cell given the
265 degradation of starch in the Salt 60°C samples. The longer incubation time used in this study
266 for the intestinal phase may have resulted in cell wall swelling and thereby facilitating the
267 diffusion of larger molecules through the cell wall. Another recent study also found that
268 chickpea proteins were hydrolysed despite being encapsulated into intact cells at the time of
269 ingestion (Perez-Moral, Saha, Pinto, Bajka, & Edwards, 2023). Given that pectin is the main
270 polysaccharide thought to influence cell wall porosity, it is not surprising that the EDTA
271 treatment increased the ability of the enzymes to diffuse inside the cell, and consequently
272 protein hydrolysis (McCann & Knox, 2011). The preparation of isolated cells would have
273 therefore disrupted the cell wall integrity and, throughout digestion, solubilised their
274 components (pectin and hemicellulose while the cellulosic backbone remained intact).

275

276 *3.3. Effect of pea cell wall and its components on protein digestion*

277 In order to investigate the role of isolated cell wall components on protein hydrolysis,
278 polysaccharides of different chemical natures as well as solubilised pea dietary fibres were
279 utilised in the digestibility experiments. Therefore, the extent of protein hydrolysis was
280 monitored in the presence of three main classes of polysaccharides making up the cell wall
281 composition (i.e., cellulose, hemicellulose and pectin) and dietary fibres obtained after the
282 incubation of pea fibres and flour. These experiments demonstrated that rhamnogalacturonan

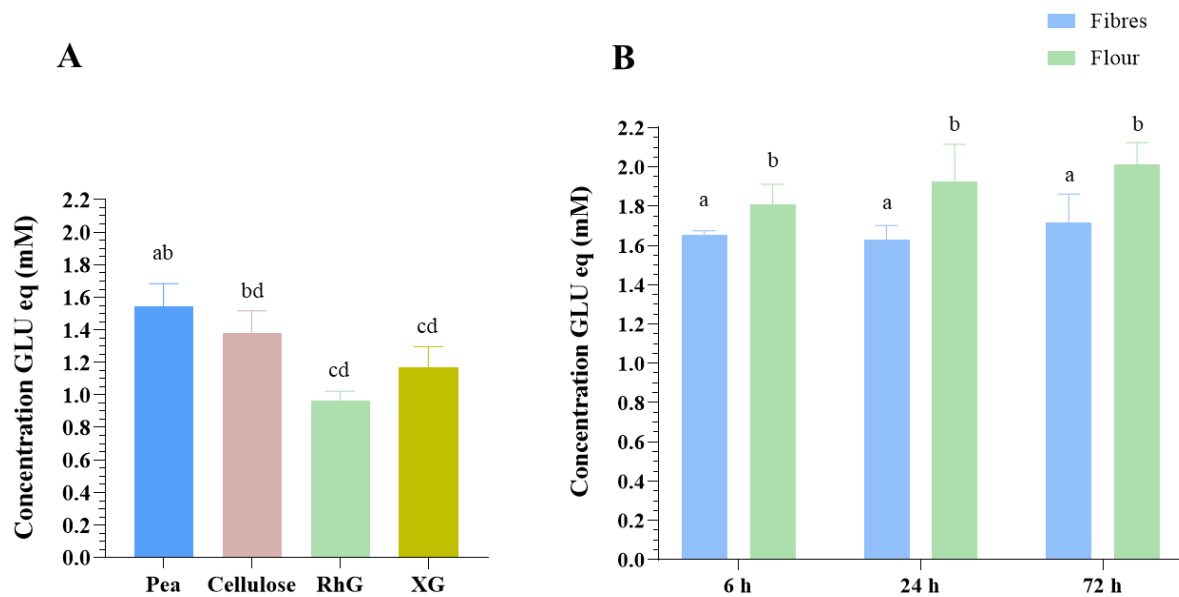
283 I (pectin, RhG) and xyloglucan (hemicellulose, XG) reduced pea protein hydrolysis
284 significantly compared to the pea cell walls (Pea). The intestinal digesta containing RhG and
285 XG looked like a “gel” that was generated from these viscous polysaccharides (Fig. 3S image
286 B).

287 Polysaccharides extracted from pea are not commercially available. Therefore, even though
288 similar in their structure it is likely that the polysaccharides used in this study, especially RhG
289 and XG, had different physico-chemical properties to the pea pectin and hemicellulose
290 solubilised following incubation. Also, pea cell walls may contain polysaccharides of different
291 sizes and chemical structure (branching and sugar composition) (Brillouet & Carré, 1983;
292 Talbott & Ray, 1992). For this reason, pea fibres (consisting of cell wall fragments) and flour
293 were left to incubate in order to solubilise the pea polysaccharides. This enabled us to study
294 the effect of these polysaccharides “outside” the cell wall on protein hydrolysis. Thus, solutions
295 obtained from the incubation of fibres also reduced protein hydrolysis, but to a lesser extent
296 than RhG and XG. Incubation of flour had even less of an impact on protein digestion.
297 Incubation time did not modulate any of these effects therefore the “active” compounds
298 (probably polysaccharides but maybe also phenolic compounds) must have been released at
299 the early stage of the incubation. Rupture of pea cells appeared to be necessary to release
300 polysaccharides and other cell wall components (e.g., phenolic compounds) that could interact
301 with either the proteases or the proteins to influence protein hydrolysis.

302 The functionality of extracted, purified compounds is often different to when the same
303 compounds are still present within the food matrix. Indeed, the extraction process is likely to
304 change their chemical composition, molecular size, their (micro)environment and as a
305 consequence, their biological activity. This is well known in the case of β -glucan, which can
306 lose its properties once extracted (Wang & Ellis, 2014). In this study, we also revealed that cell

307 wall polysaccharides did not have the same impact on protein hydrolysis as part of the cell
 308 wall, solubilised in the aqueous environment or extracted.

309



310

311 **Fig. 6.** Protein digestibility expressed as glutamic acid equivalents (in mM) corresponding to
 312 the free amino groups quantified by the OPA method after intestinal digestion of pea protein
 313 in the presence of polysaccharides (A) or incubation solutions of fibres or flour (B). Different
 314 letters indicate significant differences as determined by one-way ANOVA and Tukey's post-
 315 hoc test ($P < 0.05$). Pea: cell wall fragments from pea, RhG: rhamnogalacturonan I, and XG:
 316 xyloglucan.

317

318 Therefore, the hindering of protein hydrolysis by encapsulation mechanism is time dependent,
 319 with the cell wall being degraded as the food travels through the gastrointestinal tract. In
 320 particular, swelling and solubilisation of cell wall components (pectin) can increase porosity,
 321 thereby allowing the penetration of proteases. Longer incubation time was selected in this work
 322 based on *in vivo* data (Grundy et al., 2023). The Infogest protocol has been developed primarily
 323 for dairy sources of proteins, however plant-based foods can be difficult to digest due to the

324 presence of tissue structure containing intact cells, the presence of antinutritional factors and
325 the structure of the proteins (Grundy et al., 2022; Sousa, Portmann, Dubois, Recio, & Egger,
326 2020).

327

328 **4. Conclusion**

329 The current study demonstrated that different treatments applied to peas to obtain isolated cells
330 has consequences on the cell wall structure and porosity, which in turn affects the kinetics of
331 protein hydrolysis. Therefore, the way in which a plant tissue is transformed to generate food
332 products is crucial for its behaviour in the gastrointestinal tract and its subsequent health
333 outcome. Specifically, modulating the porosity of cell walls can be a strategy to improve the
334 postprandial absorption of certain nutrients. However, as far as pulses are concerned, it may be
335 of interest to reduce encapsulation of proteins and improve their digestibility. We demonstrated
336 that even though the cell walls appear to maintain their apparent structure, most of the cell
337 contents were hydrolysed after intestinal digestion. Questions still remain regarding the fate of
338 those cell walls in the colonic compartment and the subsequent impact on the composition and
339 functionality of the gut microbiota.

340

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343

344

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