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## Impact of soaking process on the microstructure of cowpea seeds in relation to solid losses and water absorption

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1                   **Impact of soaking process on the microstructure of**  
2                   **cowpea seeds in relation to solid losses and water**  
3                   **absorption**

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**Abstract**

A cytohistological investigation was performed to better understand the structural alterations of cowpea seeds in relation with soaking. Thin sections obtained from seeds soaked at 30°C, 60°C and 95°C were treated to specifically visualize starch, proteins, cellulose and pectin. Micropyle behavior as well as water uptake and dry matter loss were also monitored. A Soaking at 30°C induced slight alteration of parenchymatous cells of cotyledons, whereas drastic alterations were observed at 60°C and intense alterations at 95°C. All these structural modifications of cells could explain losses of nutrients and antinutritional factors at the highest soaking temperature (95°C). The size of the apertures in the micropyle sections varied depending on both soaking temperature and time. At 30°C and 60°C, the micropyle aperture enabled notable water uptake by seeds. At 95°C, water was entirely taken up through the testa. These results show that models describing nutrients losses and water uptake should account for these structure changes during soaking of legumes.

**Keywords**

Microscopy; legumes; cell wall; micropyle; cooking;  $\beta$ -elimination

43 **1. Introduction**

44 Legume seeds such as cowpea (*Vigna unguiculata*) are widely cropped and are of vital  
45 importance to the livelihood of several million people in West Africa (Achigan-Dako et al., 2011).  
46 Legume seeds are important in human nutrition (Graham & Vance, 2003) and unlike animal-based  
47 products, benefit the environment (Tscharntke et al., 2012). Cowpea seeds are rich in proteins (20-  
48 25 %), starch (45-55%) and micronutrients including vitamins and minerals (Sreerama, Sashikala,  
49 Pratapa, & Singh, 2012). Cowpeas are used in many different traditional African dishes (Dovlo,  
50 Williams, Zoaka, & others, 1976) whose preparation often requires a preliminary soaking step.  
51 Soaking usually involves immersing the seeds in water at room temperature overnight. After soaking,  
52 the seeds are usually cooked in boiling water for 40 to 60 min (Madodé et al., 2013). During the  
53 soaking-cooking process, due to both seed heating and hydration, the seeds partially or totally  
54 gelatinize and proteins may undergo thermal denaturation. All these combined phenomena strongly  
55 affect seed structure and confer a softer texture..

56 In terms of structure, cowpea seeds are composed of a testa with a hilum and a micropyle, two  
57 cotyledons and an embryonic axis (epicotyl + hypocotyl + radicle) (Stanley & Aguilera, 1985). The  
58 cotyledon is the main nutritional component of legume seeds and previous studies have shown that  
59 cotyledons are rich in starch and proteins (Swanson, Hughes, & Rasmussen, 1985). Heat treatment  
60 between 60°C and 75°C during soaking causes the starch to gelatinize thereby enhancing its  
61 digestibility (Coffigniez et al., 2018; Stanley & Aguilera, 1985). It also enhances protein digestibility  
62 by increasing protein solubility (Alonso, Aguirre, & Marzo, 2000; Hoover, Hughes, Chung, & Liu,  
63 2010). The structural integrity of cotyledons is also affected by heating. For example, Hung,  
64 McWatters, Phillips, & Chinnan, (1990) showed that soaking at 50°C, 70°C and 90°C dissociated the  
65 cotyledon cells. This is mainly due to pectin depolymerization and  $\beta$ -elimination (Njoroge et al.,  
66 2016). Heating has also been shown to induce nutritional and anti-nutritional losses through diffusion  
67 and thermal degradation (Ibrahim, Habiba, Shatta, & Embaby, 2002; Coffigniez et al., 2018). A

68 previous study by our team showed a low diffusion of alpha-galactosides during soaking at 30°C,  
69 which was enhanced at higher soaking temperatures (60°C and 95°C) (Coffigniez et al., 2018).  
70 However, the cytohistological mechanisms involved in this heat-induced destructuring remain poorly  
71 understood.

72 Heat treatment also affects the water uptake capacity of the seeds. The testa can act as a barrier  
73 to water uptake and affect the extent of interlocking with the cotyledons (Sefa-Dedeh & Stanley,  
74 1979). In cowpea and lupin, most water absorption occurs via the micropyle and the testa of the  
75 seeds (Sefa-Dedeh & Stanley, 1979; Robles-Díaz, Flores, & Yáñez-Espinosa, 2016). The size of the  
76 micropyle determines the quantity of water absorbed by the seed (Sefa-Dedeh & Stanley, 1979). The  
77 degree of starch gelatinization also determines the water uptake capacity of the seed (Briffaz,  
78 Bohuon, Méot, Dornier, & Mestres, 2014). However, the impact of heat treatments on the behaviour  
79 of the micropyle and consequently, on its role in regulating water uptake remains to be established.

80 The aim of the present study was thus to better understand the impact of seed structural  
81 modifications at different levels (including cotyledons and micropyle) on water uptake, dry matter  
82 losses and on the biochemical behavior of some macro constituents (starch, proteins, cell walls)  
83 during soaking of cowpea seeds using microscopic and cytohistological techniques.

## 84 **2. Materials and methods**

### 85 ***2.1 Material***

86 The variety of cowpea used for this study was the *Wankoun* brownish one provided by IITA in  
87 Benin. Cowpea seeds were sown in November 2014 and harvested in February 2015. The seeds were  
88 kept in a vacuum pack and stored at 4°C in the dark until use.

89 **2.2 Soaking experiments**

90 Thirty grams of cowpea seeds were soaked in 120 mL deionized water using the method  
91 described by Coffigniez et al., (2018) with a resulting seed-to-water ratio of 1:4 (w/w). The  
92 temperatures and time ranges investigated were 30°C/0.5-38 h; 40°C/0.25-8 h; 60°C/0.16-8 h;  
93 70°C/0.16-6 h; 80°C/0.16-6 h and 95°C/0.25-3 h.

94 In an additional experiment, prior to soaking at 30°C for 3 h or 60°C for 1 h, the hilum and  
95 micropyle were both obstructed with superglue (cyanoacrylate) to assess their role in water uptake.

96 **2.3 Microscopy studies**

97 **2.3.1 Seed overall morphology**

98 To analyze the overall structure of the seeds, symmetric longitudinal half seed sections were cut  
99 using shape razor blades and stained with lugol (to reveal the starch). Observations were performed  
100 with a Nikon AZ100 stereomicroscope (Champigny sur Marne, France).

101 **2.3.2 Cytology**

102 Raw (control) and soaked seeds (see section 2.2 for soaking procedure) were immersed in a cold  
103 fixative solution consisting of 4% (v/v) glutaraldehyde and 4% (v/v) paraformaldehyde in 0.2 M  
104 phosphate buffer (pH=7.2). To facilitate the penetration of the fixative products, samples were placed  
105 in a vacuum for 20 min. After 48 h of fixation at 4°C, the specimens were rinsed in distilled water  
106 and stored in 70% ethanol at 4°C until required. They were then cut into 5×5 mm fragments from  
107 different regions of the seed: cotyledons, testa with hilum and micropyle. All the samples were  
108 dehydrated in increasing concentrations of ethanol (80-100% (v/v)) and embedded in methacrylate  
109 resin (Kit Technovit 7100, Heraeus-Kulzer GmbH, Wehrheim, Germany) according to the  
110 manufacturer's instructions. Sections (3 µm thick) were serially cut using a retraction microtome  
111 (Supercut 2065, Reichert-Young, Wien, Austria) and deposited on microscope slides. For routine

112 observations, sections were stained with toluidine blue, a metachromatic dye (Clark, 1981). Sections  
113 were also cytochemically stained to specifically visualize major cell components using the following  
114 cytochemical reagents: periodic acid-Schiff's reagent (PAS) for starch and cellulose (pink); naphthol  
115 blue-black (NBB) protein (blue) (El Maâtaoui & Pichot, 1999), Ruthenium Red (RR) (Sigma,  
116 Germany) for pectin (red) and Fluorescent Brightener (FB) (Sigma, St Louis) for cellulose (Clark,  
117 1981). In this latter case, the observations were made using UV illumination (365 nm) (Clark,  
118 1981). All the observations were performed using a Leica DMR (Leica, Wetzlar, Germany)  
119 photomicroscope equipped for bright field, dark field, phase contrast and UV illuminations. Images  
120 were captured using a Leica DFC 300 FX digital camera and processed using LAS (Leica, Wetzlar,  
121 Germany) and image J software (<https://imagej.net/>). For each treatment, approximately 60 sections  
122 were analysed, and the best images were selected to illustrate our investigation.

### 123 *2.3.3 Micropyle aperture measurements*

124 Using the same experimental configuration as explained above, the aperture of the micropyle was  
125 measured on 50 microscopic cross sections obtained from seeds soaked in different time and  
126 temperature conditions (see section 2.2) and is expressed as an average length ( $\mu\text{m}$ ).

### 127 *2.4 Dry matter losses*

128 For each soaking experiment, around 20 mL of soaking water was collected, weighed and dried  
129 at 100°C for 48 h. The dry matter loss  $DML(t)$  (kg / 100 kg db) in soaking water was calculated  
130 against time  $t$  (h) as follows:

$$131 \quad DML(t) = \frac{m_{d,sw}(t)}{m_{d,s}(0)} * 100 \quad (1)$$

132 where  $m_{d,sw}(t)$  and  $m_{d,s}(0)$  are the mass (kg) of the 20 mL of soaking water after drying and the  
133 mass of dried seeds at initial time respectively (kg).

## 134 **2.5 Water uptake kinetics**

135 Water content  $WC(t)$  (kg of water/kg db) of soaked seeds was measured as a function of soaking  
136 time  $t$  (h) using the method described by (Coffigniez et al., 2018) and the following equation:

$$137 \quad WC(t) = \frac{m_{w,s}(t) - m_{d,s}(t)}{m_{d,s}(t)} \quad (2)$$

138 where  $m_{w,s}(t)$  and  $m_{d,s}(t)$  are the mass of the wet seeds and dried seeds versus time  $t$  (h)  
139 respectively (kg).

## 140 **3. Results and discussion**

141 Observations of symmetric longitudinal seed sections showed that the gross structure of cowpea  
142 seed is consistent with the organisation of exalbuminous *Fabaceae* (Fig. 1). The structure comprises  
143 an embryo with massive and reserve-rich cotyledons (C) as well as an embryonic axis (E) embedded  
144 in the testa (T), giving them a kidney-like appearance. The hilum (H) and micropyle (M) are located  
145 in the concave part of seeds.

### 146 **3.1 Modifications of cotyledon cells**

147 The cytohistological effects of soaking process were mainly investigated in the parenchymatous  
148 tissue of the cotyledons, which is the main component of the seed.

#### 149 **3.1.1 Modification of cell content: starch and protein**

150 The cotyledons of the control (raw seeds) were composed of an epidermal layer embedding  
151 massive, compact parenchymatous tissue containing many vascular bundles (not shown). PAS and  
152 NBB staining (Fig. 2a) revealed numerous compact, heterogeneously sized starch granules (stained  
153 pink) and a protein-rich (stained blue) cytoplasm. Proteins were dispersed in the cytoplasm  
154 (amorphous), or organised as protein bodies. The parenchymatous tissue contained sizable  
155 intercellular spaces, sometimes filled with protein.



156 Figure 2 (b, c and d) shows the impact of soaking treatments on cowpea cotyledon cells.  
157 Compared to raw seeds (Fig. 2a), sections of cotyledon obtained from seeds soaked at 30°C for 6 h  
158 showed a decrease in the intensity of the staining of the starch granules, proteins and cell walls of the  
159 parenchymatous cells (Fig. 2b). Similar alterations were observed in parenchymatous cells soaked at  
160 60°C for 2 h. However, PAS and NBB positive material accumulated in the intercellular space  
161 (Fig. 2c). Sections of cotyledons from seeds being soaked at 95°C for 1 h showed marked alterations  
162 in the structure of the parenchymatous cells (Fig. 2d). Indeed, we observed a decrease in PAS  
163 staining of starch and an increase in the size of starch granules, which doubled in size (Fig. 2d)  
164 compared to raw seeds (Fig. 2a). On the basis of Differential Scanning Calorimetry measurements,  
165 Coffigniez et al., (2018) showed that a cooking at 95°C for 1 h resulted in full gelatinization of  
166 starch. The proteins were homogeneous in the cytoplasm, but the blue color was strengthened by  
167 NBB (Fig. 2d). Dense material filled the intercellular spaces (Fig. 2d).

### 168 *3.1.2 Modifications of cell walls*

169 Given that the major constituents of plants cell walls are cellulose and pectin, we decided to use  
170 PAS, FB and RR staining reagents. Figures 2, 3 and 4 are representative micrographs illustrating the  
171 effects of soaking on the behaviour of these components. In the control, PAS resulted in intense  
172 staining of the components of the cell walls, which appeared to be compact and homogeneously  
173 organized (Fig. 2a). This was confirmed by the results of FB (Fig. 3a) and RR (Fig. 4a) treatments  
174 for cellulose and pectin respectively. After staining, the cell walls displayed a cohesive cellulose  
175 structure and a middle lamella in the form of a pectin layer. After soaking at 30°C for 6 h, the cell  
176 walls showed a reduced intensity using PAS reagent (Fig. 2b), and slight disruption causing the  
177 migration of cytoplasmic materials which accumulated in enlarged intercellular spaces. FB staining  
178 (Fig. 3b) showed loosening of the cellulose, considerably thickening the cell walls. Pectin specific  
179 staining with RR (Fig. 4b) showed a reduced intensity in the pectic middle lamella, particularly at the  
180 boundaries of the intercellular spaces. Soaking at 60°C induced similar but greater alterations of the

181 cell walls (Figs. 2c, 3c and 4c) with the accumulation of cytoplasmic material in intercellular spaces  
182 and separation of the cells. Sections of cotyledons soaked at 95°C displayed parenchymatous cells  
183 with profoundly destructured cell walls (Figs. 2d, 3d and 4d). This was confirmed by the breaks in  
184 the cell walls at several places and the onset of cell dissociation where the middle lamella had  
185 disintegrated. After this treatment, there was intense accumulation of cytoplasmic material in the  
186 intercellular spaces.

187 The cell wall degradation and accumulation in intercellular space after soaking at 60°C and 95°C  
188 (figs. 2 to 4) showed that the cell walls were permeable and that cellular content had diffused into the  
189 intercellular space. Other authors also reported similar cell disruption after heat treatment of  
190 legumes. Mwangwela, Waniska, & Minnaar, (2006) showed the accumulation of pectins in the  
191 intercellular space after hydrothermal treatment. Waldron et al., (2003) and Varriano-Marston &  
192 Jackson, (1981) reported the loss of plasmalemma and disorganization of cytoplasm after treatment  
193 at high temperature. This communication with the intercellular space probably began in the  
194 plasmodesmata and could be due to  $\beta$ -elimination and pectin depolymerization (Bernal-Lugo, Parra,  
195 Portilla, Peña-Valdivia, & Moreno, 1997; Waldron, Parker, & Smith, 2003). Buren, (1979), Liu,  
196 Phillips, & McWatters (1993) and Njoroge et al. (2016) showed that  $\beta$ -elimination was limited below  
197 60°C but total after boiling at 100°C for 30 min.

198 A very slight disruption of the cotyledon cell walls appeared after soaking at 30°C. The limited  
199 disruption could be linked to lack of  $\beta$ -elimination, but also to possible cell wall strengthening, partly  
200 due to phytate hydrolysis. In cowpea, phytate concentrations range from 0.3–1.5 mg/100 mg, mainly  
201 in the protein bodies of the cotyledon parenchyma. Phytates are associated with bivalent minerals  
202 such as calcium, magnesium, iron and zinc (Kruger, Minnis-Ndimba, Mtshali, & Minnaar, 2015;  
203 Gonçalves et al., 2016; Sandberg, 2002). Some authors have reported loss of phytates during soaking  
204 of legumes at 30°C due to hydrolysis from endogenous phytase (Alonso et al., 2000; Chang,  
205 Schwimmer, & Burr, 1977; Shi, Arunasalam, Yeung, Kakuda, & Mittal, 2004). This phytate

206 degradation triggers the release of bivalent minerals from the phytate-mineral complex and their  
207 migration towards the cell wall of the middle lamella (Phillips et al., 2003), where the pectins are  
208 usually soluble and linked to monovalent minerals. However, in this case, the released divalent  
209 minerals, especially calcium, replace the monovalent minerals and the pectin becomes insoluble  
210 (Kruger, Minnis-Ndimba, Mtshali, & Minnaar, 2015). The insoluble pectin forms a stronger cell  
211 wall, thereby preventing cell separation (Phillips et al., 2003).

212 When legumes are soaked at between 50°C and 60°C, there is competition between pectin  
213 strengthening and pectin degradation in the cell walls. However, in our cytohistological study and in  
214 this temperature range, pectin degradation predominated (Figs. 2b and 4b).

### 215 *3.1.3 Impact of cell wall degradation on mass transfer properties*

216 The observed structural changes in the cell wall during soaking can strongly affect the mass  
217 transport of several components, including nutrients and antinutrients, from the seed to the soaking  
218 medium. As shown in figure 6a, soaking temperature had a strong effect on the extent of solid losses.  
219 Indeed, over a soaking time of 3 h, mean dry matter losses were about 10 times higher at 95°C than  
220 at 30°C. The same trend was reported by Kon, 1979 in beans with a dry matter loss of 2%, 12% and  
221 12% after soaking for 5 h at 40°C, 1 h 30 at 60°C and 0.8 h at 90°C, respectively. These losses,  
222 which often include nutrients and antinutrients, are thus facilitated by high soaking temperatures that  
223 may increase cell wall degradation.

224 For instance, concerning antinutrients, a previous study by our team on the same cowpea  
225 variety (*Wankoun*) showed that 2.2%, 58% and 48% of alpha-galactosides had diffused in water after  
226 soaking at 30°C for 6 h, at 60°C for 2 h, and at 95°C for 1 h, respectively (Coffigniez et al., 2018).  
227 This diffusion of alpha-galactosides from cowpea seed was modelled using Fick's law and apparent  
228 diffusivities were identified, revealing a strong effect of temperature. For example, the apparent  
229 diffusivity of stachyose was found to be 150 times lower at 30°C than at 60°C and 1.5 lower at 60°C

230 than at 95°C. The modification in the structure of the cell wall together with pectin degradation at  
231 higher temperature may explain the resulting higher apparent diffusivities obtained at 60°C and 95°C  
232 compared to 30°C. This model could be upgraded by integrating this structural factor.

233 Similarly, other components can leach out of the legume seed to an extent which varies with  
234 the soaking conditions. Carbohydrates represent the main fraction which is lost during soaking. For  
235 instance, Sayar et al. (2011) showed in the case of chickpea that boiling for 1 h or soaking at 20°C  
236 for 15 h induced carbohydrate losses of 75% (db) and of 88% (db) respectively. For instance,  
237 soaking cowpea seeds at room temperature for 8 h resulted in a loss of tannins, phytic acid and  
238 trypsin inhibitors of less than 1%, whereas boiling for 45 min resulted in a loss of 41%, 6.8 % and  
239 87 % of the same species, respectively (Ibrahim, Habiba, Shatta, & Embaby, 2002). In beans, Kon,  
240 1979 found that increasing soaking temperature (40°C/5 h, 60°C/1.5 h, 90°C/50 min) increased the  
241 diffused fraction of thiamin by 1%, 32% and 40%, respectively). All these observations show that the  
242 soaking temperature can be optimized to preserve the nutritional value of seeds for human  
243 consumption.

244 By comparing the results of the present microscopic study and those of a quantitative study  
245 on alpha-galactosides by Coffigniez et al. (2018) using the same seed cultivar, we can confirm that  
246 there is a strong link between destructuring of the cell (disruption of the cell wall and degradation of  
247 pectin) that is enhanced by high soaking temperatures and molecular diffusion through the  
248 intercellular space. However, other seed structures could also affect the material losses from the seed  
249 during soaking such as the micropyle whose opening can facilitate diffusion, essentially at low  
250 temperatures.

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### 3.2 Water uptake by the seeds

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#### 3.2.1 Water uptake from the micropyle

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The behaviour of micropyle was monitored using cross sections stained toluidine blue. Cross sections from the control (Fig. 5a) showed a closed aperture embedded in three different structures: a spongy tissue (around 500  $\mu\text{m}$ ), a palisade layer (around 150  $\mu\text{m}$ ) and a group of tracheids (around 400  $\mu\text{m}$ ). After soaking at 30°C for 3 h and 6 h and at 60°C for 1 h and 2 h, the micropyle was open. However, the opening was twice as big after the shortest soaking time at 30°C and 60°C (Table 1). After soaking at 95°C, the micropyle remained almost closed (Table 1) at both soaking times.

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Figure 6b shows cowpea seed water uptake kinetics at different soaking temperatures ranging from 30°C to 95°C. The early water uptake rate (calculated as the linear slope from 0 to 0.5 h, data not shown) increased with soaking temperature, except at 95°C, when the lower initial water uptake rate could be due to the closure of the micropyle (Figs. 5f and 5g). These results are consistent with those obtained by Coffigniez et al., (2018) using the same cowpea material and in the same experimental soaking conditions. These authors identified apparent water diffusivities ( $D$ ,  $\text{m}^2.\text{s}^{-1}$ ) in cowpea seed through model adjustment and showed that  $D$  was two times lower at 95°C than at 60°C. In the present study, seed water content was also measured in the same experimental conditions but with the seed micropyle and hilum artificially closed using superglue (data not shown). In this configuration, the resulting water content was 39% and 33% lower after soaking at 30°C for 3 h and at 60°C for 1 h, respectively than in seeds with a free micropyle and hilum. These observations suggest that at low temperature, these structures are involved in the regulation of water uptake by the seed. Moreover, at low soaking temperatures (i.e. at 30°C and 40°C), water absorption behavior was not purely Fickian, with a short delay at the early stage of water uptake kinetics. Indeed, at 40°C for example, the initial ( $t < 0.25$  h) water uptake rate was found to be lower

275 (0.88 kg/kg db/h) than that from 15 to 30 min (1.52 kg/kg db/h). These results suggest that active  
276 transport might occur through the micropyle and hilum. The contribution of these structures to the  
277 active control of seed water absorption at low soaking temperatures was confirmed with the aperture  
278 of the micropyle at 30°C (figs. 5b and 5c).

279 Other authors also highlighted the contribution of the micropyle to the water absorption process  
280 in some legume seeds. Using a dye in lentil and lupin seeds soaked at room temperature, Robles-  
281 Díaz et al., (2016) showed that water uptake preferentially occurred through the micropyle. Gama-  
282 Arachchige, Baskin, Geneve, & Baskin, (2013) blocked the hilum and micropyle of *Cercis*  
283 *canadensis* seeds, which partly blocked water uptake. Lush & Evans, (1980) reported that the  
284 behaviour of the micropyle of cowpea seeds depended to a great extent on experimental conditions.  
285 Indeed, they showed that the micropyle is waterproof (closed) when the seed is dry (water content of  
286 10-12 %). To absorb water, a change in cell conformation and a water content threshold (24%) are  
287 required for micropyle opening.

### 288 **3.2.2 Water uptake through testa layer**

289 Despite the blockage of micropyle and hilum structures (section 3.2.1), 60% of water uptake was  
290 maintained during soaking at 30°C and 60°C. This water uptake probably occurred through the testa  
291 layer. In this case, the structure of the cowpea seed testa may play an important role in water uptake  
292 (Stanley & Aguilera, 1985). The water diffusivity through the testa increased with decreasing  
293 thickness and increasing temperature (Kaptso et al., 2008; Yildirim, Öner, & Bayram, 2011). At  
294 95°C, water was only taken up through the testa (micropyle closed), which explained the lower water  
295 uptake rate before 1 h compared with that at lower temperatures. However, the marked breakdown of  
296 cell walls observed at 95°C (Figs. 2d to 4d) could facilitate water uptake and partly explain the  
297 higher water uptake after 1 h compared with that at lower temperatures.

#### 298 **4. Conclusion**

299 Our study has advanced our knowledge of changes in major cell components and structure  
300 (including micropyle) of cowpea seeds during soaking and their impact on the diffusion of material  
301 and uptake of water. The changes in the cells were monitored by means of cytohistological  
302 investigation. During soaking at 30°C, only slight structural modifications were observed, with  
303 minimal impact on mass transfer out of the cells and water mainly taken up through the micropyle.  
304 At higher soaking temperatures (i.e. 60°C and 95°C), cell wall disruption occurred due to  $\beta$ -  
305 elimination and depolymerization of pectin. The drastic effect of high soaking temperatures on the  
306 cell walls was responsible for the loss of material in the soaking water that facilitated water uptake  
307 by the seeds. At high soaking temperatures, water uptake mainly occurred through the testa layer. All  
308 these temperature-dependent structural changes should be integrated in existing models to better  
309 describe the diffusion of alpha-galactosides and the uptake of water by cowpea seeds during soaking.

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311 The authors declare no conflicts of interest. FC, MEM and LA set up the study and performed the  
312 experiments. FC, MEM AB, CM and PB revised the manuscript. All the authors read and approved  
313 the final manuscript.

314

#### 315 **6. References**

316 Achigan-Dako, E. G., N'Danikou, S., Assogba-Komlan, F., Ambrose-Oji, B., Ahanchede, A., &  
317 Pasquini, M. W. (2011). Diversity, geographical, and consumption patterns of traditional  
318 vegetables in sociolinguistic communities in Benin: Implications for domestication and  
319 utilization<sup>1</sup>. *Economic Botany*, 65(2), 129.

320 Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods  
321 on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food*  
322 *Chemistry*, 68(2), 159–165.

323 Bernal-Lugo, I., Parra, C., Portilla, M., Peña-Valdivia, C. B., & Moreno, E. (1997). Cotyledon  
324 thermal behavior and pectic solubility as related to cooking quality in common beans. *Plant*  
325 *Foods for Human Nutrition*, 50(2), 141–150.

326 Briffaz, A., Bohuon, P., Méot, J.-M., Dornier, M., & Mestres, C. (2014). Modelling of water  
327 transport and swelling associated with starch gelatinization during rice cooking. *Journal of*  
328 *Food Engineering*, 121, 143–151.

329 Chang, R., Schwimmer, S., & Burr, H. K. (1977). Phytate: removal from whole dry beans by  
330 enzymatic hydrolysis and diffusion. *Journal of Food Science*, 42(4), 1098–1101.

331 Clark, G. (1981) *Staining procedures*, 4th edition, William-Wilkins, London, UK

332 Coffigniez, F., Briffaz, A., Mestres, C., Alter, P., Durand, N., & Bohuon, P. (2018). Multi-response  
333 modeling of reaction-diffusion to explain alpha-galactoside behavior during the soaking-  
334 cooking process in cowpea. *Food Chemistry*, 242, 279–287.

335 Dovlo, F. E., Williams, C. E., Zoaka, L., & others. (1976). *Cowpeas. Home preparation and use in*  
336 *West Africa*. International Development Research Centre. Retrieved from  
337 <http://www.cabdirect.org/abstracts/19771455627.html>

338 El Maâtaoui, M., & Pichot, C. (1999). Nuclear and cell fusion cause polyploidy in the  
339 megagametophyte of common cypress, *Cupressus sempervirens* L. *Planta*, 208(3), 345–351.

340 Gama-Arachchige, N. S., Baskin, J. M., Geneve, R. L., & Baskin, C. C. (2013). Identification and  
341 characterization of ten new water gaps in seeds and fruits with physical dormancy and  
342 classification of water-gap complexes. *Annals of Botany*, 112(1), 69–84.

343 Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E. A., ...  
344 Rodrigues, M. (2016). Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop



345 for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the*  
346 *Science of Food and Agriculture*, 96(9), 2941–2951.

347 Graham, P. H., & Vance, C. P. (2003). Legumes: importance and constraints to greater use. *Plant*  
348 *Physiology*, 131(3), 872–877.

349 Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure,  
350 properties, and modification of pulse starches: A review. *Food Research International*, 43(2),  
351 399–413.

352 Hung, Y.-C., McWatters, K. H., Phillips, R. D., & Chinnan, M. S. (1990). Effects of Pre-  
353 decortication Drying Treatment on the Microstructure of Cowpea Products. *Journal of Food*  
354 *Science*, 55(3), 774–776.

355 Ibrahim, S. S., Habiba, R. A., Shatta, A. A., & Embaby, H. E. (2002). Effect of soaking, germination,  
356 cooking and fermentation on antinutritional factors in cowpeas. *Food/Nahrung*, 46(2), 92–95.

357 Kaptso, K. G., Njintang, Y. N., Komnek, A. E., Hounhouigan, J., Scher, J., & Mbofung, C. M. F.  
358 (2008). Physical properties and rehydration kinetics of two varieties of cowpea (*Vigna*  
359 *unguiculata*) and bambara groundnuts (*Voandzeia subterranea*) seeds. *Journal of Food*  
360 *Engineering*, 86(1), 91–99. <https://doi.org/10.1016/j.jfoodeng.2007.09.014>

361 Kon, S. (1979). Effect of Soaking Temperature on Cooking and Nutritional Quality of Beans.  
362 *Journal of Food Science*, 44(5), 1329–1335. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2621.1979.tb06432.x)  
363 [2621.1979.tb06432.x](https://doi.org/10.1111/j.1365-2621.1979.tb06432.x)

364 Kruger, J., Minnis-Ndimba, R., Mtshali, C., & Minnaar, A. (2015). Novel *in situ* evaluation of the  
365 role minerals play in the development of the hard-to-cook (HTC) defect of cowpeas and its  
366 effect on the *in vitro* mineral bioaccessibility. *Food Chemistry*, 174, 365–371.

367 Liu, K., Phillips, R. D., & McWatters, K. H. (1993). Mechanism of pectin changes during soaking  
368 and heating as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food*  
369 *Chemistry*, 41(9), 1476–1480.

370 Lush, W. M., & Evans, L. T. (1980). The seed coats of cowpeas and other grain legumes: structure in  
371 relation to function. *Field Crops Research*, 3, 267–286.

372 Madodé, Y. E., Nout, M. J., Bakker, E.-J., Linnemann, A. R., Hounhouigan, D. J., & van Boekel, M.  
373 A. (2013). Enhancing the digestibility of cowpea (*Vigna unguiculata*) by traditional  
374 processing and fermentation. *LWT-Food Science and Technology*, 54(1), 186–193.

375 Mwangwela, A. M., Waniska, R. D., & Minnaar, A. (2006). Hydrothermal treatments of two cowpea  
376 (*Vigna unguiculata* L. Walp) varieties: effect of micronisation on physicochemical and  
377 structural characteristics. *Journal of the Science of Food and Agriculture*, 86(1), 35–45.

378 Njoroge, D. M., Kinyanjui, P. K., Chigwedere, C. M., Christiaens, S., Makokha, A. O., Sila, D. N., &  
379 Hendrickx, M. E. (2016). Mechanistic insight into common bean pectic polysaccharide  
380 changes during storage, soaking and thermal treatment in relation to the hard-to-cook defect.  
381 *Food Research International*, 81, 39–49.

382 Phillips, R. D., McWatters, K. H., Chinnan, M. S., Hung, Y.-C., Beuchat, L. R., Sefa-Dedeh, S.,  
383 Saalia, F. K. (2003). Utilization of cowpeas for human food. *Research Highlights of the*  
384 *Bean/Cowpea Collaborative Research Support Program, 1981 - 2002*, 82(2–3), 193–213.  
385 [https://doi.org/10.1016/S0378-4290\(03\)00038-8](https://doi.org/10.1016/S0378-4290(03)00038-8)

386 Robles-Díaz, E., Flores, J., & Yáñez-Espinosa, L. (2016). Paths of water entry and structures  
387 involved in the breaking of seed dormancy of *Lupinus*. *Journal of Plant Physiology*, 192, 75–  
388 80.

389 Sandberg, A.-S. (2002). Bioavailability of minerals in legumes. *British Journal of Nutrition*, 88(S3),  
390 281–285.

391 Sayar, S., Turhan, M., Köksel, H., (2011). Solid loss during water absorption of chickpea (*Cicer*  
392 *Arietinum* L.). *Journal of Food Process Engineering*, 34(4), 1172–1186.

393 Sefa-Dedeh, S., & Stanley, D. W. (1979). The relationship of microstructure of cowpeas to water  
394 absorption and dehulling properties. *Cereal Chemists*, 56(4), 379–386.

395 Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., & Mittal, G. (2004). Phytate from edible beans:  
396 chemistry, processing and health benefits. *Journal of Food Agriculture and Environment*, 2,  
397 49–58.

398 Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., & Singh, V. (2012). Nutrients and antinutrients in  
399 cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour  
400 functionality. *Food Chemistry*, 131(2), 462–468.  
401 <https://doi.org/10.1016/j.foodchem.2011.09.008>

402 Stanley, D. W., & Aguilera, J. M. (1985). A review of textural defects in cooked reconstituted  
403 legumes-The influence of structure and composition. *Journal of Food Biochemistry*, 9(4),  
404 277–323.

405 Swanson, B. G., Hughes, J. S., & Rasmussen, H. P. (1985). Seed microstructure: review of water  
406 imbibition in legumes. *Food Structure*, 4(1), 14.

407 Tschardtke, T., Clough, Y., Wanger, T. C., Jackson, L., Motzke, I., Perfecto, I., Whitbread, A.  
408 (2012). Global food security, biodiversity conservation and the future of agricultural  
409 intensification. *Biological Conservation*, 151(1), 53–59.

410 Van Buren, J. (1979). The chemistry of texture in fruits and vegetables. *Journal of Texture Studies*,  
411 10(1), 1–23.

412 Varriano-Marston, E., & Jackson, G. M. (1981). Hard-to-Cook phenomenon in beans: Structural  
413 changes during storage and imbibition. *Journal of Food Science*, 46(5), 1379–1385.

414 Waldron, K. W., Parker, M. L., & Smith, A. C. (2003). Plant cell walls and food quality.  
415 *Comprehensive Reviews in Food Science and Food Safety*, 2(4), 128–146.

416 Yildirim, A., Öner, M. D., & Bayram, M. (2011). Fitting Fick's model to analyze water diffusion  
417 into chickpeas during soaking with ultrasound treatment. *Journal of Food Engineering*,  
418 104(1), 134–142.

419 <https://imagej.net/>, 04 July 2018

**Fig 1:** Micrograph showing the gross morphology of cowpea seeds. T, testa; H, hilum; M, micropyle; C, cotyledon E, embryonic axis. Lugol staining (dark blue) reveals accumulation of starch in the cotyledon. Embryonic axis appears to be starch free. Scale bars = 1630  $\mu\text{m}$ .

**Fig 2:** Effects of soaking on the parenchymatous cells of cowpea cotyledon. Sections were stained with PAS-NBB. (a) Control (raw seeds). (b) After soaking at 30°C for 6 h. (c) After soaking at 60°C for 2 h. (d) After soaking at 95°C for 1 h. Note the decrease of stain intensity of starch and proteins in (b) and (c) compared to (a), and the presence of amorphous materials in intercellular spaces in (c) and (d) (arrows), and the intense blue colour of the cytoplasm in (d). CW: cell wall; IC: intercellular space; S: starch; Cy: cytoplasm; PB: protein body; N: nucleus. Scale bars = 28  $\mu\text{m}$ .

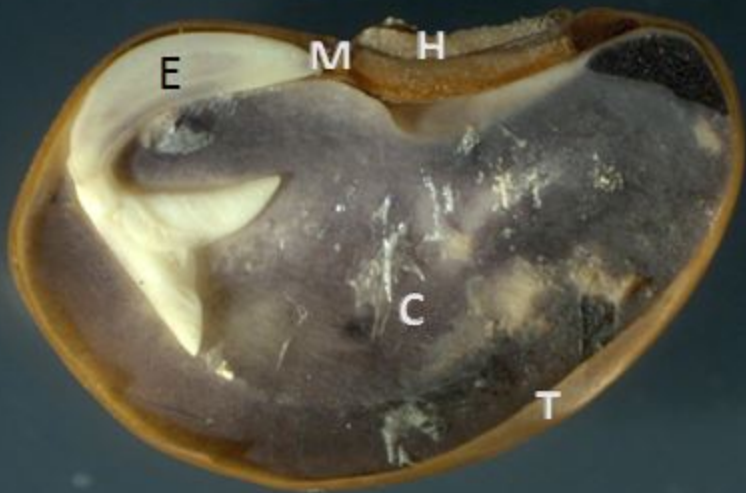
**Fig 3:** Effects of soaking on cellulose in the cell walls of cowpea cotyledons. Sections were stained with FB. (a) Control (raw seeds). (b) After soaking at 30°C for 6 h. (c) After soaking at 60°C for 2 h. (d) After soaking at 95°C for 1 h. Note the disruption of the cell wall in (c) and (d) (arrows), and cell dissociation in (d) (star). Scale bars = 18  $\mu\text{m}$ .

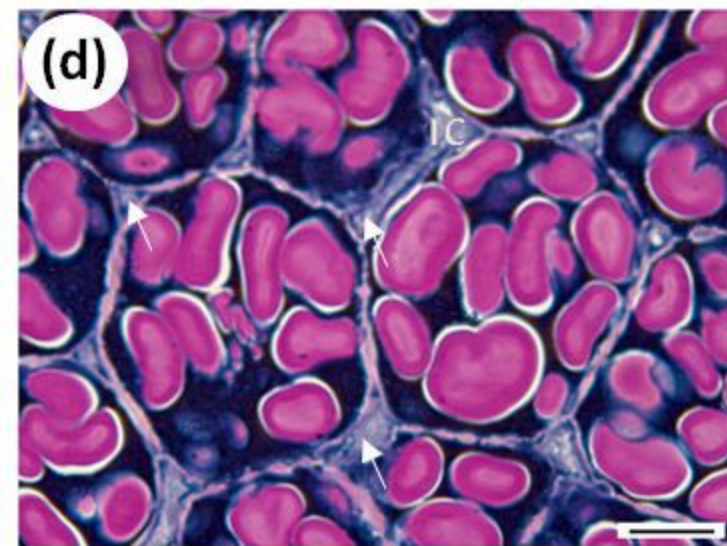
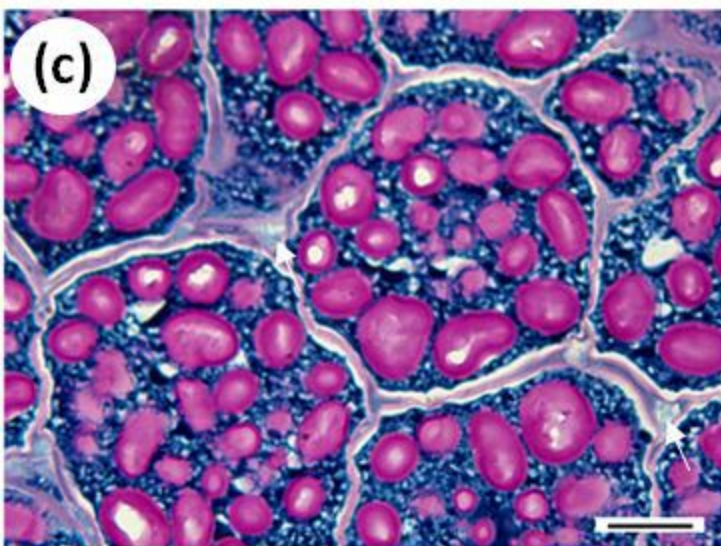
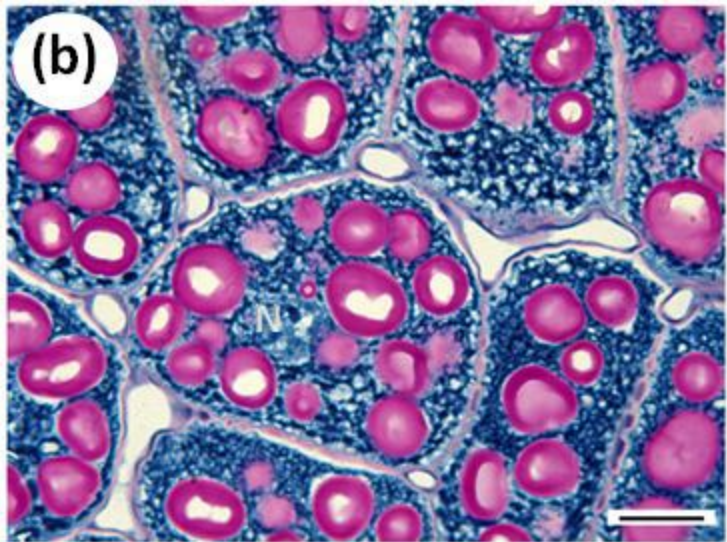
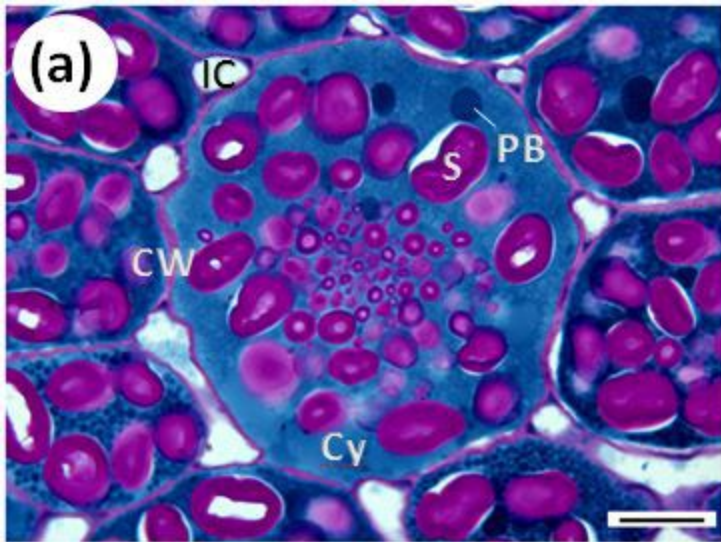
**Fig 4:** Effects of soaking on pectin in the cell walls of cowpea cotyledons. Sections were stained with RR. (a) Control (raw seeds). (b) After soaking at 30°C for 6 h. (c) After soaking at 60°C for 2 h. (d) After soaking at 95°C for 1 h. Note the presence of pectin in the intercellular spaces in (b), (c) and (d) (arrows), and the fusion of starch granules in (d). S, starch. Scale bars = 15  $\mu\text{m}$ .

**Fig 5:** Effects of soaking on cowpea micropyle. Sections were stained with toluidine blue. (a) Control (raw seeds). (b) After soaking at 30°C for 3 h. (c) After soaking at 30°C for 6 h. (d) After soaking at 60°C for 1 h. (e) After soaking at 60°C for 2 h. (f) After soaking at 95°C for 0.5 h. (g) After soaking at 95°C for 1 h. Note the presence of the aperture in (b), (c) and (d). Ap: aperture; Pa: palisade; TI: tracheid island. Scale bars = 18  $\mu\text{m}$ .

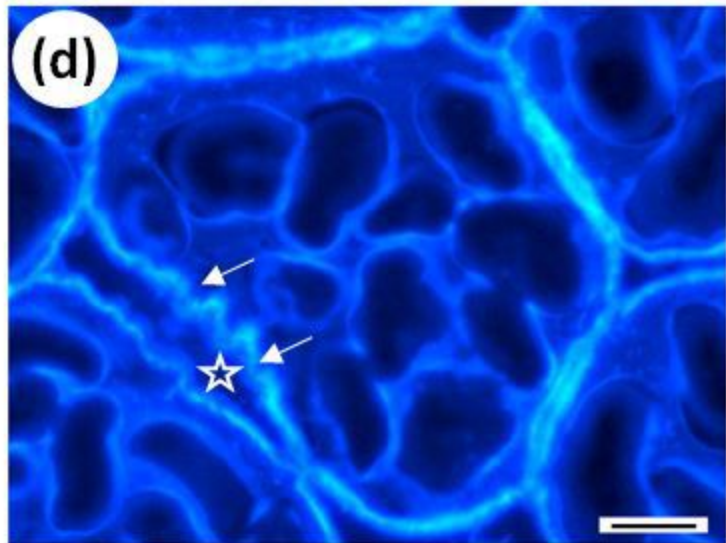
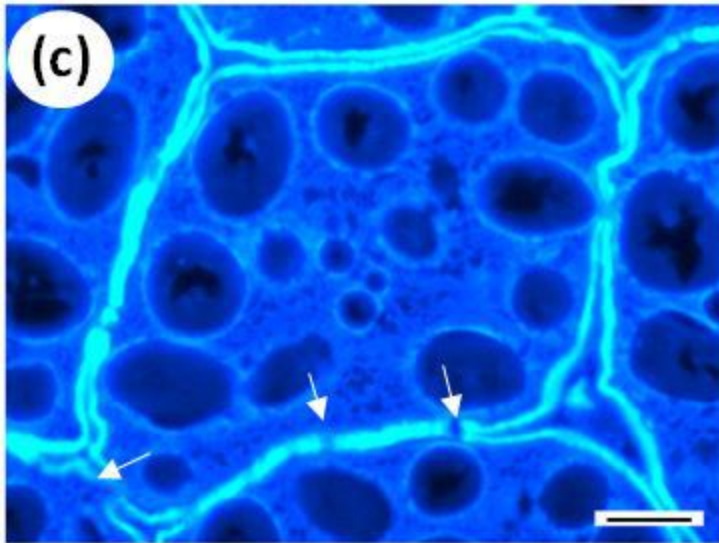
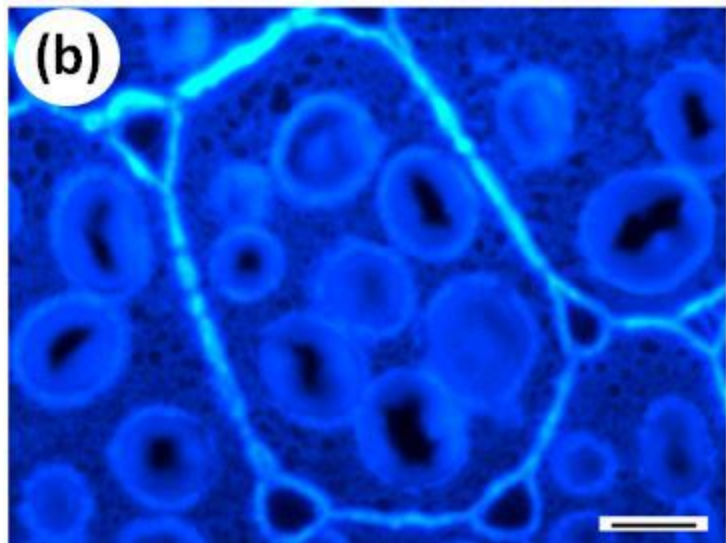
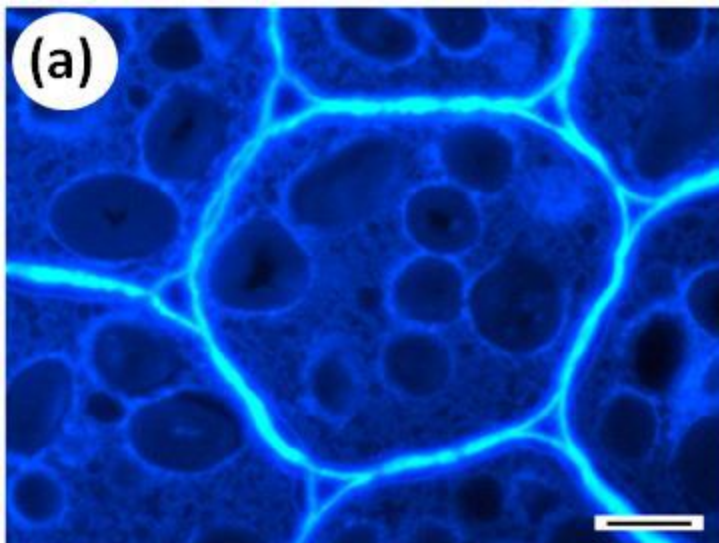
**Fig 6:** (a) Dry-matter loss kinetics (kg/100 kg db) of cowpea seeds during soaking at 30°C (black), 60°C (blue) and 95°C (brown). (b) Water uptake kinetics (kg of water/kg db) of cowpea seeds during soaking at 30°C (black), 40°C (red), 60°C (blue), 70°C (green), 80°C (orange) and 95°C (brown). Error bars represent the standard deviation (n=4)

**Table 1.** Changes in the micropyle aperture after soaking at 30°C, 60°C and 95°C. The mean aperture was measured on the whole micropyle presented in Figure 5. (mean values  $\pm$  standard deviations determined with n=6 measurements)

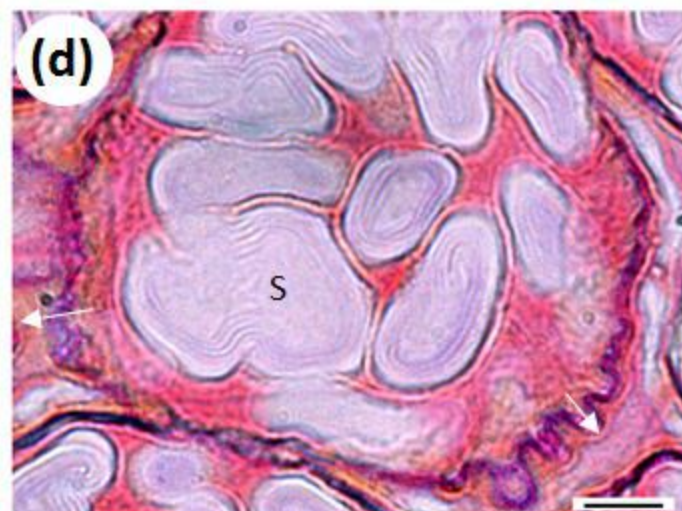
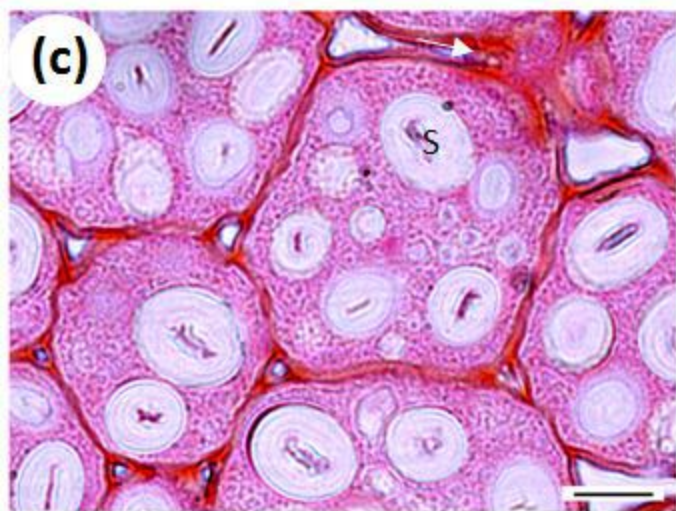
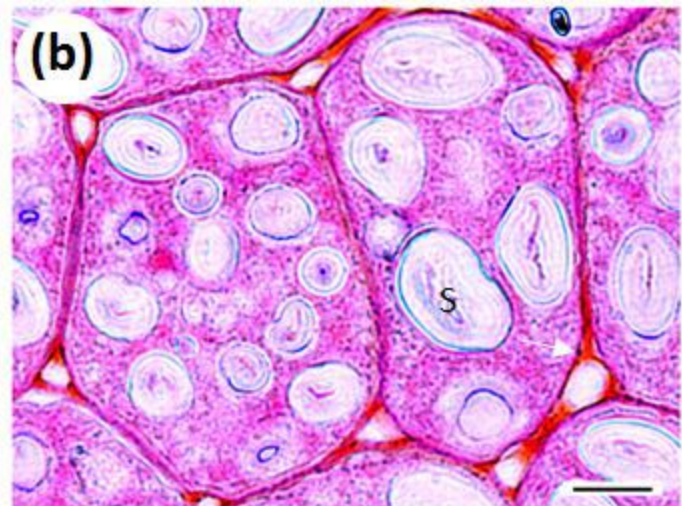
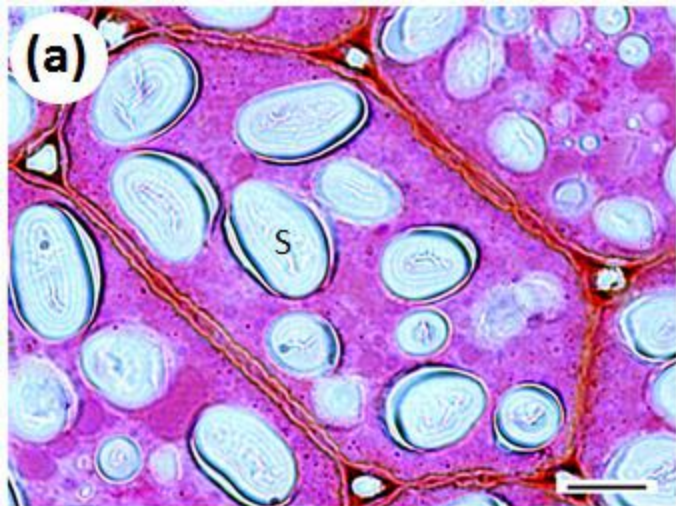


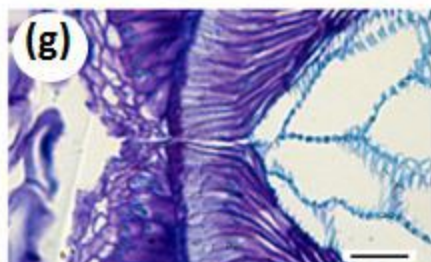
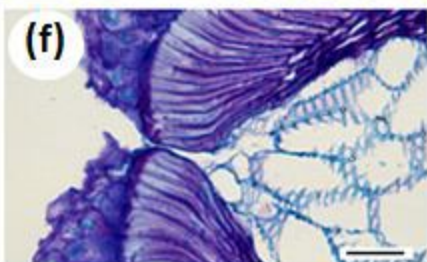
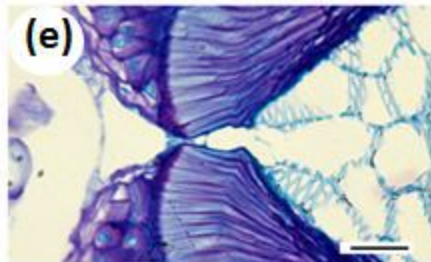
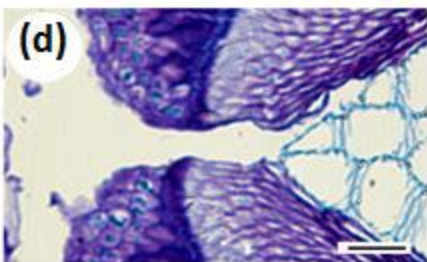
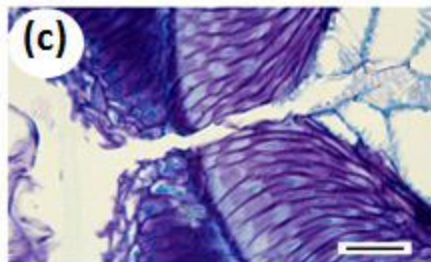
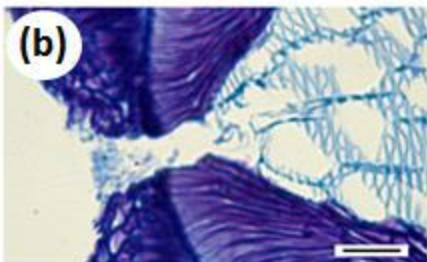
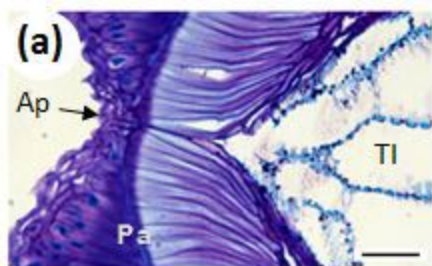


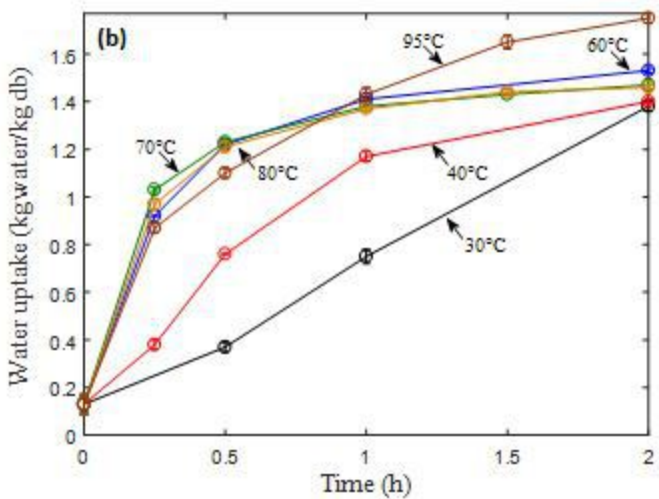
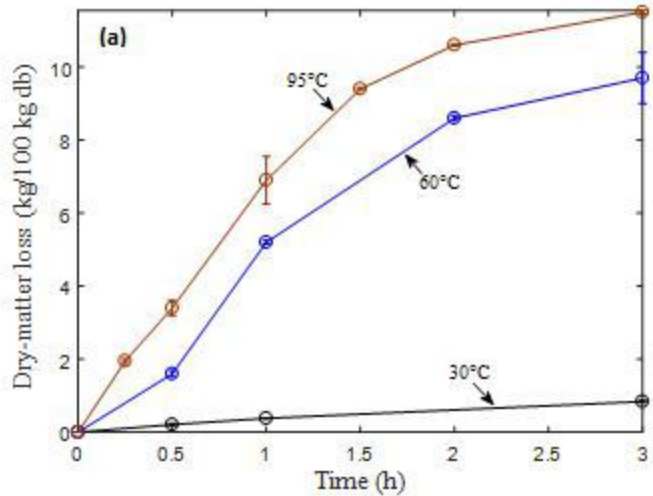












Temperature (°C)	Time (h)	Micropyle thickness (μm)
0	0	0 ± 0 <sup>a</sup>
30	3	12.2 ± 1.3 <sup>b</sup>
	6	5.8 ± 0.8 <sup>c</sup>
60	1	11.7 ± 2.0 <sup>b</sup>
	2	5.4 ± 2.6 <sup>c</sup>
95	0.5	1.8 ± 0.7 <sup>d</sup>
	1	1.3 ± 0.2 <sup>d</sup>

Values with different letters (a–b–c–d) are significantly different ( $p < 0.05$ ).