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## 1 **Morphology and mechanical behaviour of pea-based starch-protein composites obtained** 2 **by extrusion**

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### 16 **Abstract**

17 Starch-legume protein composites were obtained by extrusion of pea flour and pea  
18 starch-protein blend at various specific mechanical energies (100-2000 kJ/kg) and a  
19 temperature low enough to avoid expansion. The morphology of these composites displayed  
20 protein aggregates dispersed in a starch matrix, revealed by microscopy. Image analysis was  
21 used to determine the median width of protein aggregates ( $D_{50}$ ), their total perimeter and  
22 surface, from which a protein/starch interface index ( $I_i$ ) was derived. The mechanical  
23 properties of composites were determined by a three-point bending test. The pea flour  
24 composites had a higher interface index  $I_i$  (1.8-3.1) with lower median particle width  $D_{50}$  (8-  
25 18  $\mu\text{m}$ ) and a more brittle behaviour than the blend composites that had a lower  $I_i$  (1-1.1) and  
26 higher  $D_{50}$  (22-31 $\mu\text{m}$ ). For both materials, rupture stress and strain were negatively correlated  
27 with  $I_i$ . This result suggested that there was a poor interfacial adhesion between the pea starch  
28 and proteins.

### 29 **Keywords**

30 Bending test, composite, interface, microstructure, protein aggregates, starch

### 31 **Abbreviations**

32  $a^*$  redness in CIELAB colour space

33  $b^*$  yellowness in CIELAB colour space

34  $C$  torque (N.m)

35 CLSM confocal laser scanning microscopy

36  $D_{50}$  median protein aggregate width ( $\mu\text{m}$ )

37 db dry basis

38 DSC differential scanning calorimetry

39  $E$  flexural modulus (GPa)

40  $F$  force (N)

41	$H$	die thickness (m)
42	$h$	specimen width (mm)
43	$I_i$	interface index
44	$L^*$	lightness in CIELAB colour space
45	$L$	support span in bending test (mm)
46	$MC$	moisture content (wb)
47	$N$	screw speed (rpm)
48	NF	native pea flour
49	NSPB	native starch-protein blend
50	PPI	pea protein isolate
51	$RH$	relative humidity (%)
52	$Q$	measured total mass flow rate (kg/h)
53	$Q_F$	mass flow rate of raw materials (kg/h)
54	$Q_v$	volumetric flow rate (m <sup>3</sup> /s)
55	$Q_W$	mass flow rate of water (kg/h)
56	SP blend	starch-protein blend
57	$T$	product temperature (°C), measured at die entrance
58	$T_{die}$	imposed die temperature (°C)
59	$t_h$	specimen thickness (°C)
60	$T_m$	melting temperature (°C)
61	$T_5$	imposed temperature in the fifth barrel (°C)
62	$SME$	specific mechanical energy (kJ/kg)
63	$W$	die width (m)
64	wb	wet basis
65	$\Delta P$	pressure drop inside the die (Pa)
66	$\sigma$	engineering stress (Pa)
67	$\varepsilon$	engineering strain (%)
68	$\eta$	apparent viscosity (Pa.s)

## 69 **1. Introduction**

70 Despite their abundance and high nutritional value, pulse legumes (pea, lentil, faba  
71 bean, etc.) have not been widely adopted in the human diet. The last decades have been  
72 marked by a rising number of research studies in pulse texturisation by extrusion. Low  
73 moisture extrusion is often used to produce cereal-based expanded foods whose  
74 characteristics owe much to their starch content and macromolecular content. Moreover,  
75 supplementation with pulse flour or protein isolates may contribute to the health benefits of  
76 these foods thanks to the complementary amino acid profiles of legumes and cereals (Day &  
77 Swanson, 2013), while attempting to understand the structural changes of the flour  
78 components. During extrusion, after mixing with water, starchy powder becomes molten by  
79 solid friction, viscous dissipation and heat conduction from the barrel. The viscous melt is  
80 forced through a die where the vapour expands the material to a porous structure, referred to  
81 as a solid foam. The texture is governed by density, cellular structure and the mechanical  
82 properties of the intrinsic material. The intrinsic material can be envisioned as a dense  
83 composite consisting of a blend of starch and protein (Guessasma, Chaunier, Della Valle, &

84 Lourdin, 2011). In addition to its composition, its mechanical properties depend on the  
85 morphology created during extrusion.

86 High input of thermo-mechanical energy leads to structural modification of the  
87 biopolymers, including starch melting and depolymerisation (Logié, Della Valle, Rolland-  
88 Sabaté, & Descamps, 2018), protein denaturation and aggregation by non-covalent  
89 (hydrophobic) and covalent (disulphide S-S) bonds (Mession, Chihi, Sok, & Saurel, 2015), as  
90 well as browning due to Maillard reactions. Shear energy can also favour the unfolding and  
91 re-association of protein aggregates (Della Valle, Quillien, & Gueguen, 1994; Fang, Zhang,  
92 Wei, & Li, 2013). With increasing melt temperature and specific mechanical energy (*SME*),  
93 the starch depolymerisation and its hydrosolubility increase and the protein solubility in the  
94 buffer decreases due to protein aggregation by disulphide bonds and increased product  
95 browning (Kristiawan et al., 2018).

96 Most research on the morphology of starch-protein composites has focused on cereal-  
97 based systems (Chanvrier, Della Valle, & Lourdin, 2006; Habeych, Dekkers, Goot, & Boom,  
98 2008; Muneer et al., 2016). The effect of protein concentration on the morphology of corn  
99 starch-zein composites obtained by extrusion and thermo-moulding was reported by  
100 Chanvrier et al. (2006). Habeych et al. (2008) studied the effect of shear rate on the  
101 morphology of blends of wheat starch and zein (0-20% db). They found that shear flow  
102 changed co-continuous starch-protein morphology into matrix/particle systems with zein  
103 aggregates as a dispersed phase. Recently, Kristiawan et al. (2018) showed that the  
104 morphology of the intrinsic material of pea flour foams changed from dispersed to bi-  
105 continuous system. These changes depended on the structural modifications of biopolymers  
106 that were governed by extrusion variables, and temperature in particular. It is therefore  
107 necessary to better understand the mechanisms of morphology evolution in legume starch-  
108 protein composites.

109 Several studies have investigated the effect of composition and microstructure  
110 (morphology) on the mechanical properties of particulate-biopolymer composites (Chanvrier  
111 et al., 2006; Fu, Feng, Lauke, & Mai, 2008; Hashin & Shtrikman, 1962; Leclair & Favis,  
112 1996; Verbeek, 2003). Several empirical or semi-empirical models have been proposed to  
113 predict Young's modulus and ultimate strength from composite microstructure, taking the  
114 strength of interfacial adhesion, aspect ratio and volume fraction of particles into account  
115 (Hashin & Shtrikman, 1962; Nicolais & Nicodemo, 1974; Verbeek, 2003). The mechanical  
116 properties of glassy cereal-based starch-protein composites were shown to be weakened by  
117 the presence of incompatible dispersed protein aggregates (Chanvrier et al., 2006, Habeych et  
118 al., 2008, Muneer et al., 2016). In the case of films of peanut protein isolate blended with pea  
119 starch, the decrease of tensile strength and the increase of strain at rupture were attributed to  
120 the interaction between swollen pea starch granules and proteins, leading to the formation of a  
121 flexible network (Sun, Sun, & Xiong, 2013). To our knowledge, none of the previous studies  
122 has determined the relationship between mechanical properties and morphological features of  
123 legume composites.

124 The aim of this work is to determine the relationship between morphological features and  
125 mechanical properties for legume composites. To do this, pea flour and a blend of pea starch  
126 and pea protein isolate were selected as a model system. The extrusion variables were varied  
127 in order to obtain dense composites with a wide variation in starch-protein morphology. The

128 biopolymer transformations and the morphology and mechanical properties of composites  
129 were analysed.

## 130 2. Materials and methods

### 131 2.1. Raw materials

132 Yellow pea grits (*Pisum Sativum* L.) were purchased from Sotexpro (France) and  
133 ground (SARL Giraud, France) in order to obtain pea flour with a median diameter of 480  
134  $\mu\text{m}$ . Pea starch (amylose content: 35%) and pea protein isolate PPI (Nutralys<sup>®</sup> F85F) were  
135 supplied by Roquettes Frères S.A. (Lestrem, France). The median diameter of pea starch and  
136 PPI, determined by laser diffraction (Partica LA-960, HORIBA, Japan) was 27 and 72  $\mu\text{m}$ ,  
137 respectively. A starch-protein (SP) blend was obtained by mixing pea starch and PPI using a  
138 Kenwood mixer for 20 min. The flour and SP blend had a similar ratio of starch and protein:  
139 1.94 and 1.99 dry matter, respectively. The chemical composition of the raw materials  
140 (Table1) was determined by standard methods described by Kristiawan et al. (2018).

141 Table 1. Chemical composition of raw material (% db)

	Starch	Proteins	Ash	Lipids	Others*
Pea starch	98	0.5	0.1	-	1.6
PPI	0.4	88.3	4.5	-	6.8
Pea Flour	46.3	23.9	2.1	2	25.7
Starch-PPI blend	63.3	31.6	1.6	-	3.5

142 (\*) Fibre and other components, determined by difference method (Kristiawan et al., 2018; Li,  
143 Kowalski, Li, & Ganjyal, 2016).

### 144 2.2. Extrusion

145 Composites were obtained as strips by extrusion of the pea flour and SP blend using a  
146 laboratory scale co-rotating twin-screw extruder (Thermo Scientific<sup>™</sup> Process 11, Germany)  
147 equipped with a plate die (section: 26 x 1 mm<sup>2</sup>; length: 70 mm). The screw diameter was 11  
148 mm and the ratio of length to diameter was 24.5. The barrel was divided into five sections  
149 heated separately at 30, 40, 60, 80 and  $T_5$  (92-164) °C. The screw profile included conveying  
150 elements with a pitch of 11 mm, followed by four kneading discs (length: 2.75 mm each) that  
151 consisted of alternate discs with 0° and 90° on the hexagonal shaft orientation, and one  
152 reverse element (length: 5.5 mm) located in the fifth barrel. The die temperature was  
153 regulated at 90-95 °C in order to obtain dense composites. The raw material was fed into the  
154 first barrel by a volumetric feeder and the water was added to the second barrel using a  
155 volumetric pump.

156 In order to obtain composites with a wide range of starch-protein morphologies and  
157 mechanical properties, the operating parameters were varied in the following ranges: moisture  
158 content (25-35% wb), screw rotation speed (120-700 rpm) and the temperature of the last  
159 barrel of the extruder  $T_5$  ( $T_m-20$  °C,  $T_m$ ,  $T_m+20$  °C), where  $T_m$  is the melting temperature of the  
160 raw material. Product pressure and temperature ( $T$ ) were measured at the die entrance with an  
161 accuracy of  $\pm 200$  kPa and  $\pm 1$  °C, respectively. *SME* (J/g) was calculated as follows:

$$162 \quad SME = \frac{C \cdot N}{Q} \quad (1)$$

163 where  $C$  is the measured torque (N.m),  $N$  the screw speed (rad/s), and  $Q$  the mass flow rate  
164 (g/s). Apparent viscosity of the melt ( $\eta$ , Pa.s) at the die exit was calculated using Poiseuille's  
165 equation through a rectangular channel:

$$166 \quad \frac{\Delta P}{L} = \frac{12\eta Q_v}{WH^3} \quad (2)$$

167 where  $\Delta P$  is the pressure drop inside the die (Pa),  $Q_v$  the volumetric flow rate (m<sup>3</sup>/s),  $W$  the die  
168 width (m) and  $H$  the die thickness (m).

169 Immediately after extrusion, the composites were dried at 40 °C for 24 h in order to avoid  
170 starch retrogradation and to obtain a final  $MC$  of less than 10% wb.

## 171 **2.3. Starch transformation**

### 172 *Melting transition*

173 In the presence of small amounts of water, as in the case of extrusion ( $MC \leq 35\%$  wb),  
174 the starch transition is the 'melting' of crystallites in the starch granule. Data for the melting  
175 temperature ( $T_m$ ) of pea flour and the SP blend at various moisture contents is essential to  
176 establish the barrel temperature profile ( $T_5$ ) in order to obtain composites with amorphous  
177 starch.  $T_m$  values of the SP blend were assumed to be the same as those of pea starch.

178 Moisture content adjustment was performed by the addition of appropriate quantity of  
179 water followed by overnight equilibration or by conditioning pea starch and flour under  
180 controlled relative humidity ( $RH$ : 58-98%) over saturated salts at 20 °C for one month. The  
181  $T_m$  value of native pea starch and flour was determined using a differential scanning  
182 calorimetry apparatus Q100 DSC (TA Instruments, USA) according to the method of Logié et  
183 al. (2018). The samples were heated from 20 to 200 °C at a heating rate of 5 °C/min. Melting  
184 temperature ( $T_m$ ) was defined as the offset temperature of the melting endothermic peak  
185 (Appendix: Fig. A1). The measurements were done in duplicate (relative error: 10%) and  
186 were compared to data in the literature (Appendix: Fig. A2).

### 187 *Crystalline structure*

188 DSC analysis in excess water was performed on the extruded products in order to  
189 verify if all starch crystallites were molten by extrusion process. The existence of residual  
190 crystalline structure can be indicated by the presence of so-called residual gelatinization  
191 endotherm in DSC thermogram. The composites were ground into a fine powder (< 250 µm)  
192 using a cryogrinder. The sample (10 mg) and deionised water (40 mg) were directly weighed  
193 in a pan and hermetically sealed. The DSC scans were run at 3 °C/min from 20 to 100 °C.

## 194 **2.4. Colour measurement**

195 The colour of composite surfaces was determined using a Chromameter (Konica  
196 Minolta CR-400, France) with a standard D65 illuminant (natural daylight) and an observation  
197 angle of 2°. The colour was expressed in CIE-Lab colour space ( $L^*$ ,  $a^*$  and  $b^*$ ).  $L^*$  represents  
198 the lightness of colour (0 black, 100 white),  $a^*$  and  $b^*$  represent the green-red (-100, +100)  
199 and blue-yellow (-100, +100) colours, respectively. The reported values were the means of ten  
200 measurements (relative error: 10%).

## 201 **2.5. Morphology**

### 202 **2.5.1. Confocal Laser Scanning Microscopy (CLSM)**

203 The organisation of proteins and starch in pea composites was determined using  
204 CLSM.

#### 205 *Sample preparation and labelling*

206 Before cryosectioning, the composites were hydrated by conditioning at 20 °C and RH  
207 98% for 4 days, and then cut into pieces of 5 mm in length. For protein labelling, one part  
208 (mass) of 0.01% (w/v) fuchsin acid in 1% (v/v) acetic acid was mixed with one part (mass)  
209 of Kaiser's glycerol/gelatine solution at 40 °C. Kaiser's solution was used to improve the  
210 adhesion of specimen slices on microscope slides (Zweifel, Handschin, Escher, & Conde-  
211 Petit, 2003). The specimen slices (thickness: 20 µm) were obtained by cutting the embedded  
212 pieces within the freezing medium tissue (Tissue-Tek O.C.T) perpendicularly to the extrusion  
213 flow using a cryotome at -20 °C. The slices were mounted in the frozen state onto microscope  
214 slides, covered with stained Kaiser's solution and a glass coverslip. The slides were stored for  
215 24 h (20 °C) to insure optimum diffusion of the markers.

#### 216 *Image acquisition*

217 Images were acquired using CLSM (Nikon A1) with an attached NIS imaging system  
218 (Nikon, Germany). Samples were examined in the epifluorescence mode of the microscope,  
219 excited by a green laser beam at 561 nm. The emitted light was selected by a long-pass filter  
220 at 570-620 nm. Images of 512×512 pixels were taken with a magnification of 20 and a  
221 resolution of 1.24 µm/pixel.

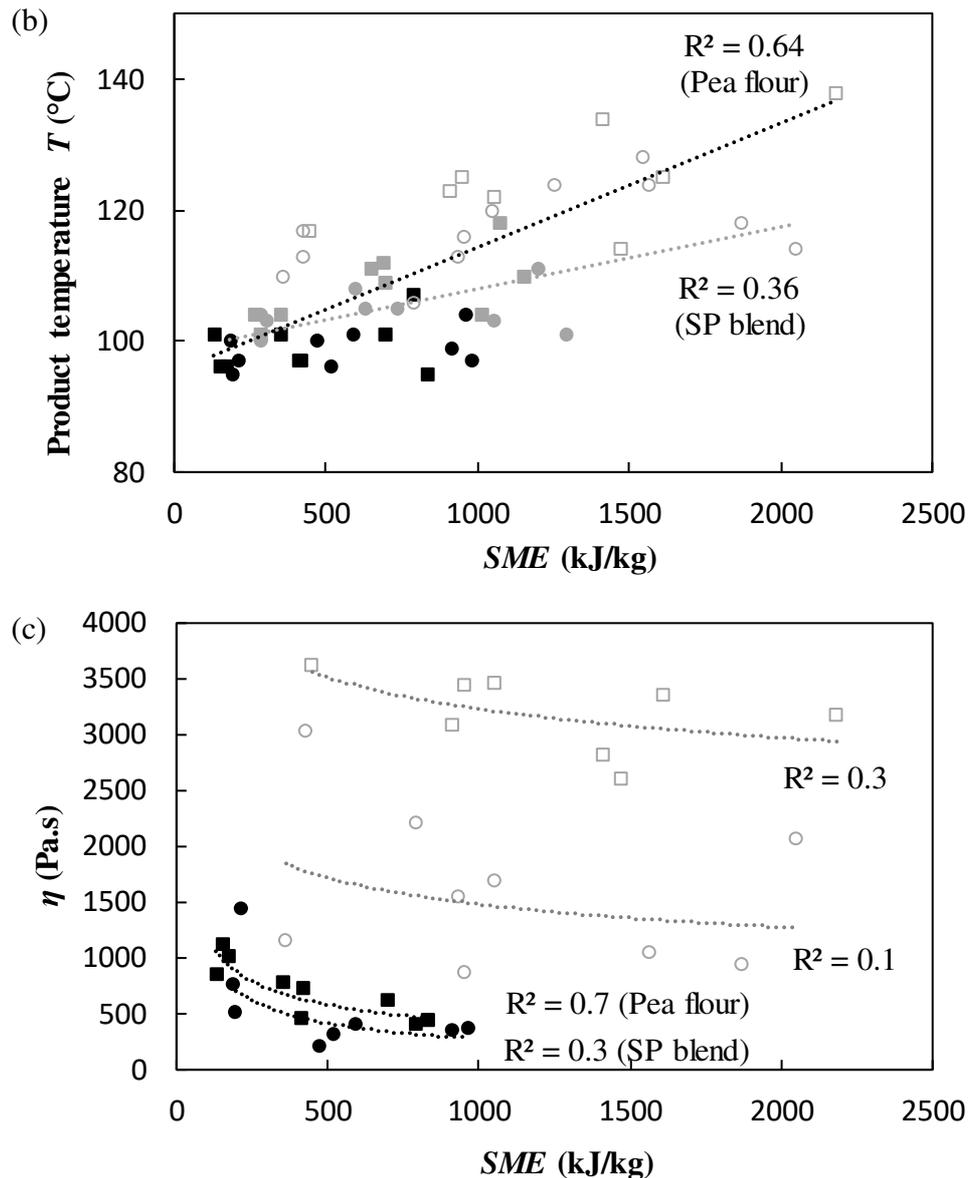
#### 222 *Image analysis*

223 Image analysis was done for at least three plane projection images of three spots in the  
224 composites. It was performed using Matlab software to determine the median particle width  
225 ( $D_{50}$ ), total area and total perimeter of protein aggregates. Firstly, CLSM images were  
226 digitised by applying a grey level threshold obtained with a k-mean algorithm (Jain, 2010).  
227 The total area of the particles and the total perimeter of their interface with the matrix were  
228 then determined. The starch-protein interface index ( $I_i$ ) was computed as the ratio of the total  
229 aggregate perimeter to the square root of the total aggregate area. Granulometry analysis was  
230 subsequently performed using mathematical morphology operations (Devaux, Bouchet,  
231 Legland, Guillon, & Lahaye, 2008; Le Bleis, Chaunier, Montigaud, & Della Valle, 2016). A  
232 curve of the cumulated area of the particles (proteins) was then built according to the width of  
233 the openings. This curve was fitted by the Gompertz function (Dehaine & Filippov, 2016):

$$234 \quad y = c \times \exp^{-\exp(-k(x-x_c))} \quad (3)$$

235 where  $y$  is the cumulative fraction of particle width less than or equal to the opening width  $x$ ,  $c$   
236 is the amplitude of the cumulative surface (100),  $k$  is the constant describing the fragment  
237 uniformity, and  $x_c$  is the central particle width. The median particle width  $D_{50}$  was computed  
238 from the Gompertz fit at 50% of the cumulated surface.





266 Figure 1. Control of extrusion variables at different moisture contents: 0.25 (white), 0.30  
 267 (grey) and 0.35 (black) for pea flour (square) and starch-protein blend (circle): (a) Variation  
 268 of specific mechanical energy ( $SME$ ) with the ratio of total feed rate ( $Q$ ) to screw speed ( $N$ ),  
 269 (b) Variation of product temperature ( $T$ ) with  $SME$ , (c) Variation of melt viscosity ( $\eta$ ) with  
 270  $SME$  for moisture contents of 0.25 and 0.35. The dotted line represents data fitting using a  
 271 power (a, c) and linear (b) function.

272  $SME$  increased with increasing screw speed and, conversely, decreased when feed rate  
 273 and moisture content  $MC$  increased. A negative correlation ( $R^2 = 0.83$ ) between  $SME$  and the  
 274 ratio of total feed rate to screw speed ( $Q/N$ ) was obtained for any  $MC$  and raw material. This  
 275 result suggested that  $SME$  can be well controlled by the tuning of these extrusion parameters.  
 276 Overall, the larger values of  $SME$  obtained with pea flour might be due to its higher fibre  
 277 content than that of SP blends (26% versus 3%, in db).

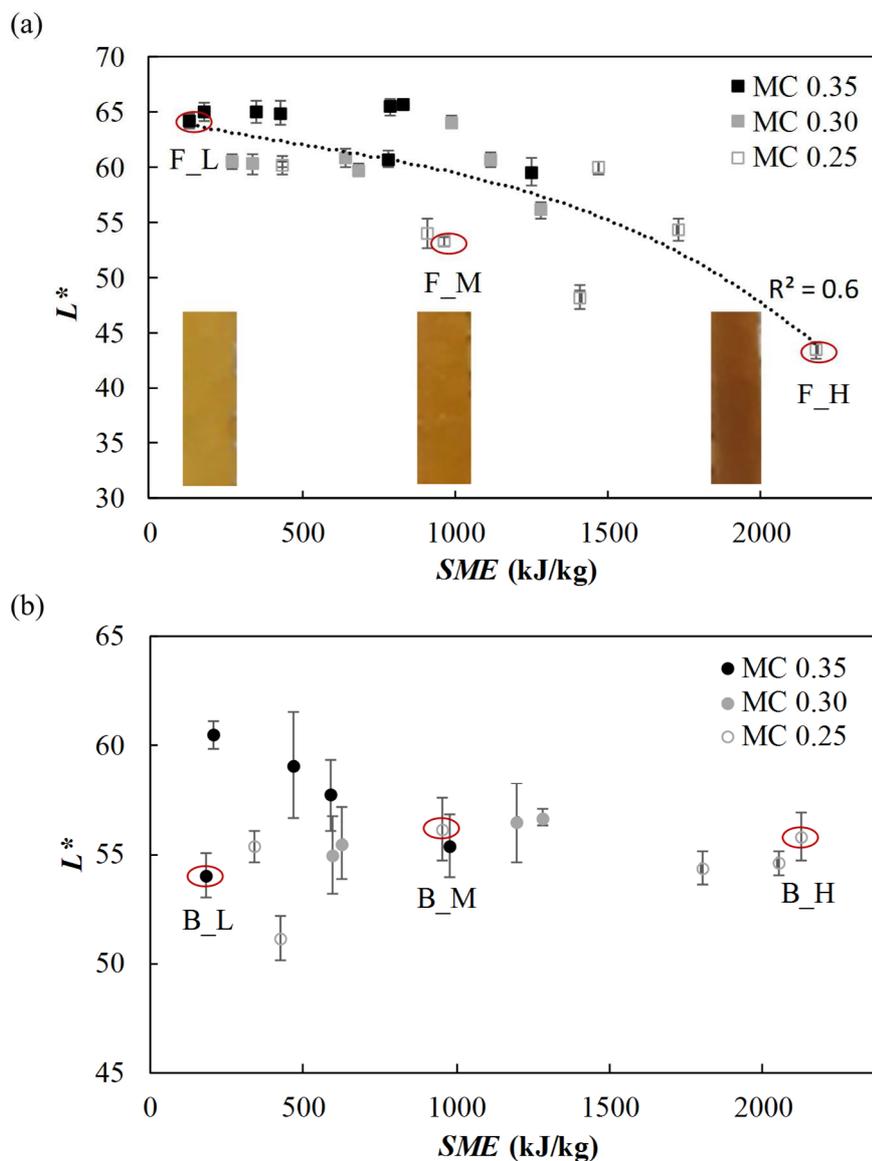
278 The effect of  $SME$  on product temperature  $T$  was positive, regardless of the raw  
 279 material (Fig. 1b). This trend could be attributed to solid friction between particles and to  
 280 viscous dissipation in the molten phase, both phenomena explaining why product temperature

281 is higher than barrel temperature. Higher viscous dissipation suggested that pea flour melt has  
 282 higher viscosity than that of SP blends, possibly because of its larger fibre content (Fig. 1c).

283 For all extrusion trials, the melt shear rate maintained the same value ( $\approx 11\pm 2 \text{ s}^{-1}$ )  
 284 because the total mass flow rate  $Q$  did not vary much (Table 2). The variations of apparent  
 285 melt viscosity with  $SME$  reflected biopolymer structural changes during processing, whereas  
 286 the dispersion may be attributed to the influence of temperature on viscosity. For instance, it  
 287 is well known that starch melt viscosity decreases with  $SME$  because of macromolecular  
 288 degradation.

### 289 Colour and starch changes

290 The  $L^*$  values of extruded composites (Fig. 2) were lower than that of raw materials  
 291 ( $L^* 90\pm 1$ ), probably due to Maillard reactions.



292 Figure 2. Variation of composite lightness ( $L^*$ ) of pea flour (a) and the SP blend (b) with  
 293 specific mechanical energy ( $SME$ ). The  $L^*$  value of raw materials was  $90\pm 1$ . The symbols  
 294 refer to data at different extrusion temperatures and the curve represents data fitting using a  
 295

296 third-order polynomial function. The red circles represent the samples selected for further  
 297 analysis.

298 Concerning pea flour, the darkness of extruded materials increased with *SME* ( $R^2 =$   
 299  $0.60$ ) (Fig. 2a). The samples also appeared to be redder (higher  $a^*$ , result not shown). No  
 300 significant  $L^*$  colour difference was detected among SP blend composites ( $L^* = 55 \pm 5$ ) (Fig.  
 301 2b, ANOVA,  $p = 0.5$ , at 5% confidence level).

302 During extrusion, Maillard reactions occur between protein amino groups and  
 303 reducing saccharides derived mainly from depolymerised starch. The increased darkness of  
 304 flour composites at  $SME > 1500$  kJ/kg may be due to additional Maillard reactions between  
 305 proteins and reducing saccharides present in the pea flour fibre (Santillán-Moreno, Martínez-  
 306 Bustos, Castaño-Tostado, & Amaya-Llano, 2011).

307 Based on the trend of variation of composite colour with *SME*, three samples of flour  
 308 composite at different *SME* were selected for further analysis. For the purpose of comparison,  
 309 three representative composites of SP blend, extruded at similar *SME* levels ( $\approx 200, 1000,$   
 310  $2000$  J/g) were also selected. The name of selected samples was given in Fig. 2 (F: pea flour,  
 311 B: SP blend; L: low; M: medium; H: high *SME*), and the corresponding extrusion variables  
 312 were reported in Table 2.

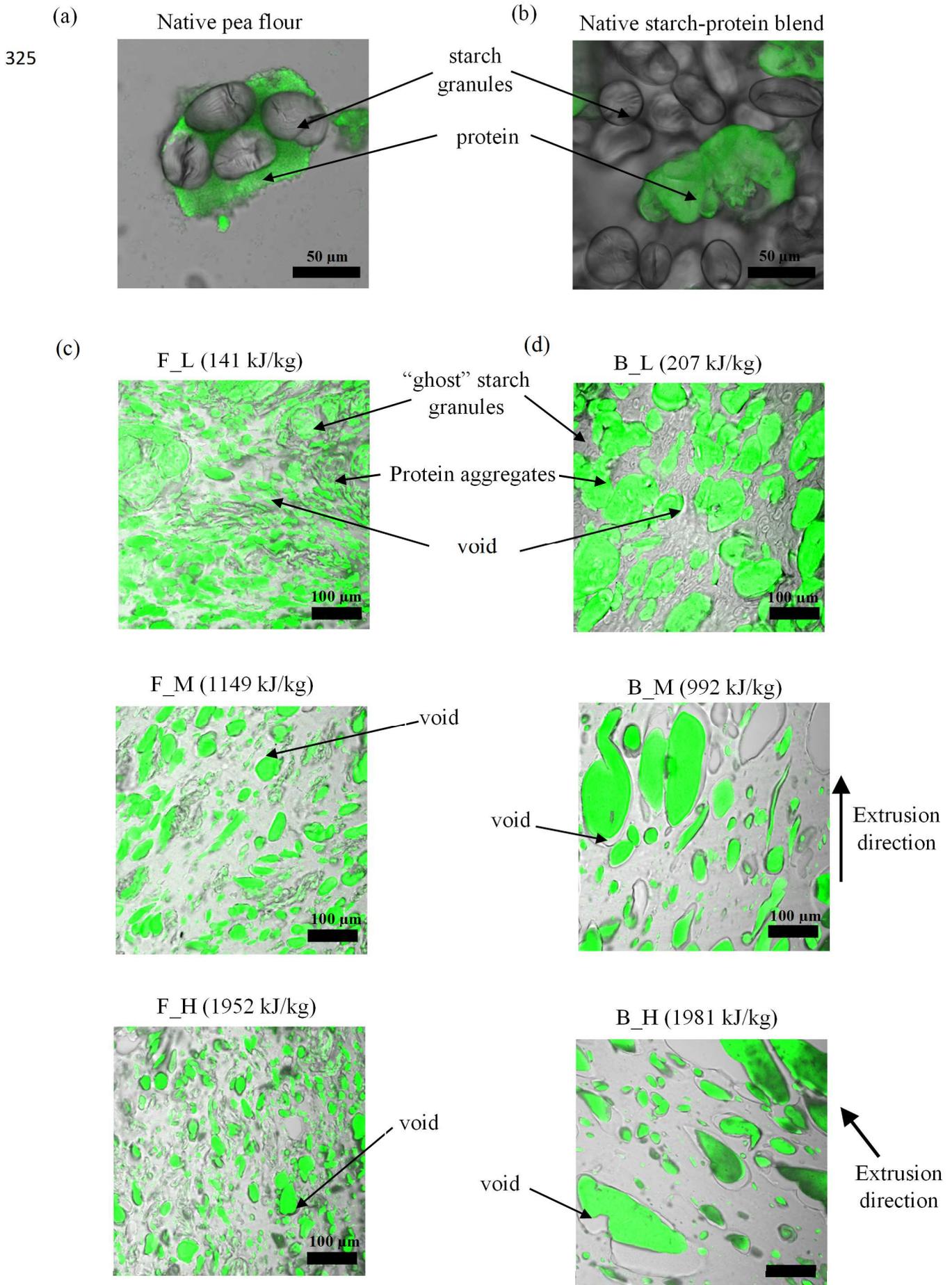
313 Table 2. Extrusion variables of pea composite

Samples	Operating conditions							Measured variables			
	<i>MC</i> % wb	<i>Q<sub>F</sub></i> (kg/h)	<i>Q<sub>w</sub></i> (kg/h)	<i>N</i> (rpm)	<i>T<sub>die</sub></i> (°C)	<i>T<sub>m</sub></i> (°C)	<i>T<sub>5-T<sub>m</sub></sub></i>	<i>Q</i> (kg/h)	<i>T</i> (°C)	<i>SME</i> (kJ/kg)	Die pressure (MPa)
Pea flour											
F_L	35	0.24	0.07	120	95	112	20	0.32	101	141	1.2
F_M	25	0.21	0.02	300	95	121	20	0.3	124	1149	7.9
F_H	25	0.21	0.03	650	95	121	0	0.31	138	1952	4
Pea starch-PPI blend											
B_L	35	0.22	0.06	120	90	112	-20	0.33	92	207	3.4
B_M	25	0.24	0.02	300	95	134	20	0.27	134	992	5.7
B_H	25	0.2	0.02	500	95	134	-20	0.26	126	1981	3.5

314 The starch transformation of selected composites was investigated by comparing their  
 315 DSC residual gelatinisation enthalpy to the gelatinisation enthalpy of native raw materials  
 316 (Appendix: Fig. A4). In the excess water (80% wb), the native SP blend and pea flour showed  
 317 an endothermic transition, reflecting starch gelatinisation, with enthalpies of 5.7 J/g and 3.7  
 318 J/g, respectively, at the same peak temperature (69-70 °C). No residual gelatinisation enthalpy  
 319 was detected for the selected composites, indicating complete melting of starch crystals  
 320 during extrusion. Therefore, for all extruded composites, starch was considered amorphous.

### 321 3.2. Morphology

322 The organisation of starch and proteins of raw material and selected composites was  
 323 investigated using CLSM and presented in Figs. 3a, b and c. The grey areas correspond to the  
 324 starch phase and the green areas depict the protein phase.



326 Figure 3. The morphology of raw material and composites observed by CLSM:

- 327 - Starch granules and protein bodies in raw pea flour (a) and starch-protein SP blend (b).  
328 - Amorphous starch and protein aggregates in pea flour (c) and SP blend (d) composites  
329 extruded at three levels of *SME*. The sample B\_H was slightly rotated on the  
330 microscopic slide.

331 The proteins were stained green with fuchsin acid. Unstained amorphous starch was in grey.  
332 The white space at the interface of starch and protein aggregates indicated the void.

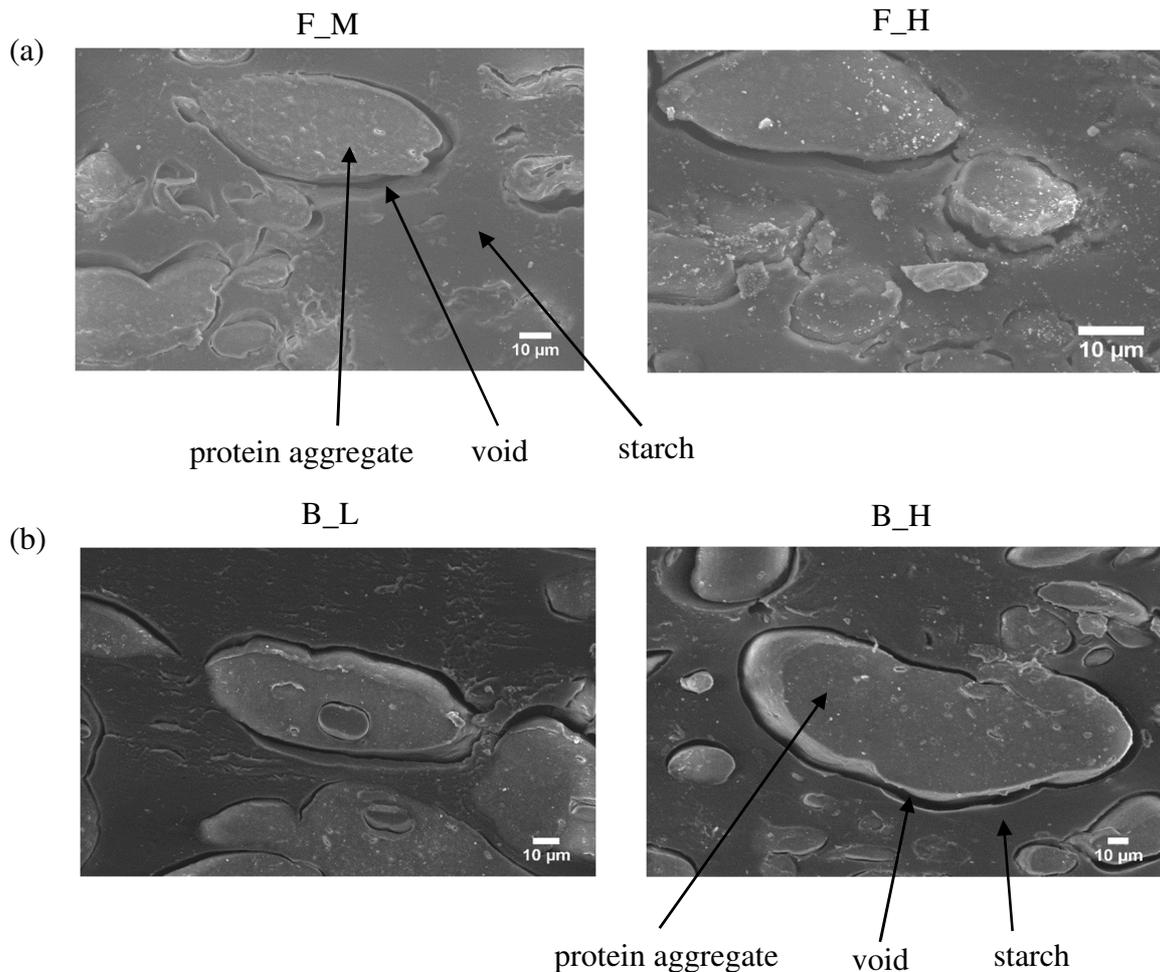
### 333 *Image description*

334 In native pea flour, proteins surrounded starch granules, whereas proteins formed an  
335 independent structure in native SP blends (Fig. 3a). In all composites, the morphology showed  
336 protein aggregates dispersed in a continuous matrix of amorphous starch for various *SME*  
337 values (Figs. 3b, c). Chanvrier et al. (2006) also observed similar morphology, namely  
338 matrix-particle, for thermo-moulded or extruded corn flour and starch-zein blends, when zein  
339 concentration was lower than 20% (db). They suggested that the protein aggregates derived  
340 from the protein bodies were disrupted, denatured and cross-linked due to high mechanical  
341 energy and temperature. In our case, protein aggregates observed in SP blend composites  
342 were clearly larger than in flour composites, likely due to the wet extraction process of PPI  
343 that results in assembled protein particles (Fig. 3b). For both composites, starch and protein  
344 domains were separated by voids, as precisely observed by scanning electron microscopy  
345 (Fig. 4).

346 These voids can result from interfacial debonding during melt cooling after leaving the  
347 extruder die, as well as from sample cryosectioning for microscopy analysis. The interface  
348 voids indicating poor interfacial adhesion were also observed for starch-zein blend composites  
349 formed by simple shear flow (Habeych et al., 2008). Utracki (2002) reported that the presence  
350 of voids in polymer blends was due to poor compatibility of the phases. Hence, regardless of  
351 the explanation, it is likely that composite morphology is due to poor adhesion of starch and  
352 proteins in the glassy domain.

353 The CLSM images showed that the size and shape of protein aggregates was not  
354 uniform within composites, and this non-uniformity was more marked in SP blend composites  
355 than in those of flour. The composites extruded at  $SME < 200$  kJ/kg (F\_L, B\_L) exhibited the  
356 highest protein area. Protein aggregates in flour composites did not present any orientation,  
357 whereas they were oriented in the extrusion direction in SP blend composites extruded at  $SME$   
358  $\geq 1000$  J/g (B\_M, B\_H). Starch granules were transformed into a homogenous matrix of  
359 amorphous starch with the exception of F\_L and B\_L composites. Indeed, these composites  
360 presented deformed ghost starch granules (size  $\approx 22$   $\mu\text{m}$ ) that were entrapped by proteins in  
361 F\_L, and were dispersed and elongated in continuous phase in B\_L. Similar flour composite  
362 morphology was observed by Kristiawan et al. (2018) in the intrinsic material of expanded  
363 pea flour. These observations clearly revealed that the variation of extrusion variables (*SME*)  
364 led to various composite morphologies, which required morphological analysis for further  
365 quantification.

366 The total area of protein aggregates decreased from 130 to 80  $\text{mm}^2$  with increasing  
367 *SME* ( $R^2 = 0.97$ ; Fig. 5a). This result may be attributed to the increase in starch transformation  
368 with *SME*, reflected, for instance, by the increase in water swelling (Kristiawan et al., 2018).  
369 These changes would cause the starch to swell up and disperse more easily, leading to a larger  
370 area of the continuous phase at the expense of the dispersed phase, protein aggregates in this  
371 case.

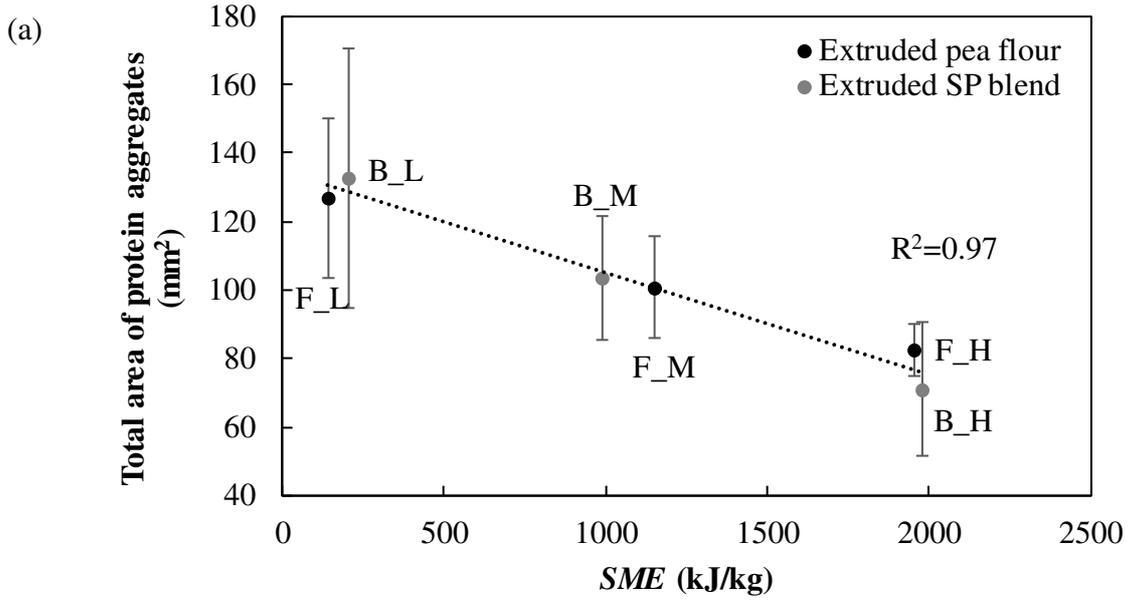


372  
 373 Figure 4. Scanning electron microscope images of pea flour (a) and starch-protein blend (b)  
 374 composites. Voids were observed at the interface of amorphous starch and protein aggregates.

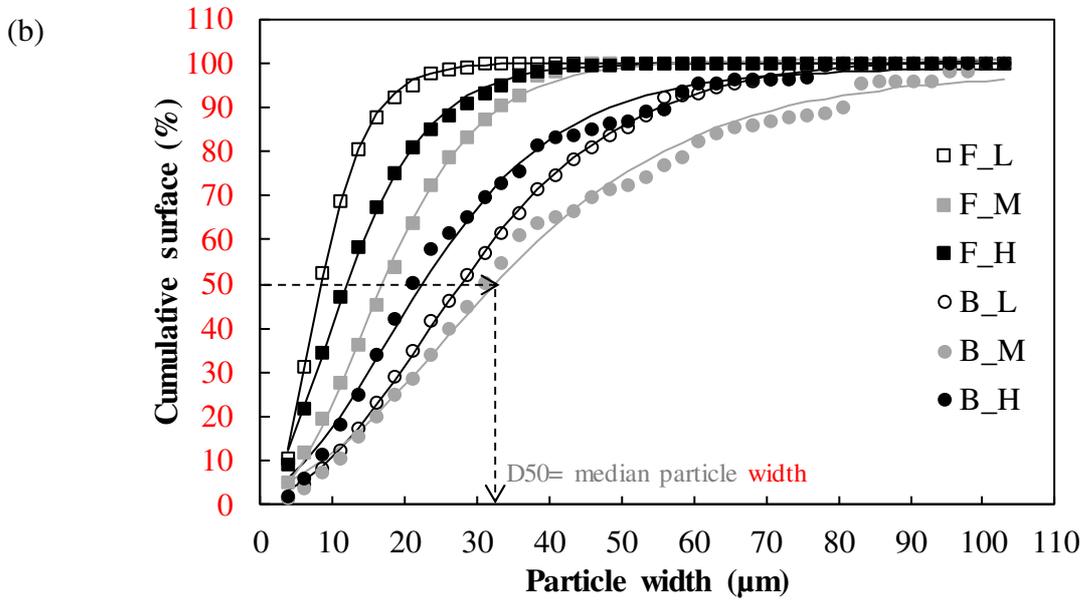
375 *Image analysis*

376 The distribution curves of the width of protein aggregates were presented as the  
 377 cumulated percentage of the total area occupied by aggregates (Fig. 5b). The good curve  
 378 fitting by the Gompertz function allowed accurate determination of median aggregate size  
 379 ( $D_{50}$ ). The SP blend composites presented larger protein aggregates ( $D_{50} = 22-31 \mu\text{m}$ ) than  
 380 flour composites ( $D_{50} = 8-18 \mu\text{m}$ ). The relative standard deviation of  $D_{50}$  values was between  
 381 11 and 15% for all samples, except for B\_L that was 28%.

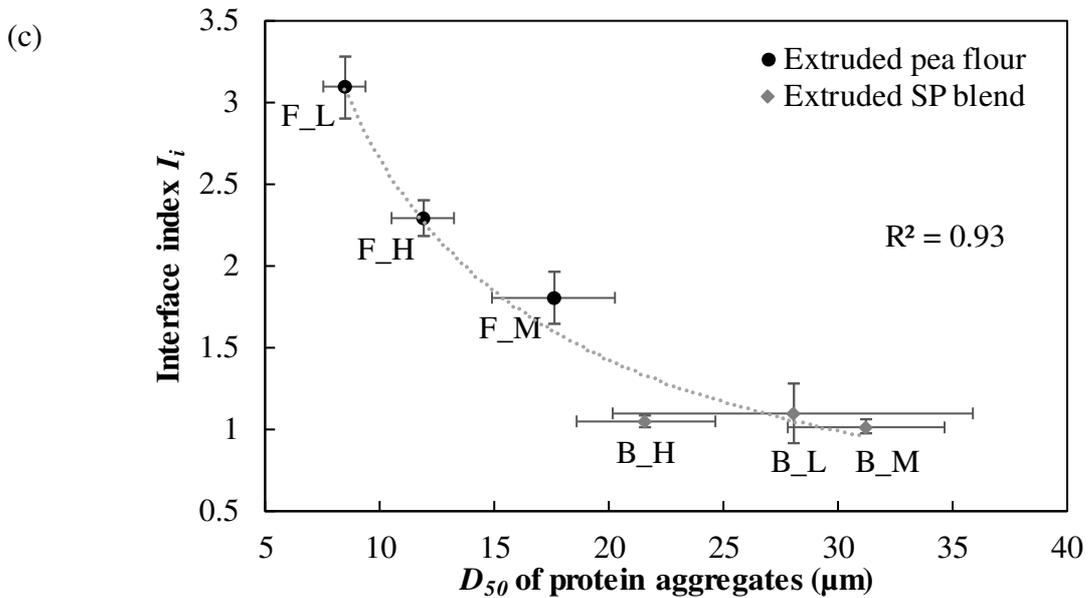
382 The interface index ( $I_i$ ) of pea flour composite, defined as the ratio of the total  
 383 aggregate perimeter to the square root of the total aggregate area, varied from 3 to 1, the lower  
 384 value being obtained for SP blend composites. Not surprisingly, it was strongly and  
 385 negatively correlated with  $D_{50}$  ( $R^2 = 0.93$ ): the smaller the aggregates were, the larger the  
 386 relative interface between proteins and starch was (Fig. 5c). This result confirms the  
 387 qualitative trend observed from CLSM images (Fig. 3): many small protein aggregates (F\_L)  
 388 have a larger total perimeter than a few large and sparse aggregates (B\_H). Since both  
 389 features, interface index and median width are correlated, the following results will be  
 390 compared using only  $I_i$ .



391



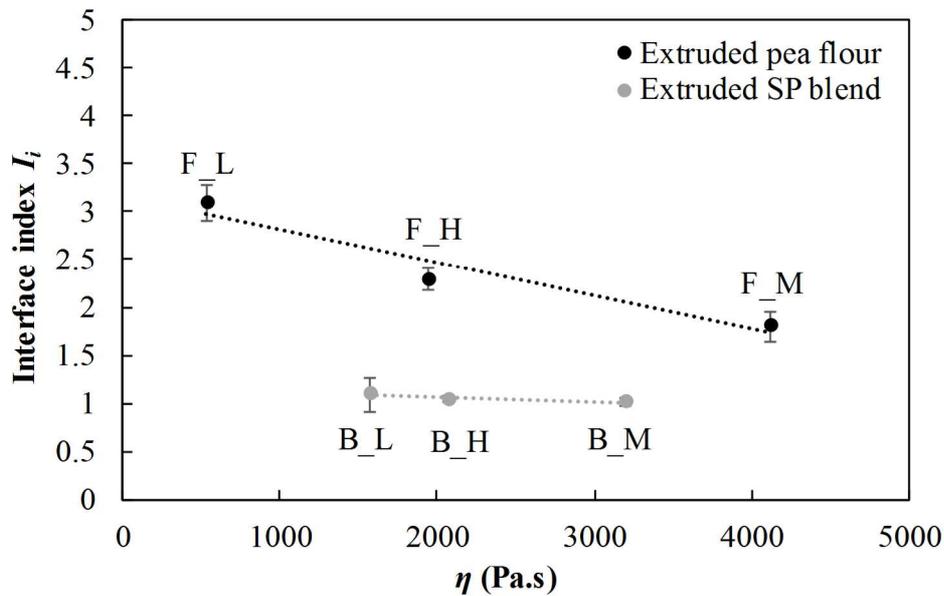
392



393

394

(d)



395

396 Figure 5. Variations of composite morphology features: total area of protein aggregates with  
397 specific mechanical energy (*SME*) (a); cumulative distribution of protein aggregate width for  
398 pea flour (square) and starch-protein blend (circle) composites (b); solid lines represent data  
399 fitting using the Gompertz function (Eq. 3,  $R^2 > 0.98$ ); interface index  $I_i$  with median protein  
400 aggregate width  $D_{50}$  (c); interface index  $I_i$  with apparent viscosity  $\eta$  (d). The dotted lines  
401 represent the data fitting using appropriate mathematical, polynomial or power functions.

402

403 The interface index decreased slightly with the apparent viscosity  $\eta$  (Fig. 5d). This  
404 variation is tiny and even not significant in the case of SP blends, given the large variation of  
405 viscosity. Conversely, during shear flow, viscosity is expected to increase with interface  
406 because of interfacial effects between the molten phases, as suggested for starch-zein blends  
407 (Chanvrier, Chaunier, Della Valle, & Lourdin, 2015). Hence, this result suggests that other  
408 structural changes, possibly antagonistic, like fragmentation of protein aggregates or starch  
degradation prevail in the building of the composite morphology.

409

### 3.3. Mechanical properties

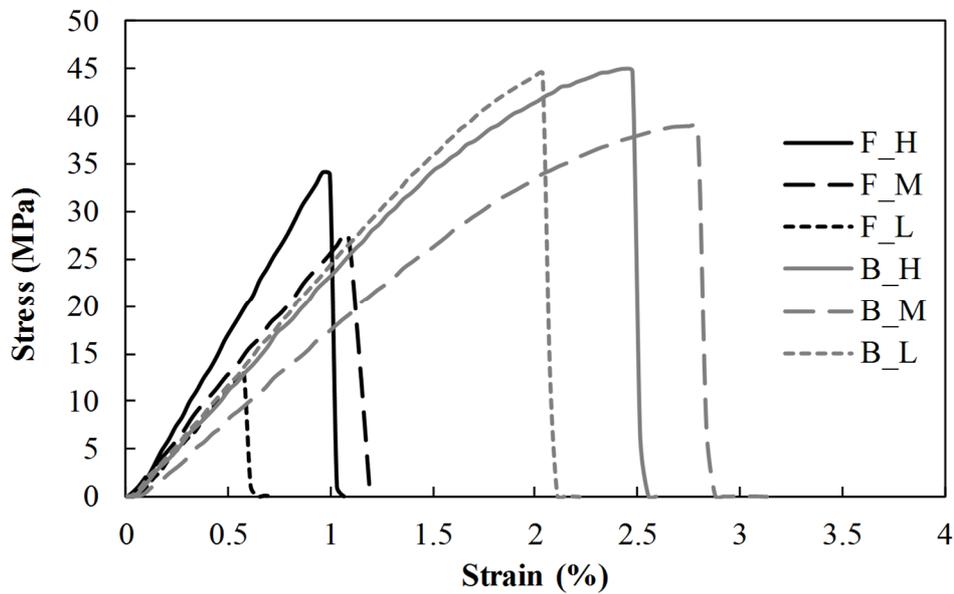
410

411 The main results of the three-point bending test of composites (*MC* 12% wb, 20 °C)  
412 are illustrated in Fig. 6. Stress-strain curves revealed that flour composites exhibited brittle  
413 behaviour with the rupture of specimens in the elastic domain. SP blend composites were less  
414 brittle because their rupture took place beyond the elasticity stage. At similar *SME*, SP blend  
415 composites had higher values of stress and strain at rupture and lower values of flexural  
modulus than flour composites (Table 3).

416

417 The rupture stress of pea composites was in the range of extruded and thermo-  
418 moulded corn flour (18 MPa) and starch-zein composites (~25 MPa) (Chanvrier, Chaunier,  
419 Della Valle, & Lourdin, 2015; Chanvrier, Colonna, Della Valle, & Lourdin, 2005). The higher  
420 fibre content of pea flour (26% db) may partly explain the difference in the mechanical  
421 behaviour of the composites (Muneer et al., 2018). According to Robin, Dubois, Curti,  
422 Schuchmann, & Palzer (2011), the rupture could occur either by fibre fracture or by fracture  
at the interface of fibre and continuous starch.

423 The flexural modulus was found to be independent of morphological characteristics  
 424 (Appendix: Fig. A5). Chanvrier et al. (2016) reported that zein content had no significant  
 425 effect on the storage modulus of starch-zein composites at room temperature. This may be due  
 426 to close values of the storage modulus of glassy starch and proteins. The mechanical  
 427 properties were defined only by stress and strain at the rupture point.



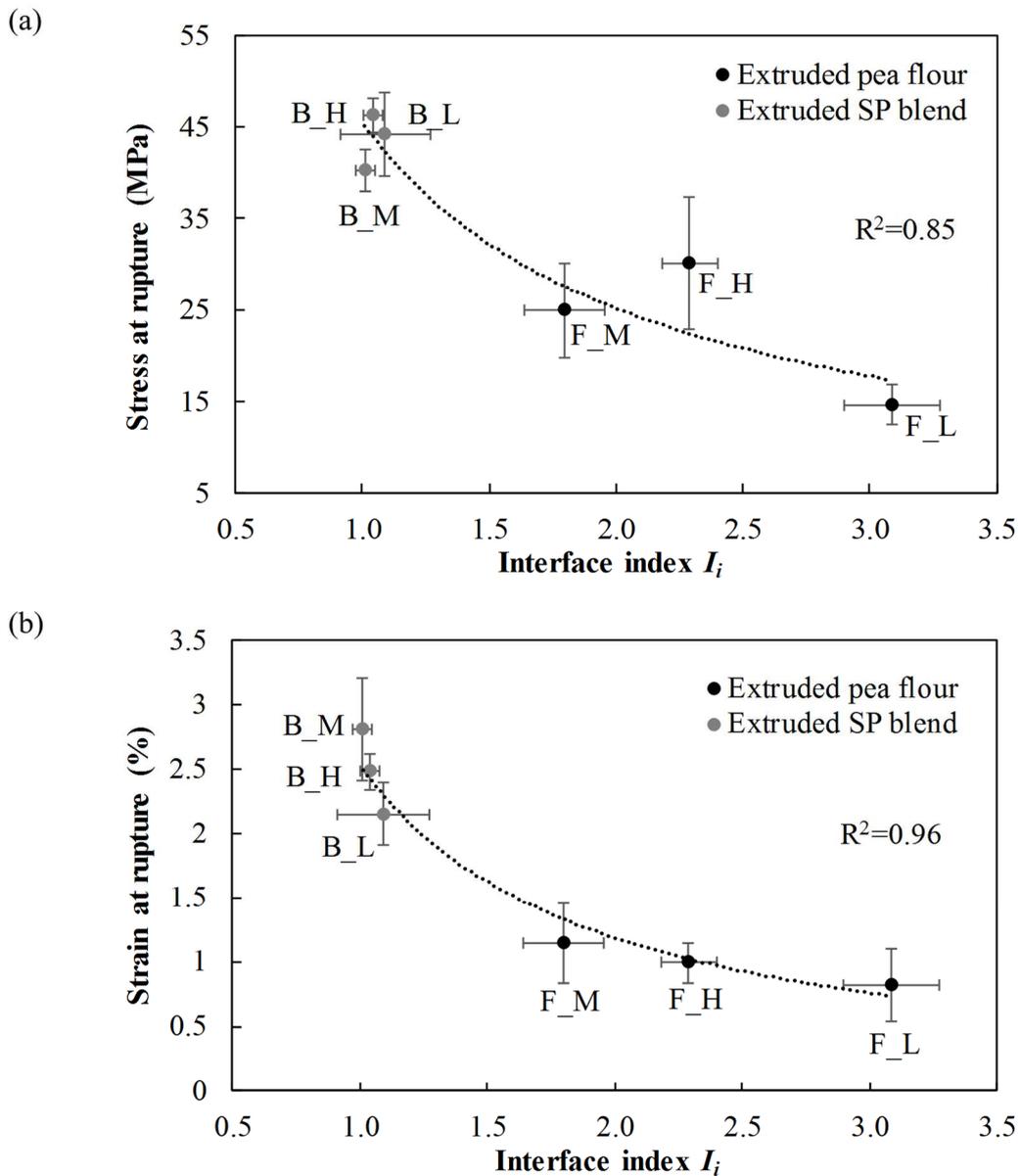
428  
 429 Figure 6. Engineering stress-strain curve obtained by a 3-point bending test for pea flour  
 430 (black lines) and starch-protein blend (grey lines) composites (20 °C, MC 12 ± 0.5% wb).

431  
 432 Table 3. Morphological features and mechanical properties of pea composite

Samples	Morphological features			Mechanical properties		
	Total area (mm <sup>2</sup> )	$D_{50}$ ( $\mu$ m)	$I_i$	$\sigma_{rupture}$ (MPa)	$\epsilon_{rupture}$ (%)	$E$ (GPa)
Pea flour						
F_L	127±23	8±1	3.1±0.2	15±2	0.8±0.3	2±0.6
F_M	101±15	18±3	1.8±0.2	25±5	1.1±0.3	2.4±0.5
F_H	82±8	12±1	2.3±0.1	30±7	1±0.2	3.1±0.5
Pea starch-PPI blend						
B_L	133±38	28±8	1.1±0.2	44±5	2.1±0.2	2.4±0.2
B_M	104±18	31±3	1±0.04	40±2	2.8±0.4	1.9±0.1
B_H	71±19	22±3	1±0.04	46±2	2.5±0.1	2.4±0.1

433  
 434 The interface index ( $I_i$ ) negatively influenced the composite breaking stress and strain  
 435 ( $R^2 \approx 0.90$ ; Fig. 7). This result can be explained by the poor interfacial adhesion between the  
 436 starch matrix and protein aggregates, as suggested from microscopy images. The larger  $I_i$  was,  
 437 the higher the interfacial debonding created under stress and the more brittle the composite  
 438 were. The strength of composites strongly depends on the stress transfer between the particles  
 439 and matrix (Fu et al., 2008). For a poorly bonded particle/matrix, the stress transfer at the  
 440 interface was inefficient. As shown in Fig. 5c, the flour composites had smaller  $D_{50}$  and larger

441  $I_i$  than SP composites. Combined with less effective stress transfer mechanisms, these factors  
 442 made flour composites more brittle than those of SP blends.  
 443



444  
 445 Figure 7. Effect of the interface index on stress (a) and strain (b) at rupture. The dotted lines  
 446 represent data fitting using power functions.

447 In the field of polymer/material science, it is assumed that the incorporation of micro-  
 448 and nanoparticles reinforces composite mechanical properties, i.e., increases Young's  
 449 modulus, breaking strength and fracture toughness. The challenge for the food sciences is the  
 450 opposite. Increased brittleness of extruded foods is considered to be advantageous for  
 451 chewing and nutrient release during digestion. Expanded leguminous snacks, obtained by  
 452 extrusion, were distinguished by uniform and relatively small pore structure (Li et al., 2016).  
 453 For the same density, starch foams with a fine cellular structure were found to be more  
 454 resistant to rupture than coarser structures (Babin, Della Valle, Dendievel, Lourdin, & Salvo,

455 2007). A fine cellular structure with crispy texture can be obtained by controlling the fragility  
456 of cell walls, which depends on starch-protein morphology, i.e., interface index and strength  
457 of interfacial adhesion. In turn, morphology can be controlled by tuning the extrusion  
458 variables (*SME*) and formulation.

### 459 **Conclusions**

460 Pea composites were obtained by extrusion in a large interval of *SME* which was  
461 particularly tuned by the ratio of total flow rate to screw speed. Different morphologies were  
462 obtained by modifying the *SME*. The structure of pea flour and starch-protein (SP) blend  
463 composites consisted generally of dispersed protein aggregates in an amorphous starch  
464 matrix. The presence of voids between the phases indicated poor interfacial adhesion. The  
465 total area of protein aggregates decreased with increasing *SME* due to starch swelling leading  
466 to larger area of the continuous phase (starch) at the expense of dispersed protein aggregate.  
467 Pea flour composites exhibited brittle behaviour with rupture in the elastic domain, whereas  
468 SP blend composites exhibited higher breaking stress and strain with rupture in the plasticity  
469 stage. The interface index, which was defined as the ratio of the aggregate perimeter to the  
470 square root of the total aggregate area, explained the variation of mechanical properties,  
471 regardless of the composite formulation. Increasing the interface index with poor  
472 compatibility between the phases weakened the composites. Finally, it is possible to modulate  
473 the mechanical properties by tuning the composite morphology through extrusion variables.  
474 These results will contribute to the determination of constitutive laws of starch-protein  
475 composites. These laws will be integrated into multi-scale numerical models to predict the  
476 mechanical properties of solid foams based on knowledge of the cellular structure and  
477 morphology of intrinsic material.

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### 482 **Appendix**

483 Supplementary material related to this article can be found in the online version.

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