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1 **Legume enriched cereal products: A generic approach derived from material**
2 **science to predict their structuring by the process and their final properties**

3

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11

12 **Highlights**

- 13 • Composition and behavioral differences in the components of wheat and legume flours
- 14 • Texture, sensory and nutritional properties of legume enriched cereal products
- 15 • Impact of adding legume flour on the multi-scale structure of cereal products
- 16 • Modifying processing to control the quality of legume enriched cereal products

17 **Abstract**

18 Enriching cereal products with legume flour has nutritional advantages, notably that of improving the
19 amino acid balance, but also modifies their processability and properties. Being able to predict such
20 modifications is a major issue for industry.

21 In this review, we focus on the properties of the main components of cereal and legume flour
22 (proteins and starch), and their functionality during processing of five typical cereal products (snacks,
23 pasta, biscuits, cakes and bread), and pay particular attention to their role in structuring the product
24 throughout processing. The nutritional and other properties (color, texture, structure, and sensory
25 acceptability) of the legume-enriched cereal products are described.

26 Adding legume flour changes the structure of the product and results in a different texture. As the
27 structure is usually designed during processing, a good knowledge of the product-process
28 interactions is required. State diagrams of starch and protein in both cereals and legumes are
29 compared. The gelatinization and the denaturation temperatures of starch and protein in legumes
30 are slightly higher than those of cereals. These differences may affect the structuring dynamics and
31 the final structure of the products. Process modifications are proposed to limit such effects and are
32 discussed in light of several results in the literature.

33 Applying an integrative approach enabled us to demonstrate the expression of the functional
34 properties throughout processing, from the composition of the different flours to the end products.
35 We propose a generic representation that can be used by all those who wish to rationally formulate
36 legume enriched cereal products.

37 **Key words:** Food; chemical-physics; functional properties; protein; starch; state diagram; structure

38 **Introduction**

39 Meat is a major source of protein in the human diet and in 2013, accounted for 40% of the world's
40 total protein supply (FAO, 2016). Meat consumption reached 34 kg/capita/year in the 2013-2015
41 period (OECD/FAO, 2016) and is projected to increase by ~70% by 2050 (FAO, 2011). However, a
42 decrease in meat consumption is recommended due to growing concerns about the environmental
43 impact of livestock and the health risks associated with high meat intake. This leads to the need for
44 more plant based diets and more energy efficient processing.

45 Cereal and legume grains are ancestral staple foods that are rich in carbohydrates, fibers and
46 micronutrients. They also provide a fair amount of proteins (9-18 and 20-40 g/ 100 g of grains wb,
47 respectively). When combined, protein efficiency is improved thanks to complementary essential
48 amino acid profiles (Young & Pellett, 1994). Both kinds of whole grain also play a key role in the
49 prevention of cardiovascular disease, type 2 diabetes, some cancers, digestive tract diseases,
50 overweight and obesity (Duranti, 2006; Mellen, Walsh, & Herrington, 2008; Ye, Chacko, Chou,
51 Kugizaki, & Liu, 2012).

52 In another connection, agricultural research has shown the potential advantages of cereal-legume
53 intercropping that makes better use of natural resources at crop or rotation scale (Pelzer *et al.*,
54 2012). Considering that wheat based baked products are widely consumed and accepted (more than
55 4 million metric tons consumed in Europe in 2015 (CAOBISCO, 2016)), improving the formulation of
56 pulse enriched products could be a step towards more sustainable food chains.

57 In the literature, a wide variety of studies focus on the formulation of cereal products with legume
58 flours or isolates (Noorfarahzilah, Lee, Sharifufin, Mohd Fadzelly, & Hasmadi, 2014). The first boom in
59 this research started in the late 1970s driven by the quest for more competitive and high quality
60 protein sources in many parts of the world. Special programs were conceived to promote the use of
61 composite flours, i.e. binary or ternary mixtures of flours and fractions (starch, proteins) of different

62 botanical origin that would enable the formulation of fortified products, especially valuable for
63 developing countries. This led to studies of the nutritional, structural and sensory properties of
64 fortified products for example by (D'Appolonia, 1977; Fleming & Sosulski, 1977a, 1977b; Mcwatters,
65 1978).

66 A second boom began in 2000 due to the strong resurgence of concerns about protein in a context of
67 forced nutritional transitions at global scale. In line with pioneer works, recent studies have focused
68 on the impact of replacing wheat flour with legume flour on nutritional, structural and sensory
69 properties of enriched products (Noorfarahzilah *et al.*, 2014). Such experiments are underway
70 worldwide. However, among studies on the use of legumes in cereal or cereal-like products, only a
71 few have focused on the flours and on the effect of partial or total replacement of cereal flour by
72 legume flour on the product structure at different spatial scales and throughout processing (Laleg,
73 Barron, Santé-Lhoutellier, Walrand, & Micard, 2016; Laleg, Cassan, Barron, Prabhasankar, & Micard,
74 2016; Petitot, Barron, Morel, & Micard, 2010).

75 Processing such products raises technological questions due to differences in functionality between
76 wheat and legume flours, at first sight the most obvious being the type and functionality of proteins.
77 Foschia, Horstmann, Arendt, & Zannini (2017) recently reviewed the uses of legumes (mainly as
78 concentrate and isolate) as an ingredient in different gluten free bakery and pasta products. These
79 authors described the resulting changes in the properties of the end product in an attempt to link
80 them with the functional properties of proteins, and underlined the difficulty in drawing any generic
81 conclusions concerning the effect of legume ingredients on product quality due to the variation in
82 their formulas and in processing conditions. To be able to properly monitor processing and to
83 achieve constant product quality, changes in the structure of the product need to be better assessed
84 at each stage of processing.

85 The present review investigates the impact of adding varying amounts of legume flour to wheat flour
86 on the multi-scale structure of five standard cereal products: bread, pasta, biscuits, soft cakes and

87 extruded snacks. In line with the above mentioned review by Foschia *et al.* (2017) of gluten free
88 products enriched in legumes, particular attention is paid to the functional properties of the legume
89 components, particularly starch and proteins. Their influence on the resulting texture, color, flavor
90 and nutrition of final food products is described. The structure-properties relationships are
91 highlighted in an original way. Finally, for the first time, a generic approach is proposed to monitor
92 the transformation of material during processing of legume enriched cereal products.

93

94 **Formulation of classical wheat products likely to be complemented with legume flours**

95 Formulation offers the opportunity to tailor the structure of a food by controlling the processing
96 conditions (Aguilera & Stanley, 1999). Table 1 provides a simplified comparison of the formulas of the
97 five products based on their major ingredients. Flour content varied homogeneously over the range
98 of formulas. Three cases can be distinguished: (i) products rich in flour, whose second ingredient is
99 mainly water (snacks, pasta, bread); ii) biscuits, with an intermediate amount of flour and equal
100 proportions of sugar and fat as second ingredients; iii) soft cakes, with a small amount of flour, the
101 same amount of sugar, and smaller amounts of fat, egg, and water. Additional ingredients are yeast,
102 raising agents or emulsifiers in bread, biscuit and cake formulas. These are minor ingredients as far as
103 their concentration is concerned but they contribute to the formation of a gas phase and/or
104 influence the distribution of air and fat inside the product structure.

105 Beyond the nature of the ingredients, their components, i.e. starch, proteins of various origins,
106 water, lipids and fibers, also require attention. The balance between these components and the way
107 they come into contact (mixing order, intensity and duration) define the way they interact to form
108 one or several phases that, in turn, determine the product structure or matrix. For an overview of the
109 different balances between components, data from the National Nutrient Database of USDA (2016)
110 for the composition of an average wheat flour (11.5% protein content) and whole eggs was used to
111 deduce the approximate starch, protein, lipid, fiber composition, and total water content of all
112 formulas (Table 1). The result highlights the importance of starch and water, followed by proteins,
113 which accounted for around 10% or less. Starch and proteins are essential components in the process
114 of structure building of these products, and their action is closely linked to water availability.

115 **[TABLE 1]**

116 **Comparative composition of wheat and legume flours**

117 Replacing wheat flour with legume flour, which differs in composition, has an impact on the balance
118 of the components in the formula. Legume flours contain less starch, more protein and more fiber
119 than wheat flour and semolina, at an equal or higher lipid content.

120 Starch content accounts for between 60% and 80% db of soft and durum wheat, whereas it ranges
121 from 40% to 65% for legumes except for lupin (less than 1% db) (Ahmed, 2014; Feillet, 2000; Laleg,
122 Barron, Santé-Lhoutellier, *et al.*, 2016; Osorio-Díaz, Agama-Acevedo, Mendoza-Vinalay, Tovar, &
123 Bello-Pérez, 2008; Petitot, Boyer, Minier, & Micard, 2010; Sosulski & Mc Curdy, 1987; N. Wang,
124 Hatcher, Toews, & Gawalko, 2009).

125 Protein contents (N x 6.25) vary between 20% and 30% db in legume flours (D'Appolonia, 1977; N.
126 Wang *et al.*, 2009) except in faba and lupin flours in which protein can reach almost 40% db (Abou-
127 Zaid, Ramadan, & Al-Askany, 2011; Ahmed, 2014). In wheat flour and semolina, protein content
128 usually varies between 9% and 18% db (Feillet, 2000).

129 Fiber contents are around 2% db in wheat flour and semolina while it can reach 10% db in pea and
130 faba flours, and even 20% to 40% db in chickpea, lentil and lupin flours (Abou-Zaid *et al.*, 2011;
131 Ahmed, 2014; Osorio-Díaz *et al.*, 2008; Petitot, Boyer, *et al.*, 2010; Previtali *et al.*, 2014; Sosulski & Mc
132 Curdy, 1987). In the latter species, the flours are made from grains that have not been dehulled, and
133 the result is a higher proportion of fiber. Lipid content varies between 1% and 3% db in wheat and
134 legume flours except for chickpea and lupin flours in which it can reach 10% to 13% db (Ahmed,
135 2014; Laleg, Cassan, Barron, *et al.*, 2016; Petitot, Boyer, *et al.*, 2010; Sosulski & Mc Curdy, 1987;
136 Zhao, Manthey, Chang, Hou, & Yuan, 2005).

137 **Comparative composition and structure of proteins and starches**

138 In addition to varying in proportions, starch and proteins in wheat and legume grains also vary in
139 nature.

140 The proportion and size of the main proteins contained in wheat and legume grains vary. In wheat,
141 gluten proteins i.e. prolamins, are the dominant group, accounting for 80% of total protein (Shewry,
142 Tatham, Forde, Kreis, & Mifflin, 1986). Prolamins are storage proteins and are classified as gliadins
143 and glutenins depending on their structure and size (Shewry *et al.*, 1986). Glutenins are high
144 molecular mass polymeric proteins (> 100 kDa) composed of subunits linked by disulfide bonds.
145 Gliadins are lower mass monomeric polypeptides (25-75 kDa) that can form intra-polypeptide
146 disulfide bonds (Feillet, 2000; Shewry *et al.*, 1986). In legumes, globulins are the dominant group,
147 accounting for 50% to 70% of total protein (Boye, Zare, & Pletch, 2010). Globulins are also storage
148 proteins that can be divided into two classes according to their Svedberg sedimentation coefficients,
149 7S and 11S. Vicilins are 7S trimeric proteins with three subunits linked by weak bonds (hydrogen,
150 hydrophobic interactions) for a total molecular mass of ~175 kDa (Boye, Zare, *et al.*, 2010). Legumins
151 are 11S hexameric proteins with six subunits held together by weak bonds for a total molecular mass
152 ranging from 270 to 420 kDa (Duranti & Gius, 1997). Wheat gliadins and glutenins contain a higher
153 concentration of sulfur amino acids than legume globulins meaning they have more reactive cysteine
154 residues (Boye, Zare, *et al.*, 2010; Mann, Schiedt, Baumann, Conde-Petit, & Vilgis, 2014). Finally, low
155 molecular mass metabolic albumins account for the remaining fraction in both cereal and legume
156 grains, reaching respectively, 15% and 15-40% of total protein contents (Boye, Zare, *et al.*, 2010;
157 Feillet, 2000). In legumes, they are subdivided into a major fraction PA1 (dimeric, 50 kDa), a minor
158 fraction PA2 (dimeric, 11 kDa), lectins (tetrameric, 50 kDa) and enzyme inhibitors (Perrot, 1995).

159 Starch in both wheat and legumes is composed of starch granules containing linear amylose and
160 branched amylopectin organized in semi-crystalline and amorphous structures. However they differ
161 in their shape and amylose/amylopectin ratio (Buléon, Colonna, Planchot, & Ball, 1998). Wheat

162 starch presents a bimodal distribution of large lenticular starch granules (25-40 μm) and small
163 spherical starch granules (5-10 μm) (Buléon *et al.*, 1998). The amylose/amylopectin ratio in wheat
164 granules varies from 11/89 to 26/74 depending on the variety (Kumar *et al.* 2016). Legume starches
165 are generally composed of oval or irregularly shaped starch granules. Their size is similar, ranging
166 from 8 to 50 μm in pea, 6 to 37 μm in lentil, and 14 to 30 μm in chickpea (Hoover, Hughes, Chung, &
167 Liu, 2010). Legume starches have a higher proportion of amylose than wheat starch: their
168 amylose/amylopectin ratios range from 24/76 to 40/60 for pea and lentil starches and from 23/77 to
169 35/65 for chickpea starch (Hoover *et al.* 2010).

170 **Comparison of the functional properties of proteins and starches**

171 During the formation of the structure, proteins and starch express their functional properties in
172 response to different environmental parameters: water availability, temperature, mechanical
173 treatment, and potential chemical or physical interactions with other components. Fundamental
174 properties concern the ability to form specific structures (networks, interfaces) and to undergo
175 thermal transitions that are all closely related to the process of structure building. First, we discuss
176 the usual functional properties of proteins from both origins. We then provide insights into the
177 thermal transitions of protein and starch based on available data, as shown in figures 1 and 2.

178 Most wheat proteins are insoluble in water at neutral pH, which corresponds to standard processing
179 conditions (Shewry *et al.*, 1986). This almost makes them unsuitable for several functional properties
180 requiring solvation, i.e. emulsification and foaming. However, under specific hydration and shear
181 conditions, wheat proteins have the ability to form dough with viscoelastic properties. These
182 mechanical properties are enabled by the formation of a gluten network made of “fibers” composed
183 of glutenins and gliadins linked by inter- and intra-disulfide bonds. This is made possible by the large
184 amount of reactive cysteine residues in these proteins (Mann *et al.*, 2014). Conversely, legume
185 protein isolates exhibit a satisfactory degree of solubility in water at pH 7, and the mean values for
186 pea, lentil and chickpea are $\sim 70\%$ (Boye, Aksay, *et al.*, 2010; Karaca, Low, & Nickerson, 2011).

187 Concerning interfacial properties, legume protein isolates have low emulsifying properties (~20 m² of
188 oil covered/g of isolate) and stability (~10 to 20 min) compared to animal protein isolates (Fenn,
189 Lukow, Humphreys, Fields, & Boye, 2010; Karaca *et al.*, 2011; Withana-Gamage, Wanasundara,
190 Pietrasik, & Shand, 2011). However, they show better foaming activities (~100% to 200%) and
191 stabilities (~60% to 90% at 30 min) (Boye, Aksay, *et al.*, 2010; Toews & Wang, 2013). This ability is
192 related to their low molecular mass that enables them to rapidly diffuse to the air-water interface. In
193 any case, their interfacial properties are better than those of wheat gluten (only ~5 m² of oil
194 covered/g of isolate and 9 min of emulsifying stability, and foaming activity and stability indexes of
195 29% and 40% (J. Wang, Zhao, Yang, & Jiang, 2006)).

196 As polymers, protein and starch are subject to thermal transitions. Their transition temperature
197 depends on the available water content that is expected to vary during the course of the process.

198 Figure 1 summarizes thermal transitions in the form of state diagrams for proteins and figure 2 for
199 starch.

200 **[FIGURE 1]**

201 Proteins are subject to two kinds of thermal transition: glass transition and thermosetting. Glass
202 transition corresponds to an increase in molecular mobility enabled by an increase in temperature
203 and/or in water content. This change in organization at a molecular scale is accompanied by major
204 changes in the mechanical properties of the material, which changes from a solid brittle state or
205 “glassy state” to a soft viscoelastic state or “rubbery state” (Cuq, Abecassis, & Guilbert, 2003). Glass
206 transition is well described in wheat proteins. Changes in the glass transition temperature (T_g) of
207 wheat gluten proteins as a function of water content, as reviewed by Cuq *et al.* (2003) and in
208 supplementary data by Bengoechea, Arrachid, Guerrero, Hill, & Mitchell (2007) are shown in figure 1.
209 Only a few studies have focused on glass transition in legume proteins, considering either isolates
210 (Bengoechea *et al.*, 2007; Pelgrom, Schutyser, & Boom, 2013) or purified fractions (Mizuno, Mitsuiki,
211 & Motoki, 2000; Morales & Kokini, 1997). Changes in the T_g of pea and soy protein isolates as a

212 function of water content reported by Pelgrom *et al.* (2013) and Bengoechea *et al.* (2007) are
213 included in figure 1. This comparison shows that glass transitions in wheat gluten and legume protein
214 isolates take place in the same ranges of temperature and water content. Thermosetting refers to
215 the fact that above a certain temperature, there is a change in protein structure. This phenomenon is
216 described as the succession of protein unfolding or denaturation and protein aggregation or
217 crosslinking, both occurring in a relatively narrow temperature range. The beginning of
218 thermosetting is usually estimated by the denaturation temperature (Td) measured by differential
219 scanning calorimetry. As a result of protein crosslinking by hydrogen, hydrophobic and disulfide
220 bonds, a three-dimensional network is formed that confers new rheological properties to the matrix
221 (Mann *et al.*, 2014). Figure 1 shows the changes in the thermosetting temperature of wheat gluten
222 proteins as reported by Kokini, Cocero, & Madeka (1995). The Td of wheat gluten proteins varies
223 from ~90 °C for water contents below 20% water db to ~70 °C for water contents above 20% water
224 db, assuming that it remains constant above 43% water db (Fig. 1). Many studies have documented
225 the denaturation of proteins in different legume species and protein fractions at water contents of
226 between 80% and 94% wb and at different DSC heating rates (2, 5 or 10 °C/min). To get an idea of
227 the variation in Td in these parameters, we chose to calculate an average Td for 50 values from five
228 studies, considering 11 different legume species and three heating rates after having checked that
229 the difference in the heating rate had very low impact on the values. Averaging the legume species
230 was possible given the low standard deviation with regard to the positioning of the Td curve in the
231 state diagram (Fig. 1). The result was $88\text{ °C} \pm 7\text{ °C}$ over the 80-94% moisture range, indicating a higher
232 Td for legume proteins than for wheat gluten proteins.

233 **[FIGURE 2]**

234 As a semi-crystalline polymer, starch is subject to glass transition of its amorphous zones and melting
235 of its crystalline zones. Like for wheat proteins, data are available on the glass transition of wheat
236 starch (Cuq *et al.*, 2003). Figure 2 shows changes in the glass transition temperature (Tg) of wheat

237 starch as a function of water content, as reviewed by Cuq *et al.* (2003). In contrast, very few data can
238 be found on the T_g of legume starches. Pelgrom *et al.* (2013) documented changes in the T_g of pea
239 starch between 10% and 16% water wb (Fig. 2). On this basis, the comparison of T_g in wheat and pea
240 starches suggests that glass transition occurs in the same ranges of temperature and water content.
241 As a semi-crystalline polymer, starch is subject to the melting of its crystalline zones at the
242 temperature T_m. Crystallites melt in a three-stage process composed of the rapid melting of
243 amylopectin crystallites (T_{m1}), slow melting of remaining ones (T_{m2}) and melting of amylose-lipid
244 complexes (T_{m3}) (Roos & Drusch, 2015). The occurrence of T_{m1} and T_{m2} depends on water content;
245 usually only T_{m2} is observed at low water content (< 35% wb), both T_{m1} and T_{m2} at intermediate
246 water contents (35% to 60% wb) and only T_{m1} at high water contents (> 60% wb) (Roos & Drusch,
247 2015). Theoretically, it is only in excess water (i.e. > 60% wb) conditions that gelatinization *stricto*
248 *sensu* can occur. Gelatinization is a complex process including melting of the crystallites at T_{m1} but
249 also a group of other structural changes leading to hydration and swelling of the granules, leaching
250 and potential loss of granular integrity. In the case of wheat starch, Vanin, Lucas, & Trystram (2009)
251 summarized data from three studies on T_{m1}, T_{m2} and T_{m3}; the corresponding T_m ranges are shown
252 in figure 2. In the figure, data from the study by Eliasson (1980) show the occurrence of T_{m2} above
253 60% water wb; however, this is not standard behavior. Like in the case of legumes, the study of the
254 starch thermal transition has mostly focused on gelatinization properties with little research devoted
255 to the different melting transitions. Consequently, available data usually provide information about a
256 single transition endotherm in the 70-80% moisture range that we believe to be T_{m1}. For the same
257 reason as for protein denaturation, we calculated an average T_{m1} for legume starches based on 46
258 values taken from four studies concerning 11 legume species. In this case, all the studies used the
259 same heating rate of 10 °C/min. The result was an average temperature of 70 °C ± 5 °C over the 70-
260 82% moisture range (Fig. 2). This suggests a higher T_{m1} for legume starches than for wheat starch,
261 whose T_{m1} lies around 55 °C ± 5 °C. Foschia *et al.* linked this to the higher amylose/amylopectin ratio
262 of legume starches (Foschia *et al.*, 2017).

263 To conclude, it appears that wheat and legume components have contrasted properties. While they
264 exhibit distinct behaviors in terms of solvation and structure building abilities (e.g. the formation of a
265 network versus foam), their thermal properties appear to be similar. However in legumes, higher
266 protein denaturation and starch gelatinization temperatures (T_{m1}) may point to a tendency to a shift
267 in these reactions as a function of temperature during thermal treatment.

268 **Current knowledge about the impact of adding legume flour on the end properties of the** 269 **product**

270 A good number of studies are available concerning the impact of replacing wheat flour on the
271 textural, sensory and nutritional properties of enriched products. They are summarized in Table 2
272 according to the trends shared by the five cereal products.

273 **[TABLE 2]**

274 ***Modifications in color and texture caused by adding legume flour***

275 Concerning color, a decrease in lightness values (L) with an increase in the proportion of legume flour
276 is regularly reported. The same decrease was observed in all five kinds of product, both in the crust
277 and crumb in the case of bread and cakes. This could be related to the color of the legume flour
278 (Zhao *et al.*, 2005), and to the thermal induced protein-carbohydrate Maillard reactions which
279 involve reducing sugar and the terminal amino group of a free amino acid, in particular lysine. These
280 reactions could be more pronounced in the case of legume-enriched products, resulting in a larger
281 decrease in the lysine content in comparison with that in wheat products (-14% and -2% decrease in
282 lysine between low and very high temperature treatment of 35% faba bean pasta and wheat pasta
283 respectively) (Laleg, Barron, Santé-Lhoutellier, *et al.*, 2016). In dried pasta, lightness decreased by
284 16% in 35% split pea enriched pasta (Petitot, Boyer, *et al.*, 2010). With 100% enrichment, the extent
285 of pasta darkening has been shown to depend directly on the type of legume. The lightness
286 decreased by 43%, 60% and 26% respectively with 100% faba, lentil and black gram pasta (Laleg,

287 Cassan, Barron, *et al.*, 2016; Petitot, Boyer, *et al.*, 2010). In biscuits, lightness decreased by 10% when
288 50% of lupin flour was added (Jayasena & Nasar-Abbas, 2011). In snacks, lightness decreased by 13%
289 when 45% of bean flour was introduced compared to 15% of added bean flour (Anton, Gary Fulcher,
290 & Arntfield, 2009). In cake, crumb lightness decreased by 3% and crust lightness by 10% when 50% of
291 chickpea flour was added (Gómez, Oliete, Rosell, Pando, & Fernández, 2008). In bread crumb,
292 lightness decreased by 7% and crust lightness by 24% when 30% of chickpea flour was added
293 (Mohammed, Ahmed, & Senge, 2012). A tendency to increased yellowness-blueness (b^*) was also
294 observed in snacks, biscuits, bread crumb and crust, and cake crumb. In snacks b^* increased by 22%
295 when 45% of bean flour was added compared to 15% of added bean flour (Anton *et al.*, 2009). In
296 biscuits, b^* increased by 51% when 50% of lupin flour was added (Jayasena & Nasar-Abbas, 2011). In
297 bread, crumb b^* increased by 1,230% and crust b^* increased by 160% when 30% of chickpea flour
298 was added (Mohammed *et al.*, 2012). In cake crumb b^* increased by 72% when 50% of chickpea flour
299 was added (Gómez *et al.*, 2008). Variations in redness-greenness (a^*) depended on each case; they
300 generally depended on type of legume and the proportion of flour.

301 In terms of texture, incorporating legume flour always increases the hardness, firmness or stiffness of
302 the product, measured by TPA, other compression tests, or bending tests. For example, in snacks
303 Anton *et al.* (2009) measured a +5% and +15% increase in the breaking strength index (peak breaking
304 force over surface area measured with a three point bending test) in snacks containing 30% and 45%
305 of legume flour compared to snacks containing 15% of legume flour. A +38% and +48% increase in
306 pasta firmness has been observed in 35% faba or split pea pasta, and in 100% faba bean pasta,
307 respectively (Petitot, Boyer, *et al.*, 2010; Rosa-Sibakov *et al.*, 2016). However, the firmness of gluten
308 free 100% faba bean, lentil and black gram pasta was shown to still be 2.3, 1.7 and 1.5 fold lower
309 than a cereal gluten free pasta (Laleg, Cassan, Barron, *et al.*, 2016). In biscuits Jayasena and Nasar-
310 Abbas (2011) observed increases in hardness and brittleness (evaluated with a three point bending
311 test) ranging between +51% and +342% for hardness and +96% and +223% for brittleness in biscuits
312 containing 10% to 50% of lupin flour. In bread, using a compression test, Previtali *et al.* (2014)

313 showed an increase in firmness of + 26% and +82% in breads containing 20% and 25% of lentil flour,
314 respectively. De la Hera, Ruiz-París, Oliete, & Gómez (2012) reported increases in hardness (TPA test)
315 ranging from +18% to +80% in layer cakes containing 50% of lentil flour (different cultivars and
316 particle sizes) and between +32% and +145% in cakes containing 100% lentil flour.

317 In pasta and biscuits, the increase in hardness can only be attributed to the structure and
318 composition of the dense matrix in contrast to snacks, bread and cake (aerated products) where
319 cellular structure may also play a role. In the case of aerated products, an increase in firmness can be
320 attributed either to the cell wall material itself or to the distribution of air within the structure (size,
321 number and distribution of the cells, thickness of the cell walls). Some of these aspects have been
322 shown to be impacted by the incorporation of legume flour in some products. First, product
323 expansion and density decreased and increased, respectively, with an increasing proportion of
324 legume flour: reaching -20% of expansion in snacks containing 45% of legume flour compared to 15%
325 in snacks (Anton *et al.*, 2009); +33% density in layer cakes containing 100% lentil flour (De la Hera *et*
326 *al.*, 2012); up to +31% of density in breads containing 40% of soy flour (Shogren, Mohamed, &
327 Carriere, 2003). Secondly, in the case of enriched snacks and breads, thickening of the cell walls and
328 an increase in porosity (meaning more holes in the walls) have been reported (Fleming & Sosulski,
329 1978; Lazou, Michailidis, Thymi, Krokida, & Bisharat, 2007; Lazou & Krokida, 2010), concomitant with
330 a decrease in the number of cells in enriched snacks.

331 ***Sensory perception and acceptability of legume-enriched products***

332 Sensory results provide qualitative information on variations resulting from descriptive profiles that
333 take color, flavor, texture and structure characteristics of products into account. The few results
334 available in descriptive profiles appear to confirm instrumental observations of volume and textural
335 properties. Indeed, Shogren *et al.* (2003) reported an increase in the perceived denseness and
336 firmness of breads when the proportion of soy flour was increased from 0% to 40%. Petitot, Boyer, *et*
337 *al.* (2010) and Rosa-Sibakov *et al.* (2016) also reported an increase in perceived hardness in pasta

338 containing 35% faba bean and split pea and 100% faba bean flour, respectively, in comparison with
339 wheat pasta. These descriptive profiles also made it possible to detect the emergence of specific
340 flavors or aftertastes, with the mention of a “beany” attribute. In the same study by Shogren *et al.*
341 (2003), the increase in the proportion of soy from 0% to 40% caused an increasing tendency to a
342 beany flavor as well as an increase in bitter flavor that is described as “mildly bitter aftertaste
343 associated with soy flour”. In pasta, Wojtowicz & Moscicki (2014) reported the “poor taste” related
344 to a beany flavor in pasta containing more than 20% of legume flour.

345 Acceptability tests have also been performed. The majority of these studies are based on
346 acceptability scores based on a scale of 0 to 10. The results show a general tendency to decreasing
347 values of acceptability of certain attributes with an increase in the amount of legume flour
348 incorporated. This may concern overall acceptability as well as general appearance, color or texture.
349 Specific attributes like taste, aroma, flavor and associated persistence are also concerned and linked
350 to unusual “beany” perceptions. Such studies usually investigate the maximum amount of legume
351 flour that can be used and still ensure a product is of acceptable sensory quality. To our knowledge,
352 no study has been conducted of the effect of the incorporation of legume flour in the acceptability of
353 snacks. The 15% and 30% enriched pasta were scored as equally acceptable as the durum control,
354 with no difference in color, flavor or overall acceptability (Wood, 2009). At higher levels of
355 enrichment, the overall acceptability of pasta decreased due to a beany flavor (Wójtowicz &
356 Mościcki, 2014), and to changes in major textural attributes, e.g. an increase in hardness, elasticity
357 and fracturability (by 60%, 30–40% and 170%, respectively, for 35% legume pasta (Petitot, Boyer, *et*
358 *al.*, 2010). However, the acceptability of pasta enriched with 70-100% legume flour was still
359 equivalent to that of their high fiber or gluten free counterparts. Indeed, acceptability of 70% faba
360 bean pasta was close to or better than whole wheat or gluten free pasta, respectively, and 100% faba
361 bean pasta tended to be better appreciated than commercial gluten free pasta (Laleg, Barron,
362 Cordelle, *et al.*, 2017). For biscuits, Noor Aziah, Mohamad Noor, & Ho (2012) reported an
363 improvement in flavor, after taste, crispness and overall acceptability with the addition of 35% of

364 mung bean or chickpea flour, but a decrease in aroma acceptability. Conversely, Jayasena and Nasar-
365 Abbas (2011) and Mc Watters (1978) reported a decrease in flavor, taste, crispness and overall
366 acceptability of biscuits with 20% and 50% of added lupin, soy or pea flours. In cakes, Gómez,
367 Doyagüe, & de la Hera (2012) reported a decrease in acceptability in the appearance, texture, odor,
368 taste, persistency and overall acceptability of cakes containing 35% of pea flour. However scores for
369 the enriched cake were higher than 5 (neither like nor dislike), indicating relatively good
370 acceptability. Finally, a decrease in the acceptability of breads containing more than 10% to 20% of
371 chickpea, soy, pea and lentil flour was observed (Alam, Talukder, Rahman, Prodhan, & Huq, 2013;
372 Mohammed *et al.*, 2012; Previtali *et al.*, 2014; Raidl & Klein, 1983).

373 ***Nutritional impact of the incorporation of legume flour***

374 The optimization of the composition of all cereal products by incorporating legumes results in
375 beneficial nutritional properties (table 2) starting with an increase in protein, fiber and mineral
376 content. The increase in protein content is associated with an improvement in the essential amino
377 acid score, notably that of lysine, which depends on the type or on the amount of legume
378 incorporated (Laleg, Barron, Cordelle, *et al.*, 2017; Sparvoli *et al.*, 2016). Even if lysine essential amino
379 acid is reduced by the Maillard reactions that take place during the thermal treatment, it is still
380 higher in legume enriched products than in wheat products (21 mg/g of protein in low temperature
381 wheat pasta and 33 mg/g of protein in very high temperature mixed faba bean pasta) (Laleg, Barron,
382 Santé-Lhoutellier, *et al.*, 2016). *In vitro* protein hydrolysis is also impacted; increases of 11% in 15%
383 green pea snacks (Patil, Brennan, Mason, & Brennan, 2016) and of 24% in 100% faba bean pasta
384 (Laleg, Barron, Cordelle, *et al.*, 2017) have been reported. In the case of pasta, this could be
385 explained by the molecular weakening of the protein network structure that facilitates accessibility
386 of proteolytic enzymes to specific protein cleavage sites (Laleg, Barron, Cordelle, *et al.*, 2017). Only a
387 few studies of the effect of legume enrichment on *in vivo* protein digestion of foods can be found in
388 the literature (Fleming & Sosulski, 1977b; Torres, Frias, Granito, Guerra, & Vidal-Valverde, 2007;

389 Torres, Frias, Granito, & Vidal-Valverde, 2007). No impact of the incorporation of legume flour on
390 protein true digestibility was reported in these studies that focused on pasta and bread. However
391 increases in the protein efficiency ratio (amount of weight gain per unit of protein consumed) have
392 been reported, from +53% for bread (Fleming & Sosulski, 1977b) to +64% to +86% for pasta (Torres,
393 Frias, Granito, Guerra, *et al.*, 2007; Torres, Frias, Granito, & Vidal-Valverde, 2007).

394 The digestive behavior of the starch fraction is more widely documented. Generally, the
395 incorporation of legume flours reduces the *in vitro* glycemic response in any product (Gularte,
396 Gómez, & Rosell, 2012; Laleg, Cassan, Barron, *et al.*, 2016; Sparvoli *et al.*, 2016). However, the same
397 difference measured *in vivo* is not statistically significant in many wheat products (Fujiwara, Hall, &
398 Jenkins, 2017; Greffeuille *et al.*, 2015). Only studies on pasta by Goñi and Valentín-Gamazo (2003)
399 and on bread by Zafar *et al.* (2015) reported a significant decrease in the *in vivo* glycemic index
400 (bread and pasta), although enriched products generally still have low *in vivo* glycemic and insulin
401 indexes (Greffeuille *et al.*, 2015).

402 Legume flour enrichment is also an effective way to provide micronutrients and bioactive
403 compounds. Some of these bioactive compounds, including protease inhibitors, phytic acid and α -
404 galactosides, are considered as “anti-nutritional factors” because of their ability to reduce protein
405 digestibility, nutrient absorption and to cause intestinal discomfort, respectively (Duranti, 2006; N.
406 Wang *et al.*, 2009). The amount of bioactive compounds in legumes can be significantly reduced by
407 preprocessing (soaking, fermentation and germination) or during standard processing. Indeed, the
408 inhibitory activity of trypsin is almost completely inactivated in cooked pasta. Total α -galactosides
409 (44 to 57 mg/g db, in lentil, faba and black gram flours) are also remarkably reduced (-41% to -71%)
410 (Laleg, Cassan, Barron, *et al.*, 2016). However, no effect of these processing steps has been reported
411 for phytic acid. Germinating *Cajanus cajan* seeds led to a 58% reduction in phytic acid (Torres, Frias,
412 Granito, & Vidal-Valverde, 2007). Finally, interest has recently focused on the nutritional function of
413 the bioactive compounds supplied by legumes as intestinal, metabolic or probiotic/prebiotic factors.

414 In conclusion, the incorporation of legume flour in cereal products has a positive impact on their
415 nutritional properties. The change in color does not appear to be a problem. The off-flavor reduces
416 the acceptability score, but could be limited by the use of flavoring agents. Finally the change in
417 texture, which is linked to the structure, needs to be controlled. A good knowledge of
418 transformations that occur during the process will help control the final structure and texture.

419 **From the raw materials to the end product: structure building by the action of the process**

420 *Simplified state diagram for protein and starch of legumes and cereal and process paths of five* 421 *main cereal products*

422 In the following review, we refer to a simplified state diagram summarizing the thermal transitions of
423 both wheat and legume components (Fig. 3a) to propose hypotheses concerning the effect of
424 incorporating legume flour on the structuring and the resulting structure of each type of product.
425 The four zones in the diagram correspond to different physical states of the material. Zones 1 and 2
426 refer to the glassy and rubbery states separated by the average glass transition lines.
427 The shift from the lower part of the diagram (zone 2) to the upper part (zones 3 and 4) corresponds
428 to the general softening of the material toward the liquid state that is related to the melting of starch
429 crystallites (Cuq et al., 2003), and may be emphasized by the presence of shear. Zones 3 and 4 differ
430 in the viscosity of the liquid as a function of the variation in water content. When thermosetting of
431 the proteins occurs, leading to the formation of a network, the product in the upper part of the state
432 diagram (zones 3 and 4) can be qualified as soft solid. It should be noted that dependence of
433 transition temperatures on water content is taken into consideration for each component separately
434 as if it had free access to total water in the product with no competition from other components or
435 ingredients. This is a simplified approach to the distribution of water in the product (Lazou & Krokida,
436 2011). Furthermore, the effect of sugar on the increase in the starch melting temperatures and the
437 decrease in the starch and protein glass transition temperatures has been well described (Roos &

438 Drusch, 2015). Such an effect should be taken into account in the case of biscuits and soft cakes that
439 contain 20% and 30% sugar wb, respectively (table 1).

440 **[FIGURE 3]**

441 **[TABLE 3]**

442 Table 3 compares the water content and temperature of the products during the course of
443 processing. Processing is summarized in four steps: raw material, mixing before thermal treatment,
444 hot product and end product. For each step, the link with the appropriate zone in the state diagram
445 (Fig. 3a) is defined based on water content and temperature. This comparison illustrates the wide
446 range of water contents (from 15% to 43% wb) of the flour or semolina during the processing of five
447 main cereal products, in the maximum product temperature (from 100 °C to 250 °C) and in the final
448 water content (from 1% to 69% wb, with a difference between the core and the surface of the
449 product, or not).

450 Based on these data, the path of each product in the simplified state diagram can be drawn (Fig. 3).
451 Each process leads to the formation of a final structure that is schematically represented in figure 4.
452 We now describe the path of each product to show how closely it is related to structure building
453 during the course of processing. The potential impact of incorporating legume flour, together with
454 the above mentioned implications are also discussed. In a material science approach, the changes in
455 the products depending on variations in temperature and moisture during processing are described
456 in the order of increasing final water content of the product.

457 **[FIGURE 4]**

458 ***The temperature-hydration history and its effect on the final structure of snacks***

459 In snacks (Fig. 3b) the initial semolina is rather poorly hydrated (15% wb). In the extruder barrel, it is
460 highly sheared (SME of 300-900 kJ/kg) resulting in a rapid and large increase in pressure and

461 temperature (Della Valle, Kozlowski, Colonna, & Tayeb, 1989). The product goes through all the
462 transition temperatures (T_g , T_m , T_d) leading to the formation of a very viscous liquid called “melt”
463 with the disruption of starch granules but also the depolymerization of proteins, amylose and
464 amylopectin (Colonna, Doublier, Melcion, De Monredon, & Mercier, 1984). The melt is pushed
465 through the extruder die (zone 4 on Fig. 3b) and returns to room pressure while still very hot. Flash
466 vaporization of water occurs accompanied by rapid expansion of the product corresponding to the
467 formation of numerous large bubbles (Kokini, Chang, & Lai, 1992). Melt viscosity is a key parameter
468 in snack expansion and is influenced by the size and structure of starch and protein macromolecules,
469 even if these are depolymerized. In the case of legume enriched melts, viscosity could be reduced by
470 the lower molecular mass of legume proteins, and by the higher amylose/amylopectin ratio of
471 legume starches, even though the impact of the latter factor is still the subject of debate in the
472 literature (Moraru & Kokini, 2003). A lower melt viscosity could increase the expansion of the matrix.
473 Upon bubble growth, stress is exerted on the cell walls that open and holes appear (Fig. 4).
474 Moreover, depending on their resistance to expansion stress, some walls may completely disappear.
475 Water vapor evacuates thereby accelerating cooling and causing the stiffening of the walls as
476 proteins and starch return to a glassy state (Kokini *et al.*, 1992) (Fig. 3b). Glass transition should occur
477 at a sufficiently high temperature to prevent the bubbles collapse. Because legume enriched snacks
478 contain significantly more proteins than 100% wheat snacks, one can hypothesize that the glass
479 transition temperature of the mixed protein + starch matrix would decrease towards the T_g of
480 proteins (Fig. 1). This would delay stiffening after the opening of the structure, resulting in shrinking
481 of the snack and consequently a lower final volume. In this case, the final water content of the snack
482 is low (1-5% wb); its texture is crispy. At macroscopic scale, the snack is an open solid foam with large
483 interconnected cells (Fig. 4). At microscopic scale, the cell walls are composed of a continuous phase
484 made of a mix of proteins, amylose and amylopectin in the amorphous state.

485 ***The temperature-hydration history and its effect on the final structure of biscuits***

486 In biscuits, the dough is poorly hydrated at the mixing step (16% wb). The presence of sugar in the
487 dough reduces the glass transition temperatures of both proteins and starch (Roos & Drusch, 2015).
488 Thus, at the mixing step, proteins are nearly able to undergo glass transition whereas starch is not
489 (Fig. 3c). Biscuit dough can be qualified as a homogeneous mix of swollen flour particles and fat
490 globules in an aqueous phase with high concentrations of sugar (Chevallier, Colonna, Buléon, & Della
491 Valle, 2000). During baking, two paths can be distinguished depending on the changes in
492 temperature. The surface of the product rapidly reaches high temperatures (~200 °C) that cause glass
493 transition in proteins and in starch with no denaturation or gelatinization phenomena because of the
494 limited water content. The core of the product reaches a lower temperature (~125 °C) that only
495 allows glass transition of proteins. Given that biscuits are not subject to either protein thermosetting
496 or to starch gelatinization (Chevallier, Colonna, Della Valle, & Lourdin, 2000), replacing wheat flour
497 with legume flour would be expected to have little impact on structuring. During baking, the reaction
498 of raising agents can lead to the formation of small air bubbles. After baking, the surface and core of
499 the product return to the glassy state. The product has low water content (1-5% wb) and a crunchy
500 texture. At macroscopic scale, the biscuit is a dense matrix that is homogeneous between the core
501 and the surface. At microscopic scale, the matrix contains unpasted starch granules, fat globules, and
502 small air cells entrapped in a continuous, highly concentrated phase of sugar and proteins (Fig. 4).

503 ***The temperature-hydration history and its effect on the final structure of pasta***

504 In pasta (Fig. 3d), the combination of 32% hydration and low shear stress during the mixing step
505 transform the semolina into a sandy dough, in which both starch and proteins are in the rubbery
506 state and no gluten network is detectable (Petitot, Abecassis, & Micard, 2009) (Fig. 3d). The extrusion
507 step (SME from 70-120 kJ/kg and pressure up to 17.6 MPa in a single screw extruder) forms a dough
508 with a partial gluten network and sufficient compactness to survive cooking. Several thermal
509 treatments are possible to dry the pasta (Fig. 3d). A low temperature treatment (40-60 °C/70-80%

510 relative humidity/18-28 h) returns the protein and starch to the glassy state without causing protein
511 thermosetting. Conversely, a high temperature treatment (> 84 °C/74-90% relative humidity/2-5 h)
512 allows gluten proteins to undergo thermosetting before reaching the glassy state. In a 0% to 100%
513 legume-enriched pasta dried at low temperature, the percentage of proteins linked by weak bonds
514 (hydrogen, hydrophobic) and covalent bonds (notably by disulfide bonds) linearly increased and
515 decreased, respectively (from 70% to 94% and 30% to 5% respectively in faba bean-wheat pasta)
516 inherent to the smaller quantity of cysteine residues in legume proteins (Laleg, Barron, Cordelle, *et*
517 *al.*, 2017). When dried at high temperature, these differences between legume enriched and wheat
518 pasta due to composition persisted. In addition, the proportions of covalently linked proteins in all
519 the pasta increased when the drying temperature reached or was above the Td of wheat protein, and
520 higher than the higher Td of legume protein. Only partial gelatinization of starch (20%) may occur
521 (Güler, Köksel, & Ng, 2002) (Fig. 3d). The complete gelatinization of starch and protein thermosetting
522 will occur during the final cooking step (mentioned in table 3 but not in Fig. 3d) in boiling water, and
523 at higher temperatures when legume flour is incorporated. Ready to eat pasta is soft and viscoelastic.
524 Its microscopic structure (Fig. 4) is characterized by swollen starch granules held in a compact and
525 poorly porous protein network.

526 ***The temperature-hydration history and its effect on the final structure of soft cakes***

527 In soft cakes, at the mixing step, the batter is relatively highly hydrated (30% wb), which allows
528 proteins and starch to go into the rubbery state (Fig. 3e). Due to the low proportion of flour in the
529 formula (table 1) and to the design of the mixer, no gluten network is formed (Dewaest, Villemejeane,
530 Berland, Michon, *et al.*, 2017). Nevertheless, mixing incorporates air in the form of small bubbles that
531 are stabilized in the batter thanks to surface active compounds (Sahi & Alava, 2003). The paths at the
532 surface of the product and in the core differ during baking. The surface dries rapidly, leading to the
533 formation of the crust (Fig. 3e) that limits water transfers between the core and the oven
534 atmosphere (Lostie, Peczalski, Andrieu, & Laurent, 2002). The surface temperature reaches high
535 temperatures (180-210 °C) and the proteins thermoset but the starch granules do not gelatinize (Fig.

536 3e). The water content of the product core remains constant while the temperature slowly increases
537 up to 100 °C. Bubbles grow thanks to water vaporization, carbon dioxide formation, and gas
538 dilatation. Like for snacks, bubble growth is associated with structure opening due to the formation
539 of holes in the cell walls and to the disappearance of the cell walls (Dewaest, VILLEMEJANE, BERLAND,
540 Neron, *et al.*, 2017; Lostie *et al.* (2002)) (Fig. 4). However, no effect on expansion has been proven, as
541 opening occurs when temperature and expansion pressure are still high. Cake expansion is limited by
542 the formation of the crust and further stopped by protein thermosetting and starch gelatinization in
543 the core that set the structure (Fig. 3e). In cakes, pasted starch granules called “bricks” are
544 considered to be the first structuring element of the cell walls, with denatured proteins forming the
545 “mortar” between the bricks (Donovan, 1977) (Fig. 4). In legume-enriched cakes, starch content is
546 lower which means the proportion of structuring elements in the cell walls is lower. There is also an
547 increase in the thermosetting temperature that could delay the increase in viscosity and
548 consequently promote cake expansion before setting. The result depends on the balance between
549 expansion and stiffening kinetics. After baking, the product surface (crust) changes to the glassy state
550 while the core (crumb) remains at the rubbery state. Usually in industry, the product is rapidly placed
551 in hermetic packaging, which leads to water redistribution within the product (last arrows in Fig. 3e).
552 At macroscopic scale, the crumb can be qualified as a soft open solid foam made of small
553 interconnected cells with holes in their walls (Dewaest, VILLEMEJANE, BERLAND, Neron, *et al.*, 2017)
554 (Fig. 4).

555 ***The temperature-hydration history and its effect on the final structure of bread***

556 In bread, the phenomena are the same as in cakes with two paths (crust and crumb) in the state
557 diagram. However they differ in two important points. First, thanks to the supply of mechanical
558 energy (10-30 kJ/kg) and to the design of the mixer, gluten proteins are able to form a 3D network
559 that gives the dough higher elastic properties (Feillet, 2000). When legume flour is incorporated, the
560 presence of lower molecular mass proteins that are unable to form a network by themselves and
561 might thus affect structuring (Boye, Zare, *et al.*, 2010). Second, during the fermentation step, the

562 bubbles start increasing in size at room temperature because yeast produces carbon dioxide. During
563 baking, the bubbles continue to expand thanks to water and carbon dioxide vaporization and gas
564 dilatation. Holes appear in the cell walls but in bread, the cell walls are more resistant to deformation
565 than in cake thanks to their viscoelastic properties (Fig. 4). Loaf expansion is stopped by the
566 formation of a crust that is more rigid than that of cake, and by the setting of the core (Fig. 3f). The
567 crust goes into the glassy state and becomes brittle when it returns to room temperature. The core
568 remains in a rubbery state and becomes a soft viscoelastic crumb with relatively high water content
569 (40-50% wb). At macroscopic scale, the crumb can be qualified as a soft open solid foam, made of
570 rather large cells with holes in their walls (Fig. 4). At microscopic scale, the cell walls are made of
571 pasted starch granules and a denatured gluten network that may be diluted by legume proteins
572 (Fleming & Sosulski, 1978; Mohammed *et al.*, 2012).

573 In conclusion, this material science generic approach allows the formulation of hypotheses
574 concerning the effects of incorporating legume flour on the structure of cereal products. However,
575 analyzing such effects is no trivial task, due to the combination of various structuring phenomena at
576 different length scales. To illustrate this complexity, in the following section, we include a
577 supplementary analysis of the incorporation of legume flour in order to describe the differences in
578 behavior we observed during processing, to discuss the validity of some of the previously proposed
579 hypotheses, and to propose changes in the process to insure the quality of such products. It aims at
580 giving an additional highlight to the generic approach developed so far, for two of the five cereal
581 products.

582 **Incorporating legume flour in wheat foods requires modifying food processing: examples**
583 **of low hydrated (pasta) and hydrated (cakes) products**

584 The examples below illustrate the effects of product-process interactions on the structure of pasta
585 and cake during dough formation and thermal treatment at different length scales.

586 ***During dough formation***

587 Legumes incorporated in the pasta dough act as a diluting agent for the gluten network and slightly
588 weakened the protein interactions (from 11% in wheat to 7% in 35% legume enriched pasta) at
589 molecular scale (Petitot, Barron, *et al.*, 2010). At macroscopic scale, a formation of large sticky
590 heterogeneous lumps of dough (particle size > 1-4 mm) was observed upon hydration and mixing,
591 rendering the extrusion step difficult especially when the proportion of legume flour was greater
592 than 35% (Petitot, Boyer, *et al.*, 2010; Wood, 2009). This can be attributed to the difference in
593 particle size of legume flour and semolina that can affect their hydration properties and increase
594 dough heterogeneity. Moreover, the higher proportion of protein and enzyme in legume flour can
595 modify its granulation properties (Laleg, Cassan, Abecassis, & Micard, 2016). An increase in mixing
596 speed from 60 to 120 rpm and a decrease in dough hydration from 47% db in wheat pasta to 45-40%
597 db in pasta containing at least 35% of legume have been suggested as a way of limiting the formation
598 of aggregates and lumpy dough (Petitot, Boyer, *et al.*, 2010). New alternatives are proposed under
599 patent WO 2016097328 A1 (Laleg, Cassan, Abecassis, *et al.*, 2016).

600 In the processing of industrial soft cakes (e.g. layer cakes or pound cakes), batter can be formed by
601 multi-stage mixing of dry ingredients (flour, sugar, emulsifiers) with egg and water, and with fat in
602 the form of liquid oil. During the first step, the flour is hydrated and a foam is created. At
603 macroscopic scale, this leads to the formation of a continuous phase of hydrated proteins in which
604 air bubbles, fat droplets, and starch granules are dispersed. Proteins and starch undergo glass
605 transition. At this level, the ability of wheat or legume proteins to solvate or hydrate determines
606 batter viscosity, which is a fundamental criterion for cake expansion (Sahi & Alava, 2003). Concerning

607 foaming at mesoscopic scale, the presence of 20% to 40% of pea flour in a mix with wheat flour
608 reduced batter density (-12% and -16% of batter density respectively compared to 100% wheat
609 batter in a pound cake recipe, Monnet *et al.*, unpublished results). The better interfacial properties of
610 legume proteins enabled more efficient stabilization of the bubbles than wheat proteins. One way to
611 incorporate the same amount of air at this step would be to reduce the duration of mixing when pea
612 flour is used. During the second step of mixing, an oil-in-water emulsion is formed. Usually a partial
613 loss of air can be observed at this step due to the antifoaming properties of oil. After oil is
614 incorporated, the batter densities are equivalent (-1% or -2% with respectively, 20% or 40% of pea
615 flour). Thus, the higher surface activity of pea proteins does not seem to prevent the antifoaming
616 action of oil. However, in layer cakes made with a single-stage mixing method, Gómez *et al.* (2008,
617 2012) and De la Hera *et al.* (2012) reported significant decreases in batter density with the
618 incorporation of 50% to 100% of chickpea, pea or lentil flour (-3% to -10% of density depending on
619 the proportion, the cultivar and particle size of the legume concerned). Beyond the quantity of air at
620 macroscopic scale, incorporating legume flour may affect the size and distribution of air bubbles in
621 the batter. Gómez *et al.* (2012) showed that the distribution of air in batters containing 50% or 100%
622 of pea flour was more stable with smaller bubbles. In turn, this could impact cell structure in the cake
623 and hence its global structural and textural properties.

624 ***During thermal treatment***

625 In standard processing of wheat pasta, drying promotes the aggregation of protein polymers through
626 disulfide bonds, leading to the formation of larger protein aggregates (Petitot, Barron, *et al.*, 2010).
627 The extent of protein aggregation depends on the drying temperature (55 °C vs. 90 °C). At a low
628 drying temperature (55 °C), as there is no thermosetting of either wheat or legume proteins,
629 incorporating legume flour has no other impact than gluten dilution. The low drying program does
630 not provide the right conditions for legume proteins to interact with each other covalently or with
631 gluten via disulfide bonds. At a higher drying temperature (90 °C) additional covalent linkages (mainly
632 disulfide, isopeptide or Maillard products) involving both wheat and legume proteins can be created

633 (Laleg, Barron, Cordelle, *et al.*, 2017). A proportion of between 35% and 100% faba bean pasta dried
634 at a very high temperature was subject to fewer covalent protein interactions than wheat pasta.
635 However, the increase in covalently linked proteins at very high temperature versus low temperature
636 drying conditions was greater when the amount of faba bean proteins was increased from 0% to
637 100%, showing that very high drying temperature enables additional reactivity of faba proteins.

638 Cooking results in extensive protein aggregation through covalent bonds in both wheat and in
639 legume enriched pasta. This reduces the difference in network structuring between pasta with and
640 without legume flour even if one difference can still be observed: a significant increase in losses
641 during cooking, depending on the proportion of legume flour added, has been reported for pasta
642 enriched with different types of legumes (lentil, pea, black gram, white bean and faba bean) (Laleg,
643 Barron, Cordelle, *et al.*, 2017; Laleg, Cassan, Barron, *et al.*, 2016; Rosa-Sibakov *et al.*, 2016; Wójtowicz
644 & Mościcki, 2014). The losses were linked to several phenomena, including the weaker final protein
645 structure due to the reduced capacity of legume proteins to covalently interlink, to the disruption of
646 gluten matrix by the higher quantity of fiber provided by legume flour (Petitot, Barron, *et al.*, 2010),
647 and to the increased proportions of salt-soluble proteins resulting from the incorporation of legume
648 flour (Petitot, Boyer, *et al.*, 2010). Drying pasta at a high temperature can help counteract cooking
649 losses notably by increasing the proportion of proteins linked by covalent linkages in the pasta
650 protein network (Laleg, Barron, Cordelle, *et al.*, 2017).

651 When soft cakes are baked, wheat glutenins and gliadins bind to each other through disulfide bonds
652 and egg proteins do the same separately. It is only at the very end of baking that both kinds of
653 proteins interact through disulfide bonds (Dewaest, Villemejeane, Berland, Michon, *et al.*, 2017). This
654 interaction reduces the proportion of non-extractible proteins due to steric hindrance (Dewaest,
655 Villemejeane, Berland, Michon, *et al.*, 2017). When 20% to 40% of pea flour was incorporated in a
656 pound cake recipe, an almost linear increase in the proportion of proteins linked by weak
657 interactions was observed (+ 43% and + 81% respectively, Monnet *et al.*, unpublished results). This

658 increase was offset by a decrease in the proportion of covalently linked proteins (-7% and -13%,
659 respectively) however, with little variation in the proportion of proteins linked by disulfide bonds (-
660 3% and +1%). This is inherent to the structure of legume proteins. Thus, introducing legume proteins
661 may weaken the force of interaction between proteins, especially their capacity to strongly bind to
662 wheat and egg proteins. At macroscopic scale, incorporating pea flour increased the density of the
663 cake (+3% for 20% or 40% compared to the control). At microscopic scale, the distribution of air
664 among the cell walls appeared to be more homogeneous, and the cell walls appeared to be thicker.
665 This suggests later and/or less efficient stiffening of the structure (due to a delay in thermosetting
666 and/or reduced starch content) that may have caused shrinking of the cells and thickening of the cell
667 walls during baking.

668 These two examples show how, by changing the nature, proportion, and functionalities of the
669 components (especially the binding abilities of proteins), incorporating legume flour can result in
670 differences in the structure building process, thereby elucidating the product-process interactions
671 that occur from the raw material to the end product.

672

673 **Conclusion**

674 Mixing legume and cereal flour leads to process different nature and amounts of their two main
675 components, starch and proteins. Even though our approach, which consists in considering their
676 distinct thermal transitions independently (one from another, and from other components and
677 ingredients) may limit our overall understanding, the theoretical differences in their transition
678 temperatures enabled us to propose preliminary hypotheses concerning the multi-scale structure
679 building during baking or drying in connection with expansion properties in the case of aerated
680 products. Comparing these hypotheses with results in the literature and with our own experience
681 provided information on the effects of incorporating legume flour in standard cereal products at
682 macroscopic, mesoscopic and macromolecular scales. Yet it is clear that establishing relationships
683 between phenomena at different length scales is far from simple. Considering the information
684 collected at different length scales, it can be assumed that incorporating legume flour has a
685 significant effect on product structuring during processing. Knowledge about how to adapt process
686 parameters to counteract such effects is already partially available. For snacks, barrel temperature,
687 screw speed, feed rate and moisture content can be adjusted to modulate melt viscosity and
688 expansion with respect to stiffening. For pasta, an increase in mixing speed and a decrease in
689 hydration of the dough are recommended to limit the formation of lumps, and more drastic drying
690 conditions to compensate for the weakening of protein interactions and the dilution of the gluten
691 network. For cake, adjustments in mixing speed or in the order of incorporating the ingredients
692 during batter formation could make it possible to adjust the amount and the distribution of air, and
693 improvements could be made in structure setting by changing baking parameters (temperature, air
694 speed). Finally for bread, dilution of the gluten network could be counteracted by sequential mixing
695 to enable the formation of the protein network before the legume flour is added. The use of a
696 structuring agent, such as a gum or animal proteins (ovalbumins) to imitate gluten structure is a
697 possible alternative. In this way, wheat and legume flours could be successfully combined in a wide
698 variety of cereal products. Aside from structural aspects, the nutritional quality of legume-enriched

699 products is significantly improved. Adverse sensory effects are still an obstacle to the wider use of
700 these products, but it is believed that such effects could be counteracted by proper aromatic
701 formulation, and should thus not be considered as limiting. Finally, we propose an approach that
702 could help predict the effect of replacing wheat flour with legume flour and make it possible to
703 control the structure and texture of the product at each stage of processing, depending on the path
704 taken by the components in the state diagram. This approach should be completed by studies of
705 thermal transitions in real formula conditions, as well as by detailed studies on the structural changes
706 that occur at microscopic and macromolecular length scales in all cereal products.

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1031 **FIGURE CAPTIONS**

1032 **Fig. 1** Summary of data on glass transition temperatures (Tg) and denaturation temperatures (Td) of wheat and
1033 legume proteins from different sources in the literature.

1034 * Tg range of wheat gluten adapted from Cuq *et al.* (2003)

1035 ** Average Td value for legume proteins calculated from data from five studies on protein isolates or 7S and
1036 11S globulins between 80% and 94% water (wb), for 11 different legume species and 3 heating rates (2, 5 and
1037 10 °C/min). The error bars show the standard deviation associated with the calculation ($T_d = 88\text{ °C} \pm 7\text{ °C}$).

1038 Sources: Kitabatake, Tahara, & Doi (1990); L'hocine, Boye, & Arcand (2006); Shevkani, Singh, Kaur, & Rana
1039 (2015); Sosulski, Hoover, Tyler, Murray, & Arntfield (1985); Withana-Gamage *et al.* (2011).

1040 *** Extrapolation of Td ranges for wheat and legume proteins at high water contents (40-100% water wb)

1041 *All temperatures correspond to peak temperatures of endotherms measured by differential scanning*
1042 *calorimetry.*

1043 **Fig. 2** Summary of data on glass transition temperatures (Tg) and melting temperatures (Tm) of wheat and
1044 legume starches from different sources in the literature.

1045 * Tg range of glass transition temperatures in wheat starch, adapted from Cuq *et al.* (2003)

1046 ** Ranges of Tm1, Tm2, Tm3 in wheat starch, adapted from Vanin *et al.* (2009)

1047 *** Average Tm1 value for legume starches calculated from data from four studies between 70% and 82%
1048 water on a wet basis (wb), for 11 different legume species and 1 heating rate (10 °C/min). The error bars show
1049 the standard deviation associated with the calculation ($T_m = 70\text{ °C} \pm 5\text{ °C}$). Sources: Chung, Liu, Hoover,

1050 Warkentin, & Vandenberg (2008); Hoover and Ratnayake (2002); Singh, Sandhu, Kaur, Singh Sandhu, & Kaur
1051 (2004); Zhou, Hoover, & Liu (2004).

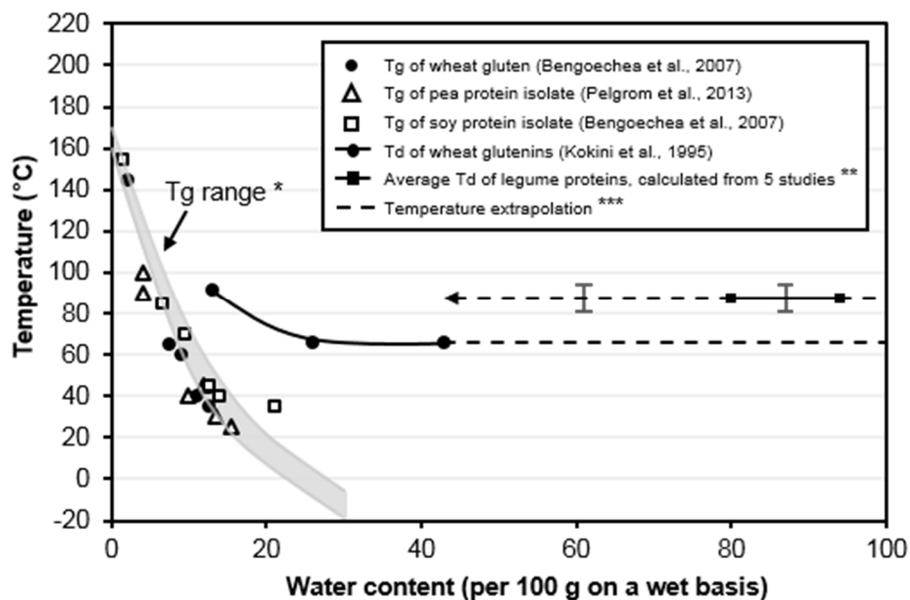
1052 *All temperatures correspond to peak temperatures of endotherms measured by differential scanning*
1053 *calorimetry.*

1054 **Fig. 3** Simplified state diagram for starch and proteins with four zones (a) and paths of the products: snacks (b),
1055 biscuits (d), pasta (d), soft cakes (e), bread (f).

1056 *Standard deviation bars in (a) show the ranges of variation of transition temperatures shown in figures 1 and 2.*

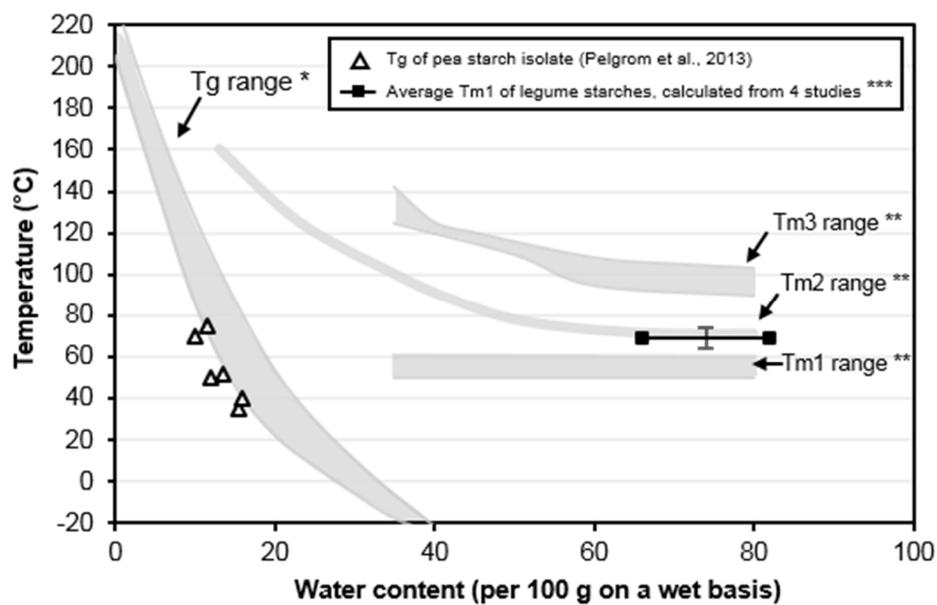
1057 **Fig. 4** Schematic representation of all structures at two length scales. Distribution of the products according to
1058 water content and type of structure (presence of air in the form of cells interconnected to varying degrees, or
1059 absence of air).
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1061 **FIGURE 1**



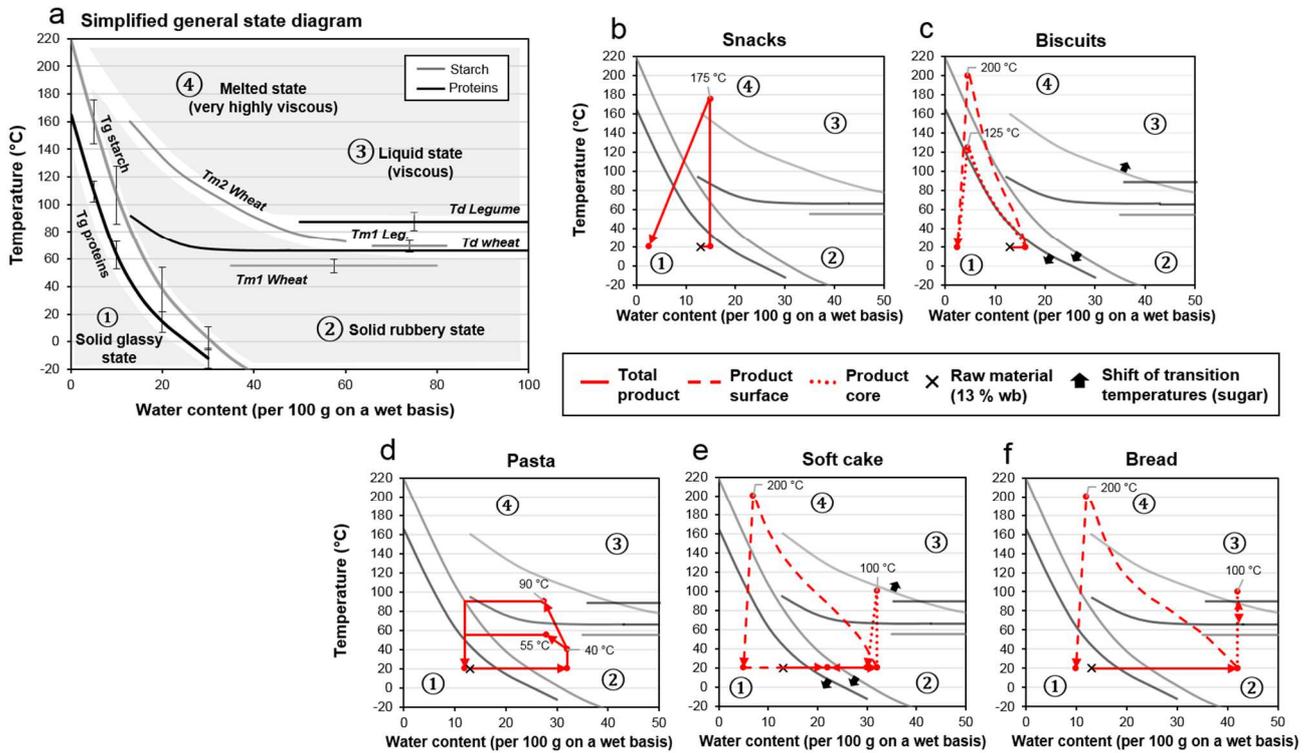
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1063 **FIGURE 2**



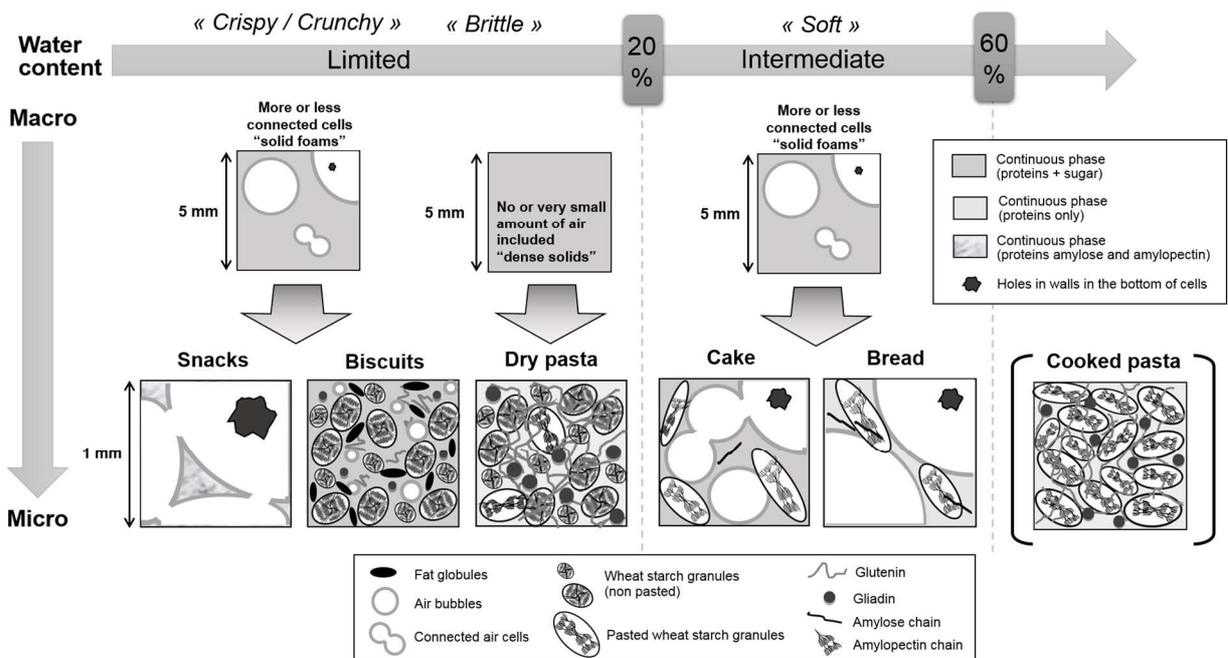
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1065 **FIGURE 3**



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1067 **FIGURE 4**



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Table 1. Main characteristics of wheat product formulas before processing						
		Snacks ^a	Pasta ^b	Bread ^c	Biscuits ^d	Soft cakes ^e
Major ingredients (g/ 100 g of total formula)	Wheat flour or semolina	97	78	60	50	29
	Sugar	-	-	*	20	30
	Fat	-	-	*	20	9
	Egg	-	-	-	-	15
	Water	3	22	35	10	17
Other ingredients				Yeast, Salt	Raising agents, Emulsifiers*,Salt	
Components (g/ 100 g of total formula)	Total water	15	32	43	16	32
	Starch	61	49	38	31	18
	Proteins	11	9	7	6	3 (wheat) 2 (eggs)
	Lipids	1	1	1	21	11
	Total dietary fibers	2	2	2	1	1
^a Kaletunç and Breslauer (1996) ^b Petitot, Brossard, <i>et al.</i> (2009) ^c Alam <i>et al.</i> (2013); Heenan, Hamid, Dufour, Harvey, & Delahunty (2009); Shogren <i>et al.</i> (2003) ^d Short type; Heenan <i>et al.</i> (2009); Rababah, Al-Mahasneh, & Ereifej (2006); Zucco, Borsuk, & Arntfield (2011) ^e Layer type; De la Hera <i>et al.</i> (2012); Gularte <i>et al.</i> (2012) * Optional ingredients <i>Composition of the wheat flour chosen for calculations: 11.5% proteins, 63% starch, 2% fibers, 1.5% lipids, 13% water (USDA, 2016).</i>						

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Table 2. Impact of replacing cereals with different legumes on textural & structural, color, sensory and nutritional properties of the food products

Legume-enriched food	% Legume flour	Food properties			
		Texture & Structure	Color	Sensory attributes	Composition & Nutrition
Snacks^a	0-90%	<ul style="list-style-type: none"> - ↓ expansion ratio - ↑ density and porosity - ↑ breaking strength index (N/mm²) and stiffness (Young's modulus) - ↓ crispness (distance of travel to F max during compression and percent deformation) - Scanning electron microscopy: ↓ number of cells, thickening of cells and cell walls 	<ul style="list-style-type: none"> - ↓ L - ↑ a* and b* 	<ul style="list-style-type: none"> - ↓ crunchiness 	<ul style="list-style-type: none"> - ↑ protein content (up to 12 fold) - ↑ antioxidant activity - ↑ phytic acid and trypsin inhibitors - ↑ <i>in-vitro</i> protein hydrolysis
Cooked pasta^b	0-100%	<ul style="list-style-type: none"> - ↓ breaking energy, cohesiveness and resilience - ↑ stickiness - ↑ firmness and chewiness - Confocal laser scanning microscopy: no change in protein network at 35% - Brightfield microscopy: denser protein film, at 100% 	<ul style="list-style-type: none"> - ↓ L - = a* and b* 	<ul style="list-style-type: none"> - ↓ general appearance - ↑ fracturability, hardness and stickiness, and ↓ in bulkiness, elasticity, surface smoothness - ↑ adhesiveness and grittiness - ↓ taste acceptability - ↑ intensity of "beany" flavor - ↓ color acceptability - ↓ overall acceptability 	<ul style="list-style-type: none"> - ↑ protein content - ↑ amino acid score - = or ↓ glycemic index - = or ↓ rapidly available glucose - ↑ <i>in-vitro</i> protein hydrolysis - ↑ <i>in-vivo</i> protein digestibility and efficiency
Bread^c	0-40%	<ul style="list-style-type: none"> - ↓ volume - ↑ density (↓ specific volume) - ↑ firmness or hardness - Scanning electron microscopy: disruption of protein-starch complex, thickening of cell walls, presence of holes in the walls 	<ul style="list-style-type: none"> - Crumb and crust: ↓ L, ↑ a* and b* 	<ul style="list-style-type: none"> - ↓ overall appearance acceptability - ↑ crumb denseness - ↑ size of the cells - ↑ crumb hardness, firmness - ↑ crust color - ↑ flavor intensity (e.g. "beany") - ↓ taste and odor acceptability - ↓ overall acceptability 	<ul style="list-style-type: none"> - ↑ protein, fiber and mineral contents - ↑ amino acid score - ↓ blood glucose response (glycemic index) - ↓ satiety - Rat growth assay : ↑ feed consumption, weight gain, net protein ratio and protein efficiency ratio
Cake^d	0-100%	<ul style="list-style-type: none"> - ↓ volume - ↑ density (↓ specific volume) - ↓ symmetry - ↑ firmness or hardness (TPA) 	<ul style="list-style-type: none"> - Crust : ↓ L - Crumb : ↓ L, ↑ a* and b* 	<ul style="list-style-type: none"> - ↓ general appearance - ↓ flavor, aroma, persistency acceptability - ↓ texture acceptability - ↓ overall acceptability 	<ul style="list-style-type: none"> - ↑ total and available protein, mineral and fiber contents - ↓ rapidly available glucose
Biscuits^e	0-100%	<ul style="list-style-type: none"> - Diameter, thickness, spread ratio: variable effect depending on type and % of legume incorporated - ↑ hardness (F max) and fracturability 	<ul style="list-style-type: none"> - ↓ L and ↑ b* above 20% - ↓ a* 	<ul style="list-style-type: none"> - ↑ color acceptability - flavor, aroma, overall acceptability: variable effect depending on type and % of legume incorporated - ↑ aftertaste (e.g. "beany") 	<ul style="list-style-type: none"> - ↑ protein, fiber and mineral contents - ↑ amino acid score - ↑ antioxidant activity - ↓ predicted glycemic index

^a Anton *et al.* (2009); Lazou *et al.* (2007); Lazou and Krokida (2010); Patil *et al.* (2016)

^b Goñi and Valentín-Gamazo (2003); Greffeuille *et al.* (2015); Laleg, Barron, Cordelle, *et al.* (2017); Laleg, Barron, Santé-Lhoutellier, *et al.* (2016); Laleg, Cassan, Barron, *et al.* (2016); Rosa-Sibakov *et al.* (2016); Wójtowicz and Mościcki (2014); Wood (2009)

^c Alam *et al.* (2013); Fleming & Sosulski (1978, 1977a, 1977b); Mohammed *et al.* (2012); Previtali *et al.* (2014); Raidl and Klein (1983); Shogren *et al.* (2003); Villarino *et al.* (2015); Zafar *et al.* (2015)

^d Campbell, Euston, & Ahmed (2016); De la Hera *et al.* (2012); Gómez *et al.* (2008); Gómez *et al.* (2012); Gularte *et al.* (2012)

^e Jayasena and Nasar-Abbas (2011); Mcwatters (1978); Noor Aziah *et al.* (2012); Patel and Rao (1995); Sparvoli *et al.* (2016); Zucco *et al.* (2011)

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Table 3. Changes in water content and in the temperature of the five products and the corresponding zone in the state diagram (Fig. 3)

	Snacks ^a	Biscuits ^b	Pasta ^c	Soft cakes ^d	Bread ^e
Flour or Semolina	① 13% wb Room temp.	① 13% wb Room temp.	① 13% wb Room temp.	① 13% wb Room temp.	① 13% wb Room temp.
After mixing/kneading or during extrusion: Dough, Batter or Mix	② 15% wb Room temp. 300-900 kJ/kg	① 16% wb Room temp. 5-10 kJ/kg	② 32% wb Mixing: Room temp. Extrusion: 40 °C single screw 70-120 kJ/kg twin-screw 900 kJ/kg	② 32% wb Room temp.	② 43% wb Room temp. 10-30 kJ/kg
During baking or drying : Hot product	④ Surface & core: 150-200 °C at the die	② Surface: 180-220 °C Core: 100-150 °C	② ③ [Drying] Surface & core: 55-90 °C	② Surface: 180-210 °C Core: 100 °C	④ ③ Surface: 200-250 °C Core: 90-100 °C
			③ [Cooking] 100 °C		
After cooling and equilibration: End product	① 1-5% wb Room temp.	① 1-5% wb Room temp.	① [Dried] 12% wb Room temp.	① 22% wb Room temp.	① ② Crust: 5-15% wb Crumb: 40-50% wb Room temp.
			② [Cooked] 69% wb Consumption temp. (~ 50 °C)		

^a Della Valle *et al.* (1989); Kaletunç and Breslauer (1996)

^b Feillet (2000); Wade and Davis (1964)

^c Abecassis, Abbou, Chaurand, & Morel (1994); Petitot, Brossard, *et al.* (2009)

^d De la Hera *et al.* (2012); Dewaest, Villemejeane, Berland, Michon, *et al.* (2017); Lostie *et al.* (2002)

^e Cuq *et al.* (2003); Feillet (2000); Vanin *et al.* (2009)

In some cases, the specific mechanical energy (SME) (kJ/kg) is given.

A standard moisture loss of 2% was used for the cooling step based on data on bread from Feillet (2000). Moisture loss during snack cooling was 12.5%. Moisture loss during pasta cooling and bread/cake crumbs during baking were considered negligible. Moisture loss during baking or drying in all other cases was calculated to obtain a water content after cooling of + 2%.