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Karima Laleg, Cécile Barron, Sylvie Cordelle, Pascal Schlich, Stéphane Walrand, et al.. How the structure, nutritional and sensory attributes of pasta made from legume flour is affected by the proportion of legume protein. *LWT - Food Science and Technology*, 2017, 79, pp.471-478. 10.1016/j.lwt.2017.01.069 . hal-01517226

HAL Id: hal-01517226

<https://hal.science/hal-01517226v1>

Submitted on 26 Sep 2017

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Accepted Manuscript

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PII: S0023-6438(17)30081-6

DOI: [10.1016/j.lwt.2017.01.069](https://doi.org/10.1016/j.lwt.2017.01.069)

Reference: YFSTL 6021

To appear in: *LWT - Food Science and Technology*

Received Date: 10 October 2016

Revised Date: 23 January 2017

Accepted Date: 25 January 2017

Please cite this article as: Laleg, K., Barron, C., Cordelle, S., Schlich, P., Walrand, S., Micard, V., How the structure, nutritional and sensory attributes of pasta made from legume flour is affected by the proportion of legume protein, *LWT - Food Science and Technology* (2017), doi: 10.1016/j.lwt.2017.01.069.

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Comment citer ce document :

Laleg, K., Barron, C., Cordelle, S., Schlich, P., Walrand, S., Micard, V. (Auteur de correspondance) (2017). How the structure, nutritional and sensory attributes of pasta made from legume flour is affected by the proportion of legume protein. *LWT - Food Science and Technology*, 79, 471-478. DOI : 10.1016/j.lwt.2017.01.069

1 **How the structure, nutritional and sensory attributes of pasta made from legume flour is**
2 **affected by the proportion of legume protein**

3
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19 Abstract

20 In this study, wheat in pasta was partially or completely replaced by faba to increase its
21 protein quantity and improve its quality. Increasing the ratio of faba:wheat protein from 0:100
22 to 100:0 (g/g) in pasta enlarged its protein network at the microscopic scale and linearly
23 diluted the covalently linked gluten network of wheat pasta by weakly linked proteins. A
24 concomitant linear increase in the cooking loss (up to 2.6 fold), a decrease in resilience (up to
25 1.4 fold) and an increase of the *in-vitro* protein digestion (up to 25%) were observed in pasta.
26 The increase in drying temperature (90°C vs. 55°C) promoted the covalent aggregation of
27 proteins in all pasta, but was more efficient in legume pasta, enhancing their resilience and
28 reducing their cooking loss, without altering the degree of protein hydrolysis. This was partly
29 explained by the reduction in trypsin inhibitory activity in all legume pasta dried at 90°C.
30 Interestingly, scores for sensory attributes such as liking attributed to pasta containing 80%
31 faba-protein were close to scores given to a commercial whole wheat pasta. Pasta made
32 exclusively from faba dried at 55°C or 90°C tended to be liked more than their commercial
33 gluten-free counterparts.

34 Key words

35 Protein network structure, cooking quality, *in-vitro* protein digestion, trypsin inhibitory
36 activity, sensory analysis.

37 Abbreviations

38 °H: degree of hydrolysis, DTE: dithioerythritol, F: faba, LT: low temperature, OCT: optimal
39 cooking time, SDS: sodium dodecyl sulphate, SE-HPLC: size exclusion-high performance
40 liquid chromatography, S-GF: gluten-free spaghetti, S-WW: whole wheat spaghetti, TDS:
41 temporal dominance of sensation, TIA: trypsin inhibitory activity, VHT: very high
42 temperature, W: Wheat.

43

44 **1. Introduction**

45 Consumer demand for an alternative to meat proteins in the diet has been increasing in recent
46 decades. The potential of legumes such as faba to partly replace meat intake in the human diet
47 was reviewed by Multari, Stewart, and Russell (2015). In addition, the association of wheat
48 and legumes in the same food helps benefit from the nutritional composition of both crops,
49 notably their complementary essential amino acid profile (Duranti, 2006). Among several
50 traditional wheat products, pasta is an appropriate base for this association because of its
51 palatability, low cost and wide consumption. Legume-wheat mixed pasta with 50% of faba
52 protein has been demonstrated to have a better essential amino acid profile than classical
53 wheat-gluten or wheat-egg enriched pasta at identical protein content (Laleg, Barron, Sante-
54 Lhoutellier, Walrand & Micard, 2016a) with a conserved low glycemic index (Greffeuille *et*
55 *al.*, 2015). However, the total amount of essential amino acids required by the body has not
56 yet been reached in pasta (Laleg *et al.*, 2016a) because of technological problems that arose
57 when more than ~50% legume protein was included in the pasta (Petitot, Boyer, Minier &
58 Micard, 2010b; Wood, 2009). Two recent studies demonstrated that it is now possible to
59 overcome the 50% threshold in legume protein in pasta and that it is even possible to produce
60 pasta with legume as the only source of proteins (Laleg, Cassan, Abecassis & Micard, 2016b;
61 Rosa-Sibakov *et al.*, 2016). These completely gluten-free legume pasta could be of interest for
62 celiac patients, or for people who wish to reduce or eliminate gluten from their diet.

63 However, including legume protein in pasta can also have unexpected nutritional and sensory
64 effects. Legumes contain some protease inhibitors such as trypsin inhibitors, which can alter
65 the digestibility of proteins (Duranti, 2006) but could be partially or totally inactivated by
66 thermal treatment of legume pasta (Laleg, Cassan, Barron, Prabhasankar & Micard, 2016c;
67 Zhao, Manthey, Chang, Hou & Yuan, 2005). In addition, it has been reported that beyond
68 28% of gluten protein substitution with legume protein, cooking properties and sensory

69 acceptance of pasta are reduced (RayasDuarte, Mock & Satterlee, 1996). This was attributed
70 to the dilution or the absence of the gluten network responsible for the pleasant organoleptic
71 and cooking quality of pasta. The use of high temperature to dry pasta has been shown to
72 prevent the alteration of cooking, textural and organoleptic properties of classical and low
73 legume protein (<50% of total protein) enriched pasta by promoting covalent links between
74 proteins (Laleg *et al.*, 2016a). However the effect of drying temperature on pasta with higher
75 level of legume protein substitution has not yet been studied.

76 The aim of this work was to study the impact of the percentage (0% to 100%) of enrichment
77 using legume protein (faba) in pasta and the impact of drying temperature (low temperature,
78 LT, vs. very high temperature, VHT) on pasta structure and its resulting textural and cooking
79 properties. The effects of changes in pasta formulation and/or processing on trypsin inhibitory
80 activity and on its protein network structure and resulting *in-vitro* digestibility were analyzed.
81 Pasta with the best textural, cooking and/or nutritional qualities was subjected to consumer
82 acceptance analyses and compared to a commercial gluten-free and a whole wheat counterpart
83 for the first time using the Temporal Dominance of Sensations test.

84 2. Material and methods

85 Wheat semolina (W) and faba flour (F) were supplied by Panzani (Marseille, France) and
86 GEMEF industries (Aix-en-Provence, France), respectively. W and F contained, on a dry
87 basis, 13.1 and 24.0 g/100 g of proteins, 77.8 and 57.6 g/100 g of starch, and 2.4 and 11.7
88 g/100 g of fibers, respectively. The particle size distribution (D50) of W-semolina and F-flour
89 was 252 and 25 μm , respectively. Whole wheat spaghetti (S-WW; Celnat, Saint-Germain-
90 Laprade, France) and gluten-free spaghetti (S-GF; Schär, Burgstall, Italy) made from maize,
91 millet and rice were purchased from a local French market and used to evaluate the sensory
92 attributes of our pasta.

93

94 1. Pasta production

95 Pasta containing 0 (F0), 50 g (F50), 80 g (F80) and 100 g (F100) of faba protein per 100 g of
96 total protein were produced using a mixture of W-semolina and F-flour at W:F (g/g) ratios of
97 100:0, 65:35, 30:70 and 0:100, respectively. Pasta formulation and composition are detailed in
98 table 1. F0 and F50 pasta were processed into spaghetti as described by (Petitot *et al.*, 2010b).
99 F80 and F100 pasta were produced according to the WO2016097328A1 patent (Laleg *et al.*,
100 2016b). F0, F50, F80 and F100 were hydrated to 47, 45, 43 and 42 g/100 g (db) respectively,
101 mixed for 20 minutes and extruded using a continuous pilot-scale pasta extruder (Bassano,
102 Lyon, France). All the pasta were dried at low temperature 55°C (LT) or at 90°C (VHT) in a
103 pilot-scale drier (AFREM, Lyon, France). The diameter of the dried pasta was 1.56 ± 0.03
104 mm for F0 and F50, 1.51 ± 0.01 mm for F80 and 1.47 ± 0.03 mm for F100 pasta. The total
105 protein content of the dry pasta was determined in duplicate using the Kjeldahl procedure (NF
106 V 03-050, 1970) with a conversion factor of 5.7 for wheat and of 6.25 for faba proteins.
107 Lysine and cysteine amino acids were determined in duplicate on dry pasta at CIRAD

108 (Montpellier, France) according to Moore, Spackman, and Stein (1958). Pasta composition is
109 detailed in table 1.

110 **2. Molecular structure of the protein network of dried pasta**

111 The extraction procedure of pasta protein was performed according to Morel, Dehlon, Autran,
112 Leygue and Bar-L'Helgouac'h (2000). Samples of dried pasta were ground and proteins were
113 extracted in triplicate from the raw mixtures used for pasta production (100% semolina, 65%
114 semolina + 35% faba, 30% semolina + 70% faba and 100% faba) and from ground pasta. The
115 first extraction was performed in sodium dodecyl sulphate (SDS, 0.1 mol/L) to disrupt the
116 electrostatic, hydrophobic and hydrophilic interactions between proteins. After centrifugation,
117 the pellet was subjected to a second extraction in SDS (0.1 mol/L) + dithioerythritol (DTE,
118 0.02 mol/L), and sonicated (Vibracell 72434, Bioblock Scientific, Illkirch, France) at 50% and
119 at a frequency of 20 kHz for 5 min to disrupt disulfide linked proteins. The protein size
120 distribution of each extract was studied by size exclusion (SE)-HPLC (Morel *et al.*, 2000).
121 Areas (in arbitrary units) of SDS-soluble and DTE-soluble proteins were added and the sum
122 (i.e. total extractable proteins) was expressed as a percent of the corresponding total area
123 calculated for W-semolina (for F0), for blends of semolina and F-flour with 50% and 80%
124 protein from F-flour (for F50 and F80, respectively), or for F-flour (for F100). The remaining
125 pellet made of non-extractable proteins represented proteins linked by covalent linkages that
126 were not affected by sonication and/or DTE (e.g.: isopeptide bonds).

127 **3. Cooking and textural properties of pasta**

128 Each pasta was cooked to its own optimal cooking time (OCT) in demineralized water
129 containing 7 g/L of salt according to the AACC approved method (66-50), and then left to rest
130 for 10 min in a covered container at 25°C in a saturated vapor atmosphere. Optimal cooking
131 time was 9.6 ± 0.2 min for F0-LT, 10.3 ± 0.5 min for F0-VHT, 9.0 ± 0.1 min for F50-LT, 9.6

132 ± 0.3 min for F50-VHT, 9.2 ± 0.1 min for F80-LT, 9.5 ± 0.1 min for F80-VHT, 9.5 ± 0.2 min
133 for F100-LT and 9.7 ± 0.1 min for F100-VHT.

134 Cooking losses were determined in triplicates according to the following equation:

$$\text{Cooking loss (\%, db)} = \frac{\text{cooked pasta (g, db)} - \text{dry pasta (g, db)}}{\text{dry pasta (g, db)}}$$

135 A TA-XTplus (Stable Micro Systems, Scarsdale, USA) texture profile analyzer was used to
136 evaluate the resilience of the pasta. A single strand (2 cm) of spaghetti was compressed, using
137 a cylindrical probe, at a constant rate of deformation (1 mm/s) to 70% of the initial spaghetti
138 thickness. The probe was then retracted. The peak of the force (N) was plotted as a function of
139 deformation (mm). Resilience was determined in 6 replicates as the ratio of the area under the
140 second half of the peak to the area under the first half of the peak (Petitot *et al.*, 2009).

141 **4. Microscopic structure of cooked pasta**

142 The microstructure of F0-LT, F100-LT and F100-VHT pasta cooked to OCT was observed
143 using bright field light microscopy. Pasta sections (8 μm) were stained for 10 min with fast
144 green (Sigma Aldrich Co., USA) and for one minute with lugol (Fluka, Buchs, Switzerland)
145 (Petitot, Barron, Morel & Micard, 2010a). Bright field images were acquired using the
146 multizoom AZ100M microscope (Nikon, Tokyo, Japan) equipped with a Nikon DSRi1
147 (Nikon, Tokyo, Japan) color digital camera. Observations were made with a plan fluor 5 \times
148 objective and a fixed optical zoom of 8, resulting in a total magnification of 40 \times .

149 **5. Trypsin inhibitory activity (TIA) and *in-vitro* protein digestion of cooked pasta**

150 TIA was determined in triplicate according to the standardized method ISO14902 (2009) on
151 raw material and on freeze-dried OCT cooked pasta. *In-vitro* protein digestion of pasta was
152 performed in quadruplet on 60 mg freeze-dried ground cooked pasta at 37 $^{\circ}\text{C}$ in a shaking
153 water bath according to the method of Pasini, Simonato, Giannattasio, Peruffo and Curioni
154 (2001) slightly modified. Samples were suspended in 4 mL of 0.2 mol/L HCl containing
155 73,400 U/g of protein of pepsin (P7125, Sigma, St. Louis, US). After 30 min, 1.15 ml of boric

156 acid buffer (pH 6.8) containing 10.4 USP/mL of pancreatin (P7545, Sigma, St. Louis, US),
 157 was added. Digestion was stopped after 30 min of pepsin and 180 min of pancreatic attack by
 158 adding one volume of trichloroacetic acid (1.2 mol/L). The amount of free amino groups
 159 (NH₂) in the digestion extracts (supernatant) were measured using the ninhydrin method
 160 (Prochazkova, Varum & Ostgaard, 1999), at the beginning of digestion (T₀), during digestion
 161 (T_x) and after a total hydrolysis of T₀ (HCl 6 mol/L, 24 h at 105°C) (T_{total}). The degree of
 162 protein hydrolysis (°H) was calculated according to the equation:

$$^{\circ}\text{H} (\%) = \frac{\text{NH}_2(\text{T}_x) - \text{NH}_2(\text{T}_0)}{\text{NH}_2(\text{T}_{\text{total}}) - \text{NH}_2(\text{T}_0)} \times 100$$

163 6. Sensory analysis of cooked pasta by Temporal Dominance of Sensations (TDS)

164 Six pasta were tested (F0-LT, F80-LT, F100-LT and F100-VHT cooked to OCT and two
 165 commercial pasta: S-GF and S-WW cooked for 11 and 8 min, respectively, as recommended
 166 by the manufacturer) by 43 consumers, 19 to 69 years old, balanced for gender and age. Of
 167 these, 21 were regular consumers of whole wheat pasta. Ten attributes (Table 2) were
 168 presented to the subjects prior to the sensory session. A dominant sensation was defined as a
 169 sensation that triggers the most attention at a specific point of time (Pineau *et al.*, 2009).
 170 Sensory sessions were organized in a sensory room lit with red light and equipped with
 171 separate booths. The subject received 45 g of each pasta, monadically presented. He placed
 172 one bite into his mouth, he focused on the dominant sensation and clicked on the
 173 corresponding button. When the dominant perception changed, the subject scored the new
 174 dominant sensation. He was free to choose the same dominant sensation several times or to
 175 not select an attribute as dominant at all. He carried on scoring dominant sensations after
 176 swallowing, until the perception ended. Afterwards, the subject scored his current liking on a
 177 linear scale labeled at the far left “I do not like it at all” and at the far right “I like it very
 178 much” (Meilgaard, Civille & Carr, 2006). He proceeded in the same way for a second, then
 179 for a third bite of the pasta. For each pasta and each bite, TDS curves were produced (Pineau

180 *et al.*, 2009). Times were standardized between 0 (first score) and 1 (“I no longer perceive
181 anything”) (Lenfant, Loret, Pineau, Hartmann & Martin, 2009).

182 **7. Statistical analysis**

183 All data (except for sensory analysis) were subjected to analysis of variance (two-way
184 ANOVA) using “formulation” and “drying” as factors. ANOVA was followed by the Fisher’s
185 least significant difference (LSD) test to compare means at the 5% significance level, using
186 Statistica 8.0 software (Tulsa OK, USA).

187 Concerning the sensory analysis, the confidence level was set to 5%. For the liking scores,
188 statistical calculations were performed using SAS system (SAS Institute Inc., Cary, NC,
189 USA). The analyses of the TDS measurements were performed using TimeSens® software.
190 The analysis of the liking scores was performed using the MIXED SAS® procedure, with
191 product, bite, type of consumer and their three interactions of order 2 as fixed effects.
192 Consumer and consumer interaction with bite and with product were the three random effects
193 in this model in which bite was a repeated factor within product by consumer with a
194 heterogeneous auto-regressive covariance structure. Subsequently, least square means were
195 calculated for the significant effects in order to analyze the differences revealed. In addition, a
196 canonical variate analysis (CVA) was computed to distinguish the products based on the
197 dominance durations of the TDS attributes. A CVA can be considered as a principal
198 component analysis of the average durations of the attributes by product using the inverse of
199 the within product covariance matrix as a metric, i.e., taking panelist heterogeneity into
200 account.

201 **3. Results and discussion**

202 **1. Structure of the pasta protein network**

203 Figure 1 shows the protein soluble in SDS (weakly linked), in DTE (disulfide linked) and
204 non-extractable (covalently, other than disulfide, linked) protein in dry (LT and VHT) pasta as
205 a function of the concentration of F-protein.

206 In LT-pasta, increasing F-protein from 0% to 100% linearly increased the weakly linked
207 protein (SDS-soluble) at a rate of 0.24 (figure 1A) and linearly decreased disulfide linked
208 (DTE-soluble) proteins at the same rate (-0.25) (Figure 1B), with no creation of other
209 covalently linked (non-extractable) proteins (Figure 1C). Laleg *et al.* (2016a) and Petitot *et al.*
210 (2010b) also detected a decrease in DTE-soluble proteins concomitant with an increase in
211 SDS-soluble proteins when they substituted 50% of wheat protein with faba or split pea
212 flours. In their study, DTE-soluble proteins also reached 15% and 20% of total proteins for
213 faba and split pea, respectively. However, here for the first time, we demonstrate the linear
214 character of the weakening of protein network when the proportion of faba protein is
215 increased in pasta. Only 4.5% of total proteins in F100-LT were disulfide bonded versus 30%
216 in F0-LT pasta. In the corresponding raw materials, (faba flour and wheat semolina), these
217 percentages were 3% and 15%, respectively (result not shown). Only wheat proteins therefore
218 appeared to have reacted through disulfide bonds under LT drying conditions. Even if the F-
219 protein contained 9.6 mg cysteine/g protein (versus 17.2 mg/g in wheat protein; table 1), they
220 only created minor disulfide linked proteins under LT drying. This fact and the linear
221 character of the decrease in DTE-soluble protein suggests that faba proteins acted as a diluting
222 agent of gluten in mixed wheat-faba pasta dried in LT conditions. The low water content (42-
223 47%, db) required for the production of pasta, the low energy input during mixing: ~ 8 kJ/kg
224 and extrusion: ~ 70 kJ/kg (Abecassis, Abbou, Chaurand, Morel & Vernoux, 1994; Icard-
225 Verniere & Feillet, 1999) and the choice of LT drying program, were probably not enough to
226 force W-F and F-F protein to interact covalently via disulfide bridges.

227 In comparison to LT drying, whatever the F-protein content of the pasta, VHT drying led to a
228 higher formation of disulfide and to the additional creation of other covalent linkages (e.g.
229 isopeptide or Maillard products) at the expense of weakly linked proteins (Figure 1). This has
230 already been reported in 100% wheat (De Zorzi, Curioni, Simonato, Giannattasio & Pasini,
231 2007) and protein enriched pasta (with 35% faba or 5% egg white) (Laleg *et al.*, 2016a). In
232 the present study, a much higher proportion of covalently linked proteins was detected in
233 F100-VHT than in F100-LT (32% vs. 4%, respectively), showing that a high drying
234 temperature enables additional reactivity of F-proteins. Under VHT drying, disulfide bonded
235 proteins were probably formed through the interaction of free sulfhydryl groups between
236 wheat-wheat proteins, like in LT-pasta, but also in faba-faba protein or even wheat-faba
237 proteins. In VHT-pasta, like in LT-pasta, the variations in all kinds of protein linkages (weak,
238 disulfide and other covalent) as a function of the concentration of F-protein were linear.
239 However, the rate of weakening of the protein network (increase in SDS-soluble proteins)
240 with the increase in the F-protein concentration was twice as high in VHT pasta than in LT-
241 pasta.

242 2. Microscopic structure

243 Figure 2 shows the microscopic structure of F0-LT, F100-LT and F100-VHT cooked pasta.
244 The central region of F0-LT presented compact starch granules (blue) with almost no clearly
245 visible protein network (green). Whatever the drying temperature, F100 presented a more
246 open structure, with highly swollen starch granules and a visibly thicker protein network.
247 Starch granules were elongated in F0 and round/oval in F100, related to the respective shape
248 of starch in wheat and faba raw materials (Petitot *et al.*, 2010a). In the internal region of
249 F100-VHT, the starch granules were slightly darker in color than in F100-LT, probably
250 related to limited gelatinization (Cunin, Handschin, Walther & Escher, 1995). The external
251 region of F0-LT presented highly swollen and disintegrated starch granules in comparison to

252 the pasta core, as already reported in the literature (Petitot *et al.*, 2010a). In F100-LT, the
253 starch granules in the external region were as swollen as in the pasta core, and coalesced to
254 form a continuous phase at the surface of the pasta excluding protein mass. In F100-VHT, the
255 starch was held within a protein network, which was more uniformly dispersed around starch
256 granules than in F100-LT.

257

258 **3. Cooking and textural properties**

259 Pasta cooking losses and resilience are presented in figure 3A and 3B, respectively. In LT-
260 pasta, increasing F-protein from 0% to 100% linearly increased the cooking loss, and linearly
261 decreased the pasta resilience. Petitot *et al.* (2010b) also reported 7% cooking loss and 0.53
262 resilience in pasta with faba accounting for 50% of its total protein. Rosa-Sibakov *et al.*
263 (2016) found 11% cooking loss in pasta made exclusively from faba, slightly less than in the
264 present study (14%), but identical pasta resilience of 0.41. The unique ability of gluten to
265 form a protein network is the primary factor responsible for the reduced cooking loss and the
266 good textural properties of wheat pasta (Matsuo, Bradley & Irvine, 1972; Matsuo & Irvine,
267 1970). The incorporation of non-gluten material, such as faba proteins, in pasta dilutes the
268 gluten network and weakens its overall structure, as seen in SE-HPLC section, at least
269 partially explaining the decrease in pasta resilience and the cooking loss. Torres, Frias,
270 Granito, Guerra and Vidal-Valverde (2007) and RayasDuarte *et al.* (1996) also attributed the
271 higher release of solids into the cooking water to the dilution of gluten network in a 5-30%
272 lupin enriched pasta. In addition, the presence in faba flour of more fibers than in wheat
273 semolina (11.7 versus 2.4 g/100g, db) may also have helped weaken the whole pasta structure
274 when increasing amounts of faba were included in the pasta. According to Padalino *et al.*
275 (2014) and Petitot *et al.* (2010b), the inclusion of pea fibers indeed promoted the formation of
276 discontinuities or cracks inside the pasta strand, resulting in a low sensory elastic recovery.

277 In comparison to LT drying, when increasing proportions of faba were included, VHT drying
278 linearly decreased the cooking loss and increased the pasta resilience in all faba enriched
279 pasta. The strengthening of pasta protein network by VHT drying (demonstrated in the SE-
280 HPLC section), which enabled improved entrapping of starch granules, could contribute to
281 this overall improvement in pasta quality as observed by Petitot *et al.* (2010b) in a
282 wheat/legume mixed pasta equivalent to our F50 pasta. We demonstrated that the reduction in
283 cooking loss and improvement in pasta resilience under VHT vs. LT drying were even more
284 pronounced with an increase in the concentration of F-protein in pasta due to the additional
285 reactivity of faba proteins notably through the creation of disulfide linked protein (see SE-
286 HPLC section) under VHT drying. Conversely, VHT drying did not reduce cooking loss or
287 enhance the resilience of F0 pasta. In addition, even if VHT allowed faba pasta to recover
288 higher resilience, this resilience did not go above the threshold value reached for a LT or VHT
289 dried 100% wheat pasta.

290

291 **4. Trypsin inhibitory activity and *in-vitro* protein digestibility**

292 The initial TIA measured in the raw blends used for pasta production was 0, 3.92, 6.35 and
293 7.84 mg/g (db) respectively in F0, F50, F80 and F100 (results not shown). In cooked pasta
294 (table 3), TIA increased significantly ($p < 0.05$) following the order of F-protein incorporation
295 to reach a maximum value of 2.28 mg/g of cooked pasta in F100. Therefore, up to the cooking
296 step, pasta processing decreased TIA dramatically (up to 6.4 fold), as already reported in the
297 literature (Zhao *et al.*, 2005). The decrease in TIA was more pronounced in F50 and F80 (both
298 84%) than in F100 (71%). Trypsin inhibitors are proteins with a compact structure stabilized
299 through disulfide bonds (Mueller & Weder, 1989). The lower free sulfhydryl in gluten-free
300 F100 pasta probably reduced its ability to initiate unfolding of the trypsin inhibitor by
301 interaction with its disulfide bonds during processing (Friedman, Grosjean & Zahnley, 1982).

302 Eighteen, 20 and 3 mg of TIA per gram of raw black-gram, lentil and faba grains
303 (respectively) are reported in the literature (Makkar, Becker, Abel & Pawelzik, 1997;
304 Vasagam & Rajkumar, 2011), which are all higher than those obtained for our cooked pasta.

305 The use of VHT drying had less effect (F-value = 12) but nevertheless had a significant effect
306 on TIA than the concentration of F-protein (F-value = 168). VHT drying decreased TIA
307 slightly but significantly ($p=0.005$) in the pasta. The activation of sulfhydryl-disulfide
308 interchanges under VHT drying probably modified the structure of the trypsin inhibitor. No
309 effect of the interaction between drying temperature and the pasta formulation was observed
310 on TIA.

311 The degree of protein hydrolysis ($^{\circ}\text{H}$) of cooked pasta is presented in table 3. Increasing F-
312 protein in pasta significantly increased ($p<0.05$) the $^{\circ}\text{H}$ of the protein in the pasta from 42% to
313 52%, in the order $F0<F50<F80<F100$. This could be related to the linear weakening of the F
314 protein network with an increase in the F-protein content in pasta observed by SE-HPLC
315 analyses. In addition to increased protein hydrolysis when 0% to 100% faba flour was
316 incorporated in the pasta, other nutritional changes were observed including an increase in
317 resistant starch (from 0.58 ± 0.01 to 1.16 ± 0.01 g per 100 g of cooked pasta) and a slowdown
318 in starch digestion, with the percentage of rapidly and slowly available glucose decreasing
319 from $67.0 \pm 0.7\%$ to $61.83 \pm 0.95\%$ (of available carbohydrates) and increasing from $32.4 \pm$
320 0.7% to $36.4 \pm 0.95\%$ (of available carbohydrates), respectively (Greffeuille *et al.*, 2015;
321 Laleg *et al.*, 2016c). The low *in-vitro* glycemic index and the higher protein digestibility
322 resulting from incorporation of legume flour could make legume enriched pasta highly
323 advantageous from a nutritional point of view.

324 VHT tended to increase the $^{\circ}\text{H}$ in all pasta containing F-protein ($p=0.054$), which was
325 surprising as it increased the percentage of covalently linked proteins in all pasta. However,
326 this could be due to the lower TIA recorded in the VHT-pasta than in LT-pasta.

327

328 **5. Sensory evaluation**

329 **5.1. Liking scores**

330

331 Analysis of variance showed that the variants of pasta were perceived significantly different
332 in terms of liking ($F = 3.56$, $p = 0.04$). Overall, the hedonic scores (table 4) were quite low,
333 which can be explained by the fact the pasta was presented with no butter or sauce. F0-LT
334 was the best liked pasta, and S-GF pasta was the least liked. The other pasta were close. F80-
335 LT and S-WW pasta tended to be more liked than F100-LT and F100-VHT. F80-LT was the
336 best compromise in terms of preferences.

337 The analysis also underlined a significant bite effect ($F = 8.99$, $p = 0.0005$). The liking scores
338 of all pasta decreased for bite (the LSM means for the first, the second and the third bites were
339 3.98, 3.85 and 3.73, respectively). However, the difference was less clear for F0-LT.

340 There was also a significant effect of the type of consumer ($F=7.33$, $p = 0.0073$): on average,
341 regular consumers of whole wheat pasta gave higher liking scores (LSMeans = 4.25) than
342 regular consumers of classical pasta (LSMean = 3.46). The product by type of consumer
343 interaction was not significant, showing that the overall ranking of the products based on the
344 liking scores was consistent between the two types of consumers. However, S-WW pasta was
345 better liked by the regular consumers of whole wheat pasta (LSMean = 5.06) than by the
346 regular consumers of classical pasta (LSMean = 3.10).

347

348 **5.2. TDS measurements**

349

350 The TDS curves (appendix 1) show very different temporal profiles. The best liked pasta (F0-
351 LT) and the least liked one (S-GF) mainly differed in texture: the S-GF pasta had a firmer and
352 more elastic attack and no stickiness, contrary to the F0-LT pasta. The best liked F-pasta
353 (F80-LT) appears to be more complex than F100-LT pasta and was not dominated by

354 firmness, contrary to F100-VHT pasta. In addition, the legume flavor was less dominant in
355 F80-LT pasta. F100-LT had a less firm and a less elastic texture than F100-VHT, probably
356 related to the lack in resilience measured by the instrumental method in F100-LT (textural
357 property section).

358 Figure 4 shows the results of canonical variate analysis. The projections of the three bites of
359 each variant are close, revealing very few differences in the perception of each product in
360 progressive bites. S-GF pasta was characterized by longer dominance duration of white cereal
361 flavor and of an elastic but firm texture. F0-LT pasta was mainly characterized by longer
362 dominance duration of white cereal flavor and of stickiness. F100-VHT pasta was
363 characterized by longer dominance of the legume flavor and by a firm texture. F80-LT pasta,
364 F100-LT and S-WW pasta were close. They were longer dominated by whole wheat and
365 legume flavors, but the variant F80-LT was perceived to be stickier longer than the two other
366 variants.

367

368 **4. Conclusion**

369 In this study, the relation between the structure of protein and the cooking, nutritional and
370 sensory properties of faba (F) enriched pasta was investigated. We demonstrated for the first
371 time that the incorporation of F-protein linearly weakened the protein network structure of
372 low temperature (LT) dried pasta, by diluting the gluten network without creating any
373 additional covalent interactions between gluten and F-protein. We also demonstrated that the
374 weakening of the protein network structure could be responsible for the increase in the *in-*
375 *vitro* protein digestion. However, it altered the integrity of the pasta during cooking as well as
376 its resilience. As a result, F-pasta were less appreciated than the traditional wheat pasta. Very
377 high temperature (VHT) drying strengthened the protein structure of pasta, resulting in
378 increased integrity and better resilience of all F-pasta without altering their *in-vitro* protein
379 digestibility. Consequently, VHT drying can be used to improve the cooking properties of

380 legume pasta. Interestingly, appreciation of legume pasta containing 80% or 100% F-protein
381 was similar to that of commercial counterparts made of whole wheat or gluten-free cereal.
382 The promising nutritional and sensory qualities of legume pasta thus make them an interesting
383 model, rich in good quality proteins, or gluten-free suitable for gluten intolerant people.
384

385 **5. Acknowledgments**

386 The authors are very grateful to A.-L. Loiseau (CSG, Dijon), and G. Conéjéro (INRA,
387 Montpellier, France) for sensory analysis and access to PHIV facilities, respectively. The
388 authors thank J. Bonicel, T.-M. Lasserre and D. Cassan (INRA Montpellier, France) for
389 their technical assistance. This work was carried out under the VEGAGE project that
390 received the financial support of the “Qualiment Institut Carnot”.

391

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483 **Figure legends**

484

485 **Figure 1.** Changes in sodium dodecyl sulphate (SDS, A), dithioerythritol (DTE, B) soluble
 486 proteins and insoluble protein (non-extractable, C) from pasta dried at low temperature (LT)
 487 or very high temperature (VHT), as a function of the percentage of faba bean (F) protein in
 488 the pasta.

489 Experimental data (means of three replicates): \diamond LT; \blacklozenge VHT. Formulation: significant effect
 490 ($p < 0.05$) on SDS and DTE-soluble protein; Drying: significant effect ($p < 0.05$) on SDS,
 491 DTE-soluble and non-extractable protein.

492 Best fit (y): --- LT; —VHT.

493 - SDS-soluble protein: $y_{LT} = 0.24x + 70.39$; $R^2 = 1.00$, $y_{VHT} = 0.42x + 22.40$; $R^2 = 1.00$.

494 - DTE-soluble protein : $y_{LT} = -0.25x + 30.69$; $R^2 = 0.99$, $y_{VHT} = -0.36x + 69.12$; $R^2 = 0.99$.

495 - Non-extractable protein : $y_{VHT} = -0.06x + 8.61$; $R^2 = 0.98$.

496

497 **Figure 2.** Light microscopy of cooked pasta containing 100% wheat protein and dried at low
 498 temperature (Top panels F0-LT); and pasta containing 100% faba protein and dried at low
 499 temperature (Middle panels F100-LT) and very high temperature (bottom panels F100-VHT).

500

501 **Figure 3.** Changes in cooking loss (A) and resilience (B) of cooked pasta dried at low
 502 temperature (LT) or very high temperature (VHT) as a function of the percentage of faba bean
 503 (F) protein in pasta. \diamond LT; \blacklozenge VHT. Cooking losses are means of three replicates. Resilience is
 504 the mean of six replicates. Formulation and drying had a significant effect ($p < 0.05$) on
 505 cooking loss and resilience.

506 Best fit (y): --- LT; —VHT.

507 - Cooking loss: $y_{LT} = 0.08x + 5.55$; $R^2 = 0.94$, $y_{VHT} = 0.04x + 5.29$; $R^2 = 0.95$.

508 - Resilience: $y_{LT} = -0.002x + 0.59$; $R^2 = 0.94$.

509 **Figure 4.** Canonical variate analysis (83.1%) based on the dominance durations of the TDS
 510 attributes.

Tables

Table 1. Formulation of pasta and protein composition of pasta made from different mixtures of wheat (W) semolina and F (faba) flour; and the lysine and cysteine contents of pasta dried at low temperature (LT) and very high temperature (VHT).

Amounts of raw materials (g/100g, db)	F0	F50	F80	F100
W-semolina	100	65	30	0
F-flour	0	35	70	100
Protein composition				
Total protein content (g/100g of pasta, db) ^a	13.1 ± 0.0	16.9 ± 0.1	20.7 ± 0.0	24.0 ± 0.1
W-protein (g/100g of pasta, db) ^b	13.1	8.5	3.9	0.0
F-protein (g/100g of pasta, db) ^b	0.0	8.4	16.8	24.0
W/F-protein ratio (g/g) ^b	100/0	50/50	20/80	0/100
Lysine amino acid (mg/g protein)^a				
LT-dried pasta	20.5 ± 0.6	38.6 ± 0.2	55.0 ± 0.5	65.1 ± 0.3
VHT-dried pasta	20.3 ± 1.8	33.4 ± 1.1	52.4 ± 3.0	60.3 ± 1.3
Cysteine amino acid (mg/g protein)^a				
LT-dried pasta	17.2 ± 0.1	14.8 ± 0.6	10.4 ± 0.5	9.6 ± 0.7
VHT-dried pasta	17.4 ± 0.2	15.4 ± 0.3	10.4 ± 0.4	8.8 ± 0.3

^aAnalyses were performed in duplicate.^bResults obtained by calculation according to the ratio of raw materials and their protein composition.

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Table 2. Temporal dominance of sensation (TDS) list of attributes with their definitions and/or references

Attribute	Type	Definition	References
Firm	Texture	Firmness, chewing resistance	/
Floury	Texture	Like texture of flour in mouth	/
Sticky, pasty	Texture	Sticks to teeth and palate	/
Elastic	Texture	Deforms without breaking	/
Rough	Texture	Irregularities perceived at the surface of the pasta (not smooth)	/
Salty	Taste	Salty taste	/
Legume	Flavor	Flavor of lentils, beans, chickpeas, etc.	Cooked lentils, cooked beans, etc.
White cereal	Flavor	Flavor of white flour, refined wheat	White flour
Whole cereal	Flavor	Flavor of whole-wheat flour, buckwheat	Whole-wheat flour, cooked buckwheat
Grilled	Flavor	Grilled and toasty flavor	/

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Table 3. Two-way analysis of variance (ANOVA) of trypsin inhibitory activity (TIA, mg/g db) and the degree of *in-vitro* protein hydrolysis ($^{\circ}\text{H}$; % of total protein) in cooked pasta containing different concentration of faba bean protein. F0, F50, F80 and F100 present pasta in which 0, 50, 80 and 100 % (respectively) of total proteins originated from faba bean.

Analysis of variance ^a			Comparison of means-LSD test at the 5% significance ^b						Pooled standard deviation	
			Effect of F-protein concentration				Effect of drying			
Effects	p-value	F-value	F0	F50	F80	P100	LT	VHT		
TIA (n=3)	A : F-protein concentration	0.000	168	ND	0.61 ^a	1.03 ^b	2.28 ^c			0.21
	B : Drying	0.005	12					1.44 ^a	1.17 ^b	0.77
	Interaction : A×B	0.480	0.8							
$^{\circ}\text{H}$ (n=4)	A : F-protein concentration	0.000	75	42 ^a	46 ^b	48 ^c	52 ^d			2
	B : Drying	0.054	4					46 ^a	47 ^a	4
	Interaction : A×B	0.416	1							

^a Two-way ANOVA was performed using F-protein concentration and pasta drying temperature as factors

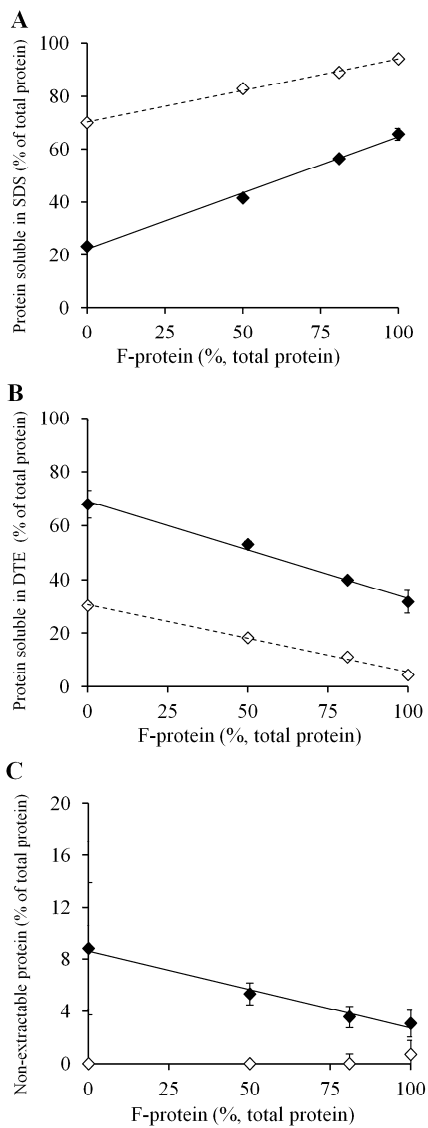
^b Values in the same row with the same lower case letters are not significantly different ($P>0.05$). For each effect analyzed, the mean value for all conditions tested for the other effect is given.

ND: not detected by the method used.

Table 4. Average liking scores for the different variants of pasta (LSMeans).

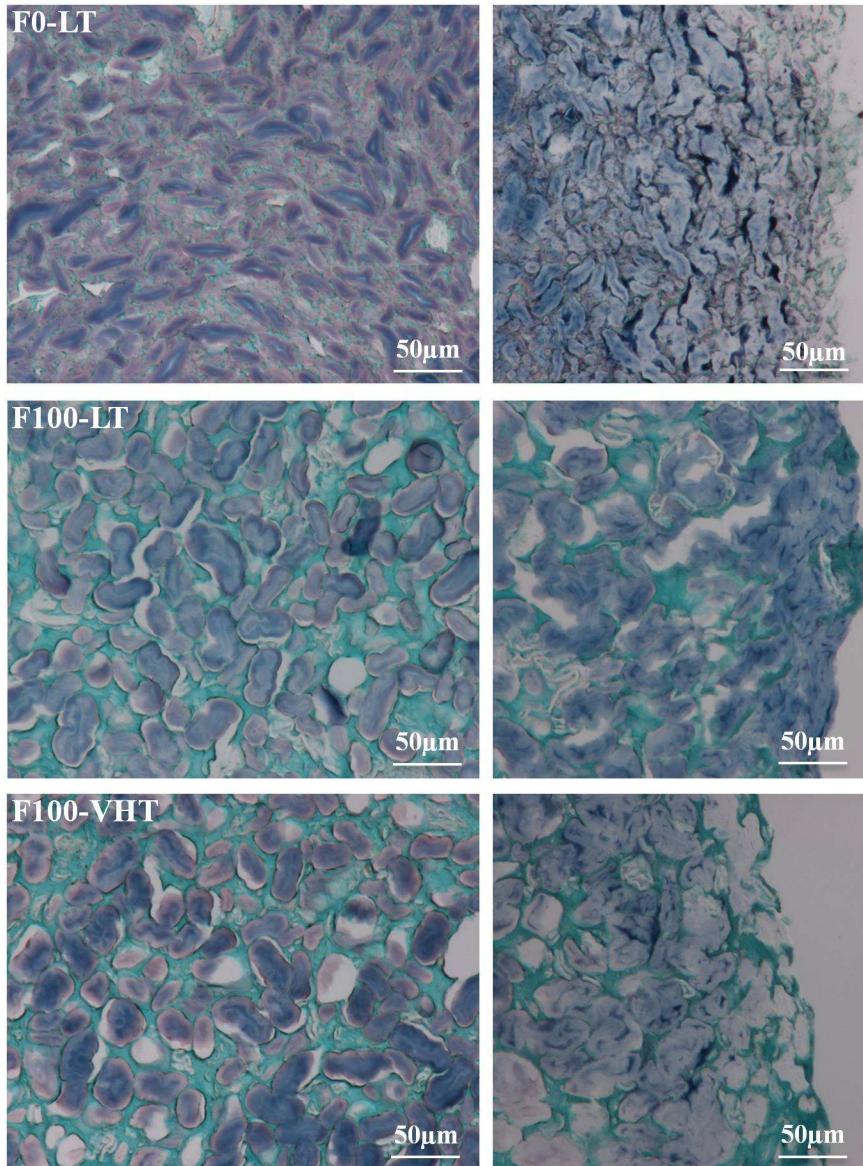
Pasta	Average liking score (n=43)
F0-LT	4.88 ^a
S-WW	4.08 ^{ab}
S-GF	2.81 ^c
F80-LT	3.95 ^{ab}
F100-LT	3.72 ^{bc}
F100-VHT	3.67 ^{bc}

Variants with the same letter are not significantly different. Pooled standard deviation: 2.33 (95% confidence intervals).



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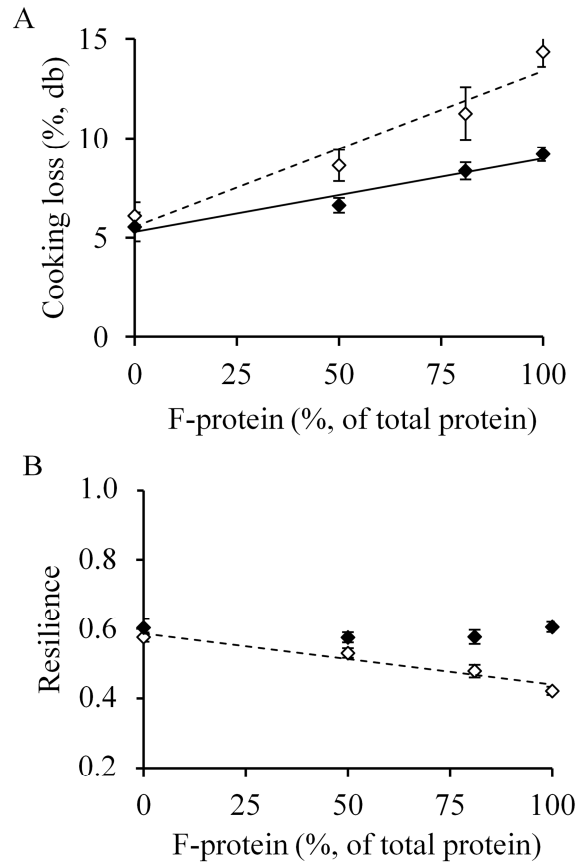


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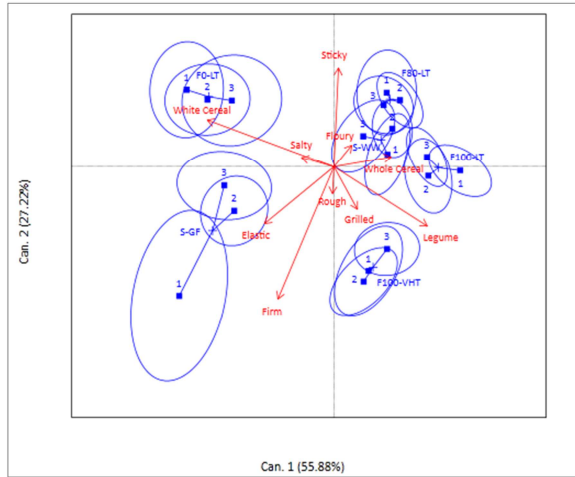
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Highlights

- Pasta enrichment in faba linearly weakens its protein network
- Increasing faba protein concentration in pasta enhances its *in-vitro* digestion
- High-temperature drying strengthens legume pasta structure
- Increasing drying temperature does not alter legume pasta protein digestibility
- Good quality protein or gluten-free legume pasta were appreciated by consumers

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