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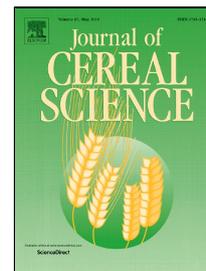
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Kinetics of gluten protein-insolubilisation during pasta processing: decoupling between time- and temperature-dependent effects.



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1 **Kinetics of gluten protein-insolubilisation during pasta processing: decoupling between**
2 **time- and temperature-dependent effects.**

3

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5

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11

12 **Keywords** - Durum wheat pasta, glutenin insolubilisation, gluten protein, extrusion.

13

ACCEPTED MANUSCRIPT

14 **Abstract**

15

16 Durum wheat gluten proteins are essential for pasta quality. Glutenin polymers undergo
17 solubilization mechanisms during pasta processing. This study aims to investigate gluten
18 proteins insolubilisation kinetics during a resting period applied to extruded. The goal is to
19 investigate the contribution of time related recovery of large glutenin polymers and temperature
20 related formation of glutenin cross-linked aggregates in the pasta quality. Extruded pasta were
21 rested for 120 minutes at 20, 30 or 40°C prior drying at high temperature (90°C). Glutenins
22 insolubilisation induced by resting and drying was assessed from the SE-HPLC elution profiles
23 of the SDS-soluble proteins from fresh, rested and dried pasta. Final pasta were characterized
24 for their organoleptic (color, surface roughness) and physical (diameter, cooking time,
25 viscoelasticity index) properties, before and after cooking. Pasta quality is discussed in relation
26 with the gluten network structure and the impact of resting time. Resting of freshly extruded
27 pasta extrusion leads to spontaneous insolubilisations of glutenins by creation of weak
28 interactions. High temperature drying induces a strong drop of solubility of all gluten protein
29 fractions by creation of covalent bonds, impacting equally rested and not rested pasta. Dry and
30 cooked pasta organoleptic and physical properties are not significantly impacted by a resting
31 time.

32 1. Introduction

33

34 Durum wheat pasta is a traditional and world-wide consumed cereal food. Pasta structure relies
35 on physical and chemical interactions that are established in between starch and proteins during
36 pasta making. Pasta processing consists in a succession of several unit operations: hydration,
37 mixing, extrusion, and drying. Durum wheat semolina is first hydrated with a specific amount
38 of water and mixed in order to obtain crumble dough of wet agglomerates. The agglomerates
39 are structured into pasta by single screw extrusion at low temperature. Fresh pastas are
40 stabilized by drying, which can be either carried out at high (70-90°C) or low (40-55°C)
41 temperature. Cooking, the final food preparation step before pasta eating, is assigned to the
42 consumer (Sicignano et al., 2015).

43 It is commonly accepted that the wheat components and their changes in structure during
44 processing and cooking control pasta final organoleptic qualities. During the first mixing step,
45 the wet agglomerates of semolina undergo shear deformation, that induces protein hydration
46 and unfolding allowing for the formation of a continuous gluten network enclosing the starch
47 granules (Guerrero et al., 2014).

48 Gluten in its hydrated form behaves like a viscoelastic cohesive mass stabilized by weak
49 hydrogen, ionic and hydrophobic bonds. Wheat gluten proteins (12 to 15% of pasta dry weight)
50 are constituted of monomeric gliadins and polymeric glutenins (Shewry & Halford, 2002). Both
51 are soluble in sodium dodecyl sulfate (SDS) buffer, fully for the former and partly for the latter.
52 Process temperature above 55°C impact gluten network structure by triggering the formation
53 of covalent links (Joubert et al., 2018; Lagrain et al., 2005; Lamacchia et al., 2007; Morel et al.,
54 2002; Singh & MacRitchie, 2004; Wagner et al., 2011; Weegels & Hamer, 1998).

55

56 When pasta extrusion is conducted above 50°C, it causes a marked decrease in the solubility of
57 salt-soluble proteins fractions (albumin, globulin). Above 96°C, extrusion results in a dramatic
58 increase of the SDS-insoluble protein fraction (Dexter and Matsuo, 1977; Ummadi et al., 1995).
59 The impact of pasta drying temperature on gluten proteins aggregation and solubility loss in
60 SDS buffer has been extensively studied. Above 55°C for glutenins and 70°C for gliadins,
61 proteins initiate disulfide/sulphydryl exchange reactions. Above 90°C, glutenin and gliadin
62 crosslink together through new disulfide bonds (Dexter and Matsuo, 1977; Morel et al., 2000;
63 Wagner et al., 2011). When reticulated during drying, the gluten protein network allows pasta
64 to resist to starch swelling mechanisms that are promoted by the final cooking stage and
65 promote a firmer texture and a better surface aspect of cooked pasta (Cubadda et al., 2007;

66 [Lamacchia et al., 2007](#); [Matsuo & Irvine, 1970](#)). Viscoelastic index of cooked pasta correlates
67 well with native semolina gluten protein content and with gluten network ability to resist to
68 mechanical stresses ([Baiano & Del Nobile, 2006](#)). When compared to low temperature drying,
69 high or very high temperature drying diagrams.

70 Recently, [Joubert et al. \(2018\)](#) indicated that the drying step of pasta making process is not the
71 only stage during which gluten protein can be insolubilized. When freshly extruded pasta is
72 rested at ambient temperature after extrusion, gluten proteins tend to spontaneously
73 insolubilize. The largest fractions of glutenin polymers are more impacted than the smallest
74 fractions. Gliadin fractions are not impacted during the resting stage. The insolubilisation
75 mechanisms of the glutenins fractions during resting at ambient temperature followed first order
76 kinetics. We aim to investigate the potential macroscopic differences in pasta characteristics
77 generated either by spontaneous insolubilisation reactions of gluten proteins at ambient
78 temperature in freshly extruded pasta or protein aggregation during drying step.

79 The objective of the present study is to investigate the impact of time during a resting period
80 after extrusion and during drying stage, on protein insolubilisation kinetics in extruded pasta.
81 The resting period was conducted under different conditions of temperature (20-40°C) and
82 followed by a drying stage conducted under low (55°C) or high (90°C) temperatures. The
83 extraction of SDS-soluble and SDS-insoluble proteins and the analyze by size exclusion high
84 performance liquid chromatography (SE-HPLC) allowed to describe the mechanisms of protein
85 reactions throughout the resting period and drying stage. The measured characteristics of dried
86 pasta (color, diameter, and cooking time) and cooked pasta (color, diameter, surface aspect, and
87 texture) were discussed in relation to the mechanisms of protein insolubilisation during pasta
88 making process, in order to evaluate the specific impact of the resting period.

89

90

91 **2. Material and methods**

92

93 **2.1. Raw material**

94 Industrial durum wheat semolina (Panzani, France) was used as raw material. Semolina was
95 stored at 4°C. Semolina water content (13.87 (± 0.12) g water/100 g wet semolina) was
96 determined according to [AACC method 45-15.02](#). Semolina total nitrogen content was
97 determined by Kjeldahl method, and the crude protein content (12.41 (± 0.26) g proteins/100 g
98 dry semolina) was calculated according to TN-5.7 ([AFNOR NF V03-050](#) method). Particle
99 diameter distribution of semolina were measured by a laser particle size analyzer (Coulter

100 TMLS 230, Malvern, England). The median diameter (d_{50}) was of 270 (± 1) μm . The diameter
101 span was $(d_{90}-d_{10})/d_{50} = 1.51 (\pm 0.42)$.

102

103 2.2. Pasta making

104 Semolina was transformed into pasta using a laboratory pasta making machine (AFREM-
105 BASSANO, France, [Abecassis et al., 1994](#)). Semolina (7.0 kg) was weighed and introduced in
106 the mixer tank. The double water-jacket of the mixing tank temperature was maintained at
107 40°C. Tap water was added to obtain a final water content of 47.0 g water / 100 g dry matter.
108 Tap water was equilibrated to prior its spreading over the mixed semolina layer. Mixing was
109 continued for 20 min, at atmospheric pressure. The wet agglomerates of semolina were extruded
110 at constant temperature (35°C) under vacuum (59 MPa). Fresh spaghettis were extruded
111 vertically from the die. Extrusion temperature and pressure were recorded along extrusion.
112 Pasta drying was conducted using a laboratory pasta drying machine (AFREM, France,
113 [Abecassis et al., 1989](#)). Extruded fresh spaghettis were immediately placed in the dryer that was
114 previously equilibrated at 35°C and 88% relative humidity (RH). Pasta drying was conducted
115 under low or high temperature conditions. The drying diagram at low temperature was: starting
116 condition at 35°C and 88% RH, 100 min to reach 55°C and 72% RH, 600 min at 55°C and 72%
117 RH, and 150 min to reach 40°C and 70% RH. The high-temperature drying diagram was:
118 starting condition at 35°C and 88% RH, 60 min to reach 90°C and 86% RH, 60 min at 90°C
119 and 86% RH, 60 min to reach 90°C and 77% RH, 60 min at 90°C and 77% RH, and 60 min at
120 35°C and 70% RH.

121 To investigate the effect of a rest period between the extrusion and drying stages, freshly
122 extruded pasta were rested in the dryer at 20, 30 or 40°C and 88% RH up to 120 minutes prior
123 to the drying phase. It was verified that at the end of the resting time no weight loss had
124 occurred.

125 Pasta samples were collected for SE-HPLC analysis immediately after the mixing and extrusion
126 steps and after different resting and drying times. Samples were immediately frozen using liquid
127 nitrogen and stored at -18°C. The frozen samples were freeze-dried, milled and kept under
128 argon until characterization.

129

130 2.3. Characterization of dried pasta

131 Diameters of dried spaghetti were manually determined using a caliper (Mituyo, CD-15DC).
132 For each product, 8 measurements were done on 8 spaghettis.

133 Color coordinates (L^* brightness, a^* redness, b^* yellowness) of dried pasta were measured
 134 using a reflectance colorimeter (Konica Minolta CR - 410, France) with daily light calibration
 135 (D65). For each product, 40 g of dried spaghetti were cut (8 cm length) and positioned inside a
 136 specific black box (14 x 10 cm) to form a homogenous layer of product (1.5 cm height). Colors
 137 measurements were carried out in triplicate.

138

139 **2.4. Cooking behavior of pasta**

140 Pasta cooking was conducted in boiling water according to the French Standard NF ISO 7303.
 141 Optimum cooking time for dried pasta was determined according to [AACC Method 66-50.01](#).
 142 Pasta were cooked at the optimum cooking time + 1 minute. After cooking, spaghetti sample
 143 was drained and rinsed with large amount of tap water. Pasta strands were kept under Petri
 144 boxes at room temperature under saturated relative humidity conditions before characterization.

145

146 **2.5. Characterization of cooked pasta**

147 Water content of cooked pasta was determined in triplicate according to the approved [AACC](#)
 148 [Method 45-15.02](#). Results are expressed in g water / 100 g dry matter.

149 Color characteristics (L^* , a^* , and b^*) of cooked pasta was measured for each product. 40 g of
 150 cooked spaghetti were arranged inside the specific black box to form a homogenous layer of
 151 product (2 cm height). Color was measured in triplicate.

152 Diameters of cooked spaghetti were manually determined using a caliper (Mituyo, CD-15DC).
 153 For each product, 8 measurements were done on 8 spaghetti.

154 Surface roughness of cooked pasta was evaluated according to ISO 7304-1:2016. Cooked pasta
 155 were disposed on a white plate and compared to standardized pictures. A grade (1 to 8 scale)
 156 was given by three different individuals after visual inspection.

157 Viscoelasticity and tensile strength of cooked pasta were measured using a texturometer TA-
 158 XT Plus (Stable Micro Systems, UK). For viscoelasticity, a flat probe (SMS P/35) was used to
 159 squeeze a 2 cm cooked spaghetti sample under 0.5 N for 40 sec, before stress relaxation (20
 160 sec). Thickness values (mm) were measured: initial diameter (E), diameter after 40 sec under a
 161 0.5N compression (e_1), and diameter after 20 sec of load release (e_2). Viscoelasticity index (IV)
 162 was calculated using [equation 1](#). For each product, five measurements were performed.

$$163 \quad IV = \frac{(e_2 - e_1)/(E - e_1)}{(E - e_1)/E} \quad (\text{Eq 1})$$

164

165 For tensile strength measurement cooked spaghettis were fixed to the tensile grips (A/SPR).
 166 The tensile test was performed at constant deformation rate (3 mm.s⁻¹) until pasta rupture. The
 167 strain and stress values were calculated considering the thinning of the sample diameter and
 168 constant sample volume between the grips (equations 2, 3).

$$169 \quad \text{Strain} = \frac{L_t - L_0}{L_0} \times 100 \quad (\text{Eq 2})$$

$$170 \quad \text{Stress} = \frac{F_t}{\frac{\pi D_0^2 \times L_0}{4 \times L_t}} \quad (\text{Eq 3})$$

171 With L_0 , sample length between the two jaws (mm); L_t , the same after time t of extension (mm);
 172 D_0 , initial sample diameter (mm); F_t , measured force at time t of extension (N). Rupture stress
 173 (MPa) and strain (%) were recorded from the stress-strain curves at the breaking point. Stress-
 174 strain curves were plotted and elastic modulus was identified as being curves' slope. Four to
 175 seven measurements were recorded for each product.

176

177 2.6. Gluten protein size distribution

178 SDS-soluble and SDS-insoluble proteins from freeze-dried and milled pasta samples were
 179 extracted and analyzed by size exclusion high performance liquid chromatography (SE-HPLC)
 180 according to Morel et al. (2000). SDS-soluble protein (F_s) were extracted in sodium phosphate
 181 buffer (pH 6.9) containing 1% SDS. SDS-insoluble proteins (F_i) were extracted by sonication
 182 of the residue re-suspended in the previous buffer supplemented with dithioerythritol (20 mM).
 183 Chromatogram of SDS-soluble proteins was divided into 5 fractions: SDS-soluble large
 184 glutenin polymers (F_1); medium range glutenin polymers (F_2); ω -type sulfur-poor prolamins
 185 (F_3); sulfur-rich α -, β - and γ -gliadins (F_4); albumin and globulins (F_5). Areas from fractions F1
 186 to F5 were expressed in % of the corresponding area from freshly extruded pasta, unless
 187 otherwise noted. In percent of total protein, fractions F_1 to F_5 of the freshly extruded pasta
 188 were: 5.7 ± 1.1 % (F_1); 26.7 ± 2.1 % (F_2); 8.6 ± 0.2 % (F_3); 37.0 ± 1.5 % (F_4) and 13.1 ± 0.4 %
 189 (F_5).

190

191 2.7. Model fitting and statistical analysis

192 Apparent first order kinetic model (Eq. 4) was used to fit the solubility changes as a function
 193 of time observed during pasta resting and drying. Areas F_1 to F_5 from extracts F_s were
 194 considered, after their normalisation to the corresponding area obtained from the freshly
 195 extruded pasta sample.

$$196 \quad y = y_0 + a(1 - e^{-bx}) \quad (\text{Eq. 4})$$

197 Where y_0 (=100) is the normalized F_i areas at $t = 0$ min. The calculated coefficients of the model
198 a and b are respectively related to the insolubilisation amplitude and characteristic reaction time
199 (C_{Time} ; $1/b$). Coefficients were determined by least-square fitting using Excel 2016 solver
200 (Microsoft, Redmond, USA). Determination coefficients (R^2) and the sum of absolute
201 deviations between experimental and calculated values were considered to gauge the goodness
202 of fit. Statistical analysis was carried out with XLSTAT (Addinsoft, NY, 2017) at 95%
203 confidence level by Student tests and p-value. The statistical significance of measured values
204 was verified using single factor analysis of variance (ANOVA) and Tukey test on replicated
205 data.

206

207

208 3. Results

209

210 3.1. Impact of drying

211 *Impact on protein insolubilisation* - Extruded pasta were dried at low (55°C) or high (90°C)
212 temperature. Solubility changes for each gluten protein fraction as a function of drying time
213 were quantified to follow protein insolubilisation dynamics (figure 1). Pasta drying promotes
214 gluten protein insolubilisation reactions.

215 Regardless of the drying temperature, non-linear solubility decreases of all SDS-soluble gluten
216 protein fractions (F_1 to F_5) were observed and were successfully fitted using an exponential
217 decay model, except for low molecular weight fractions (F_3 to F_5) that are only slightly impacted
218 by the LT drying. Low temperature (LT) drying led to specific decreases of relative solubility
219 depending on gluten protein fraction (figure 1). Fractions F_1 and F_2 underwent a fast ($C_{\text{Time}} =$
220 12.2-50.0 min) and large (27.5-34.8%) loss of solubility. LT drying mainly impact the glutenin
221 polymers fractions (F_1 and F_2).

222 High temperature (HT) drying was conducted into two phases: a heating phase from 35 to 88°C
223 in 45 min, and a high temperature treatment phase at 90°C for 255 min. HT drying led to a
224 strong decrease of protein solubility, specific to each fraction (figure 1). During the heating
225 phase, fractions F_1 and F_2 drops according to kinetic very similar to the one observed for LT
226 drying ($C_{\text{Time}} = 14.2-50.0$ min) but of larger (52.0-50.0%) amplitude. For fractions F_3 , F_4 and
227 F_5 insolubilisation was slower ($C_{\text{Time}} > 50$ min) and less pronounced (8.0-30.0%). During the
228 high temperature phase (90°C), fractions F_1 and F_2 underwent an additional very fast ($C_{\text{Time}} =$
229 5.0-11.9 min), and large (45.0-65.0%) insolubilisation process. Fractions F_3 , F_4 and F_5 loss of
230 solubility was slower ($C_{\text{Time}} = 22.2-33.3$ min) but more pronounced (38.0-55.0%) than during

231 the heating phase. HT drying amplifies gluten protein insolubilisation mechanisms. HT drying
 232 is known to increase the amount of SDS-insoluble proteins (De Stefanis and Sgrulletta, 1990;
 233 Petitot et al., 2009). Kinetic and thermal effects on high molecular weight gluten protein
 234 (F_1+F_2) insolubilisation mechanisms along drying have been depicted by a curve of protein
 235 solubility as a function of 'time*temperature' (figure 2). Regardless of drying temperature,
 236 protein solubility variations overlap for short duration.

237

238 *Impact on pasta characteristics* - As expected, drying temperature impacts pasta characteristics
 239 (table 1). Compared to LT drying, HT drying had a significant impact on pasta color. HT drying
 240 significantly increased redness (a^*) and decreased yellowness (b^*) for both dried and cooked
 241 pasta. On the other hand, opposite impacts of HT drying on brightness (L^*) were observed for
 242 dried pasta (L^* increase) and cooked pasta (L^* decrease). Compared to LT drying, HT drying
 243 had a significant impact on pasta diameter, with a significant decrease for dried pasta and a
 244 significant increase for cooked pasta. Dried pasta cooking time was not impacted by drying
 245 temperatures. Compared to LT drying, HT drying significantly improved cooked pasta surface
 246 aspect (from 6 to 8) and texture properties, with an increase of elastic modulus, breaking stress,
 247 breaking strain, and viscoelasticity index. It is admitted that compared to LT, HT drying led to
 248 pasta characterized by a higher red hue due to Maillard reaction, a firmer texture and a better
 249 surface aspect (Cubadda et al., 2007; Lamacchia et al., 2007).

250

251 3.2. Protein insolubilisation during resting of freshly extruded pasta

252 The impact of resting for 120 min at different temperatures (20, 30 or 40°C) on protein
 253 solubility and pasta characteristics was investigated. Soluble gluten proteins were quantified
 254 for time related to resting and HT drying.

255 *Impact on protein solubility in SDS buffer* – Resting led to specific solubility decrease of protein
 256 fractions F_1 and F_2 (figure 3) while fractions F_3 , F_4 and F_5 were slightly impacted (data not
 257 shown). Resting decreased the extractability of protein in SDS buffer by about 6 to 10% as the
 258 temperature rose from 20 to 40°C. In accordance with Joubert et al. (2018), only glutenin
 259 polymers were impacted, and large polymers from fraction F_1 reacted faster than those from
 260 fraction F_2 , suggesting that solubility loss resulted from an increase in the size of glutenin
 261 polymers. The rate of protein solubility loss roughly followed a first order kinetics, but a 16
 262 minutes delay 16 min was noticed for samples rested at 20°C, before any significant drop.
 263 Increase in temperature fasten solubility drop. An activation energy of 26.7 ± 7.2 kJ/moles was
 264 determined from an Arrhenius plot of the fitted rate constants. The value is far from the E_a value

265 of 75 to 95 kJ/moles reported for gliadin solubility loss beyond 100°C (Lagrain, 2011) or from
266 the activation enthalpy calculated for the oxidation of the unique thiol of bovine serum albumin
267 thiol (54-59 kJ/moles) (Bonanata et al., 2017). It approximates the E_a value of 30-40 kJ/moles
268 measured by single-molecule force clamp spectroscopy for protein disulfide bond reduction by
269 free thiol (Liang, 2011). Protein disulfide bond reshuffling, mediated by thiol/disulfide
270 exchanges would account for glutenin polymers solubility loss.

271

272 **3.3. Impact of pasta resting on protein crosslinking during drying and on pasta quality** 273 **attributes**

274 Extruded pasta were sequentially submitted to a resting period (at 30°C for 120 minutes) and
275 an HT drying (90°C). Soluble gluten proteins were quantified along resting and drying stages
276 in order to follow protein solubility variations (figure 3a, 3b).

277 *Impact on protein insolubilisation* - Resting impacted the kinetic of gluten protein solubility
278 loss kinetics induced by HT drying but not the total amplitude of the phenomenon. During the
279 early drying phase (from 35 to 88°C for 45 min) and compared to non-rested pasta, protein
280 solubility loss remained modest: levels of soluble proteins from rested and non-rested pasta
281 turned out to be equivalent at the end of the first drying step. Further HT drying at 90°C induced
282 large protein solubility loss, regarding all wheat protein classes and in a way similar to the one
283 observed for non-rested pasta (figure 1c-1d).

284 *Impact on pasta characteristics* - Compared to non-rested pasta, a resting period before HT
285 drying had significant light impacts on color properties of pasta (table 1). A resting time (120
286 min) significantly increased dried and cooked pasta yellowness (b^*) and redness (a^*), and
287 cooked pasta brightness (L^*). Resting time also induced a light decrease of dried pasta diameter,
288 but did not significantly change cooked pasta diameter. Resting time did not significantly
289 impact cooking time and texture properties of cooked pasta.

290

291

292 **4. Discussion**

293

294 **4.1. Gluten proteins insolubilisation upon high temperature treatment**

295 HT drying is a driving unit operation of changes of protein structure upon pasta processing.
296 Thermal treatment during HT drying induces a strong drop of solubility of all gluten protein
297 fractions. Glutenins (F_1 and F_2) show a stronger loss of solubility (95%) compared to gliadin
298 and cytoplasmic proteins (46-65%). HT drying leads to rapid and strong specific gluten proteins

299 insolubilisation. HT drying induces the insolubilisation of 95% of the glutenins, 60% of the
300 gliadins and 40% of the cytoplasmic proteins. Compared to gliadins, higher molecular weight
301 glutenins are highly sensitive to HT treatments. HT drying allows very fast insolubilisation
302 mechanisms, with characteristic times lower than 14 min for the glutenin fractions.

303 We proposed a phenomenological model to describe the changes in protein solubility during
304 pasta resting and drying ([figure 5](#)). Water elimination during pasta drying leads to a significant
305 reduction of pasta diameter. The generated mechanical stress could induce compaction of wheat
306 components. The protein chains get closer and could generate additional interactions. Each
307 additional bond involving glutenins reduces their solubility. The behaviour of gluten proteins
308 under HT has been extensively studied ([Icard-Vernière & Feillet, 1999](#); [Lamacchia et al., 2007](#);
309 [Lagrain et al., 2005](#); [Petitot, 2009](#); [Wagner et al., 2011](#); [Zweifel et al., 2003](#)). It is well
310 established that heating glutenins above 55°C and gliadins above 70°C allows
311 disulphide/sulfhydryl exchange reactions and the creation of new disulfide bonds. A LT thermal
312 treatment (55°C) of glutenins leads to the creation of intra-chains and inter-chains disulfide
313 bonds, as indicated by the superposed curves when plotted according to "time*temperature"
314 axis ([figure 2](#)). LT drying reinforces to a certain extent pasta protein network. A HT thermal
315 treatment (90°C) allows the creation of numerous disulfide bonds between proteins, thus
316 inducing high levels of insolubilisation. Sulfur-rich α -, β - and γ -gliadins (F₄) contain cysteine
317 residues (6 cysteines for α -type and 8 cysteines for γ -type) located at highly conserved positions
318 and all involved in intrachain disulfide bonds. Their quaternary structure might not be altered
319 during mixing and extruding stages. Thiol groups of cysteine residues might not be exposed
320 and are less able to create disulfide bonds.

321 We hypothesized that glutenin insolubilisation upon resting of extruded pasta could be due to
322 the creation of weak interactions ([figure 5](#)). After a thermal treatment during HT drying,
323 proteins insolubilisation rates for rested pasta are not significantly different from those of not
324 rested pasta. The creation of disulfide bonds during thermal treatment drives gluten protein
325 insolubilisation and the influence of weak interactions established during resting is then not
326 significant. HT drying cycle homogenizes the level of SDS-extractible proteins by forcing the
327 insolubilisation and creating large protein aggregates. [La Gatta et al. \(2017\)](#) suggested that the
328 architecture and size of protein aggregates created during drying depend on the nature and
329 proportion of the various classes of proteins. The hypothesis was made that supramolecular
330 structures derived from gluten protein aggregation could emerge during pasta drying and their
331 assembling occurs hierarchically. Due to gluten complexity, it is not always possible to predict
332 the formation of supramolecular polymeric structures.

333

334 4.2. Exchange reaction SS-SH during resting after extrusion at various temperatures

335 The water content of fresh extruded pasta is high enough (30% wet basis) to allow molecular
336 mobility of protein chains. During pasta extrusion, compression and shear forces would unfold
337 glutenin polymers facilitating their alignment and hydrophobic and H-bond pairing. Such type
338 of weak interactions, even if maintained after sample freeze-drying are going to be disrupted
339 during extraction in the SDS-buffer used here. Only irreversible changes involving covalent
340 bonds like peptide- or disulfide-bonds will be detected.

341 In this work it was found that resting at 20 to 40°C of freshly extruded pasta coincided with a
342 slight but significant glutenin polymers solubility loss. Polymers of the largest molecular weight
343 (F_1) reacted more readily than glutenin from F_2 . In less than 15 min, F_1 area decreased by 33%
344 against a drop of 16.4% in 26.9 min for the smaller glutenin polymers F_2 . An increase in resting
345 temperature (between 20 to 40°C) hastens the reaction from 30.3 min (20°C) to 11.3 min (30°C)
346 and 7.02 min (40°C). The corresponding activation energy ($E_a=26.4 \pm 7.2$ kJ/moles) lacks of
347 accuracy and additional experiments would be needed to consolidate it. Nevertheless, it remains
348 far from the E_a value reported for the gliadin solubility loss observed during pasta drying
349 (Lagrain, 2011). It is compatible with the activation energy needed to promote disulfide bond
350 reshuffling by thiol/disulfide exchange, which ranges between 30-40 kJ/moles (Liang, 2011).
351 After extrusion, stress relaxation of stretched glutenin polymers would drive this disulfide
352 reshuffling. The reaction also occurs when extruded pasta are directly placed into the
353 temperature monitored drying chamber. It accounts for the modest drop in glutenin polymers
354 observed at the beginning of LT drying. Because during this step temperature remains below
355 60°C, no further protein solubility loss induced by additional disulfide crosslinking was
356 observed. Indeed, it is well established that heating glutenins above 55°C and gliadins above
357 70°C allows for the disulphide/sulfhydryl exchange reactions that promote the creation of new
358 disulfide bonds (Lagrain, 2011).

359

360 4.3. Gluten proteins insolubilisation kinetic during resting at 30°C and HT drying

361 Resting extruded pasta leads to a specific loss of solubility of gluten protein fractions. Glutenins
362 F_1 and F_2 show a stronger loss of solubility (16.4-33.0%) compared to gliadins F_3 and F_4 (3.24-
363 7.65%) and cytoplasmic proteins F_5 (7.73%). High molecular weight glutenins (F_1) show the
364 strongest (33%) and the fastest ($C_{Times} = 11.3$ min) loss of solubility during the first 60 minutes
365 of rest. Low molecular weight glutenins (F_2) have a smaller (16.4%) and slower ($C_{Times} = 26.9$
366 min) loss of solubility upon resting. During resting, they become able to build weak interactions

367 between adjacent chains ([figure 4](#)), thus leading to a loss of solubility. This kinetic
368 insolubilisation of glutenins during resting occurs spontaneously, without mechanical nor
369 thermal stress. The water content of fresh extruded pasta is high (30% wet basis) enough to
370 allow molecular mobility of the protein chains. Glutenins insolubilisation during resting could
371 be due to the creation of hydrophobic interactions between adjacent chains, to form larger
372 aggregates. A slight increase of resting temperature (from 20 to 30°C) speeds up glutenins (F₁
373 and F₂) and gliadins (F₃ and F₄) spontaneous insolubilisation ([table 1](#)).

374 An increase of rest temperature might favor molecular mobility of protein chain in fresh pasta
375 and increase probability of efficient interactions. The contribution of covalent disulfide bonds
376 during resting is less probable, as their creation is driven by temperature higher than 55°C for
377 glutenins and higher than 70°C for gliadins ([Icard-Vernière & Feillet, 1999](#); [Wagner et al.,](#)
378 [2011](#)).

379 Gliadins (F₃ and F₄) and cytoplasmic proteins (F₅) are seldomly impacted by a resting period.
380 They experience very small losses of solubility and low insolubilisation rates during resting.
381 The sulfur-poor ω -type gliadins (F₃) show small losses of solubility while sulfur-rich α -, β - and
382 γ -gliadins (F₄) and cytoplasmic proteins (F₅), experience no loss of solubility. These low
383 molecular weight proteins are less likely to unfold during mixing and extruding stages.
384 Compared to glutenins, their open surface could be smaller, giving them few opportunities to
385 create inter-protein weak interactions and to insolubilize during resting ([figure 4](#)). Creation of
386 additional intra-gliadins weak interactions does not impact their solubility. The apparent loss
387 of solubility of cytoplasmic protein (F₅) might be induced by a linkage of proteins to other
388 components of semolina.

389 The spontaneous insolubilisation potential of gluten proteins upon resting may be linked to
390 durum wheat variety and gluten protein qualities ([Joubert et al., 2018](#)). A high glutenin/gliadin
391 ratio might be related to a strong capacity of spontaneous insolubilisation, as glutenins F₁ are
392 able to insolubilize during the resting period. It has been shown that pasta quality increases as
393 low molecular weight glutenins show a greater number of cysteines available for interactions
394 with other glutenins ([Lee et al., 1999](#)).

395

396 **4.4. Impact of a resting time on pasta quality**

397 Cooked pasta quality is not significantly impacted by resting, regardless of resting temperature
398 and time ([table 1](#)). HT drying confers identical texture qualities to rested and not rested pasta.
399 Gluten protein changes and additional weak interactions created during resting time do not have
400 a significant impact on HT dried pasta quality. Weak interactions contribution to pasta quality

401 is much lower compared to covalent bonds set up during drying. Dry pasta color and texture is
402 not impacted by a resting time before drying. Resting does not significantly impact enzyme
403 activity in fresh pasta, thus does not allow color changes.

404

405

406 **5. Conclusion**

407

408 Pasta resting after extrusion leads to gluten protein insolubilisation reactions by creation of
409 weak interactions. This spontaneous insolubilisation follows a first order kinetics and impacts
410 more glutenins compared to gliadins and cytoplasmic proteins. HT drying induces a strong drop
411 of solubility of all gluten protein fractions by creation of covalent bonds, impacting equally
412 rested and not rested pasta. Covalent bonds created during drying have a higher contribution to
413 cooked pasta quality compared to weak interactions established during resting time.

414

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420

421 **References**

422

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Table 1: Characteristics of dried pasta and cooked pasta produced by HT drying, LT drying, or coupling resting phase (for 120 min at different temperature 20, 30 or 40°C) and HT drying.

Products	Brightness L* (N/A)	Redness a* (N/A)	Yellowness b* (N/A)	Diameter (mm)	Cooking time (min)	Surface aspect (N/A)	Elastic modulus (MPa)	Rupture stress (Pa)	Rupture strain (N/A)	Viscoelasticity index (N/A)
<u>Pasta directly dried at low temperature</u>										
Dried pasta	54.1 ± 0.1 ^b	2.39 ± 0.03 ^b	42.4 ± 0.1 ^b	1.48 ± 0.04 ^a	10	N/A	N/A	N/A	N/A	N/A
Cooked pasta	79.8 ± 0.9 ^d	-0.533 ± 0.049 ^d	38.3 ± 0.3 ^d	2.68 ± 0.01 ^d	N/A	6	86.8 ± 8.0 ^a	153 ± 10 ^b	1.68 ± 0.21 ^b	8.63 ± 0.53 ^b
<u>Pasta directly dried at high temperature</u>										
Dried pasta	56.0 ± 0.3 ^a	3.43 ± 0.14 ^a	41.9 ± 0.2 ^a	1.53 ± 0.02 ^a	10	N/A	N/A	N/A	N/A	N/A
Cooked pasta	71.5 ± 0.6 ^c	0.203 ± 0.047 ^a	33.8 ± 0.3 ^c	2.38 ± 0.04 ^c	N/A	8	121.6 ± 6.4 ^b	271 ± 38 ^a	2.44 ± 0.32 ^a	14.4 ± 1.0 ^a
<u>Pasta rested for 120 min at 20°C before drying at high temperature</u>										
Dried pasta	55.2 ± 0.1 ^a	3.88 ± 0.02 ^a	39.9 ± 0.1 ^a	1.50 ± 0.03 ^a	11	N/A	N/A	N/A	N/A	N/A
Cooked pasta	81.7 ± 1.4 ^b	0.707 ± 0.076 ^c	38.0 ± 0.5 ^c	2.15 ± 0.07 ^b	N/A	8	159.4 ± 9.8 ^c	342 ± 67 ^a	2.05 ± 0.36 ^a	14.4 ± 1.1 ^a
<u>Pasta rested for 120 min at 30°C before drying at high temperature</u>										
Dried pasta	55.5 ± 1.7 ^a	5.36 ± 0.53 ^b	43.8 ± 0.7 ^b	1.47 ± 0.02 ^b	10	N/A	N/A	N/A	N/A	N/A
Cooked pasta	76.3 ± 4.6 ^b	0.803 ± 0.290 ^c		2.50 ± 0.04 ^d	N/A	8	113.2 ± 7.0 ^b	295 ± 44 ^a	2.62 ± 0.42 ^a	13.0 ± 1.7 ^a
<u>Pasta rested for 120 min at 40°C before drying at high temperature</u>										
Dried pasta	54.9 ± 0.8 ^a	4.29 ± 0.57 ^a	41.6 ± 1.4 ^a	1.47 ± 0.03 ^a	11	N/A	N/A	N/A	N/A	N/A
Cooked pasta	74.9 ± 5.1 ^c	0.988 ± 0.459 ^c	35.9 ± 0.3 ^c	2.36 ± 0.09 ^d	N/A	8	121.6 ± 8.9 ^b	263 ± 63 ^a	2.09 ± 0.38 ^a	12.4 ± 0.5 ^b

Rheological measurement and cooking times were carried out in triplicates. Eight measurements were made for color, diameter and surface aspect. N/A: Not applicable.

Figure 1: Evolution of relative contents of soluble proteins for the different wheat gluten protein fractions (F_1 to F_5) in pasta as a function of drying time during LT drying (a-b) or HT drying (c-d). *When not displayed (a;b), error bars (relative standard deviation) overlap.*

Figure 2: Evolution of relative contents of soluble glutenin fractions in pasta as a function of drying time*drying temperature during low temperature drying (LT) or high temperature drying (HT). *Error bar (relative standard deviation; not displayed) overlap.*

Figure 3: Evolution of relative contents of soluble proteins for the different wheat gluten protein fractions F_1 and F_2 in pasta as a function of time during resting at different temperatures (at 20, 30, or 40°C). *Error bars (relative standard deviation; not displayed) overlap.*

Figure 4: Evolution of relative contents of soluble proteins for durum wheat glutenin in pasta as a function of time during successive resting phase at 30°C (0 to 120 min) and HT drying (120 to 420 min). *Error bars feature the relative standard deviation.*

Figure 5: Mechanistic model of insolubilisation of wheat gluten proteins upon resting and upon high or low temperature drying in pasta.

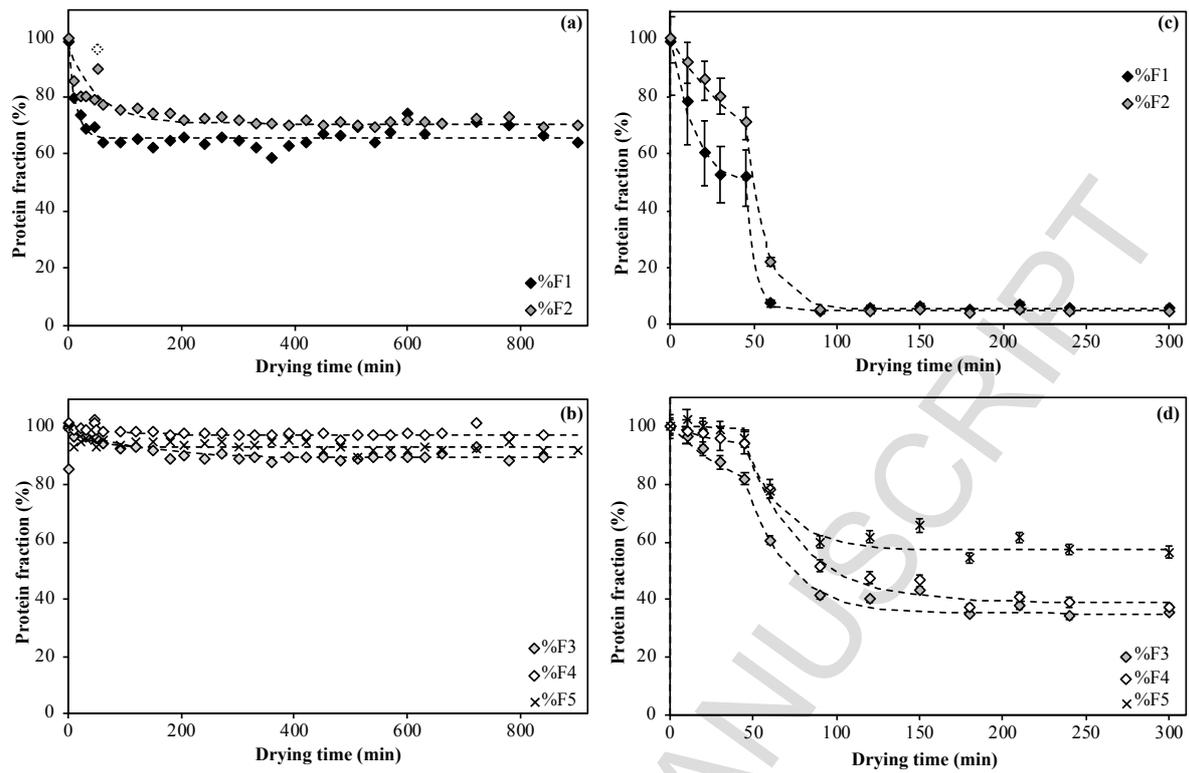


Figure 1: Evolution of relative contents of soluble proteins for the different wheat gluten protein fractions (F₁ to F₅) in pasta as a function of drying time during LT drying (a-b) or HT drying (c-d). When not displayed (a;b), error bars (relative standard deviation) overlap.

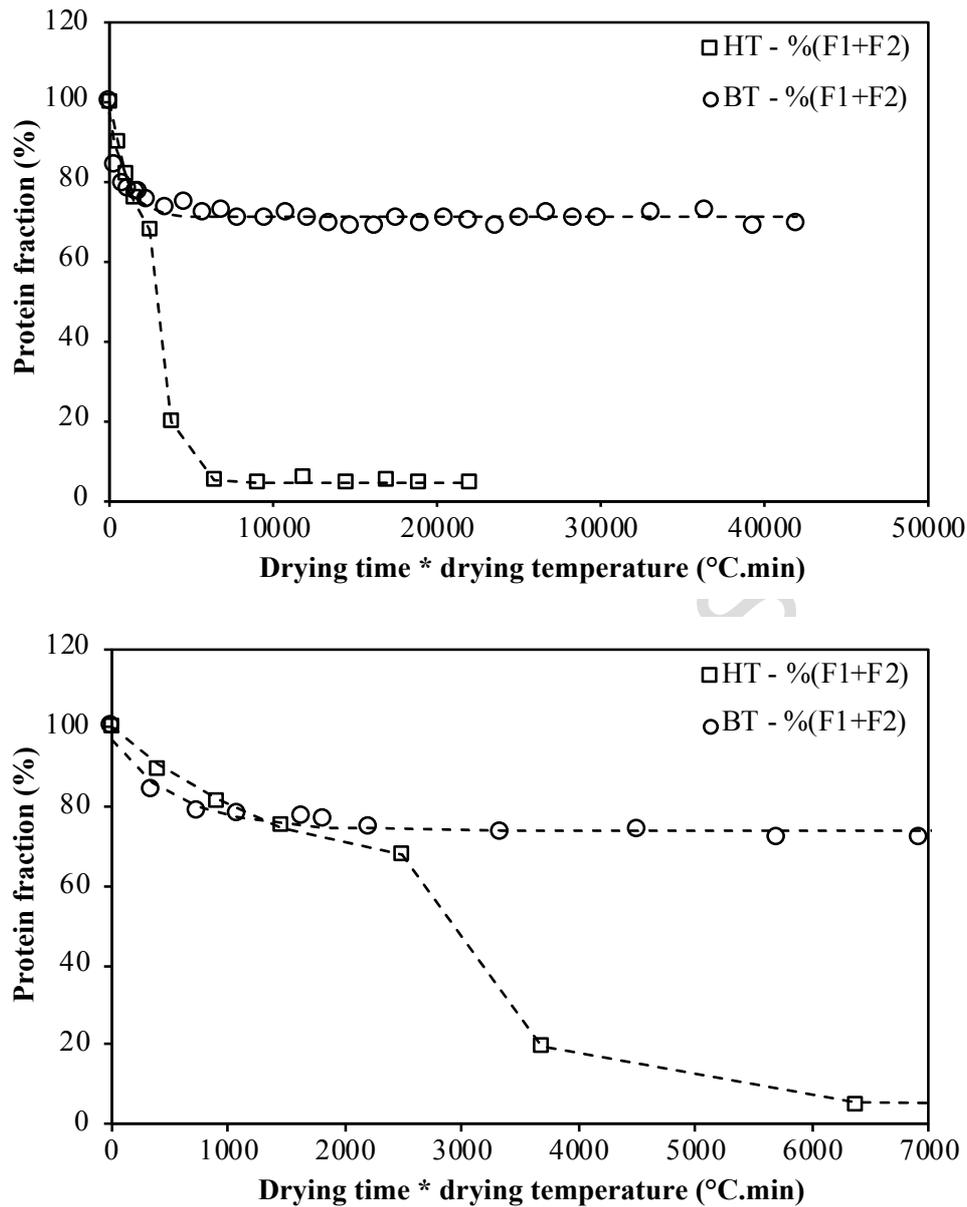


Figure 2: Evolution of relative contents of soluble glutenin fractions in pasta as a function of drying time*drying temperature during low temperature drying (LT) or high temperature drying (HT). Error bar (relative standard deviation; not displayed) overlap.

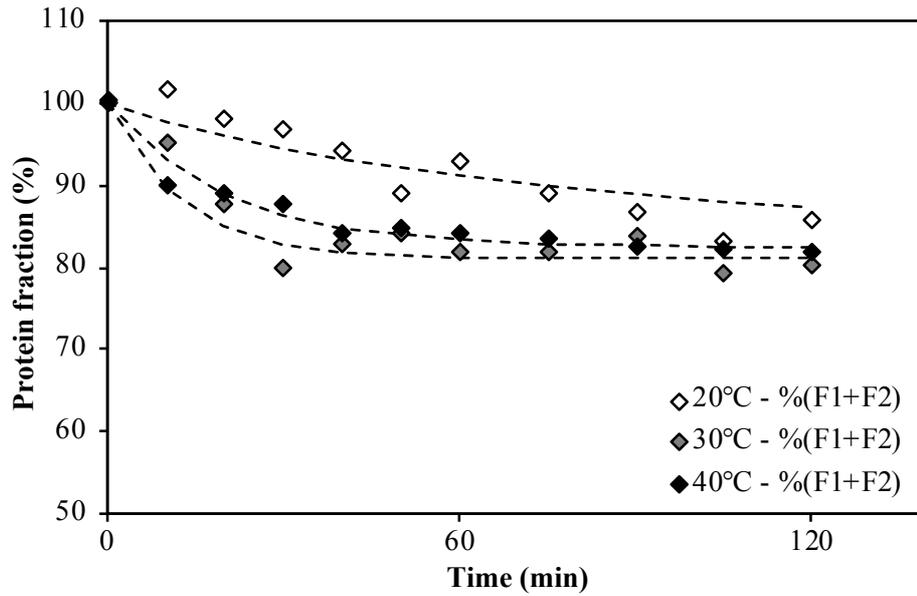


Figure 3: Evolution of relative contents of soluble proteins for the different wheat gluten protein fractions F_1 and F_2 in pasta as a function of time during resting at different temperatures (at 20, 30, or 40°C). Error bars (relative standard deviation; not displayed) overlap.

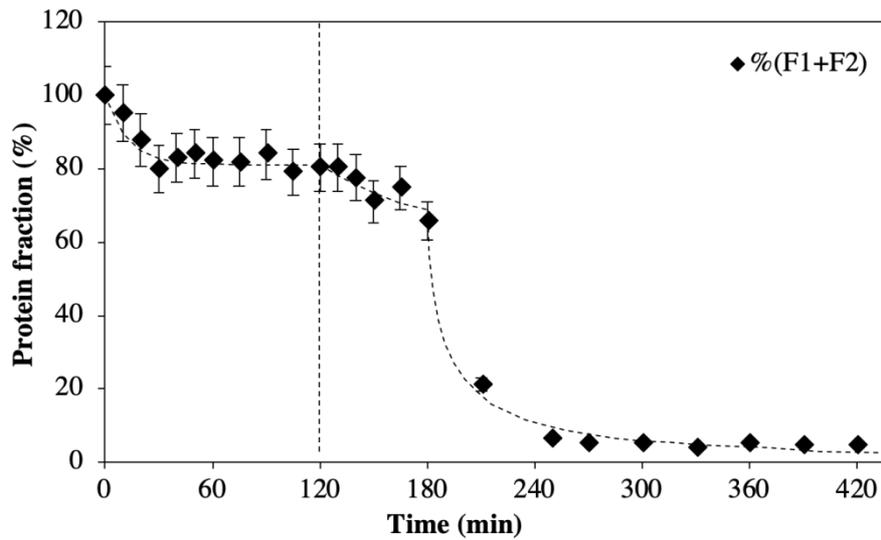


Figure 4: Evolution of relative contents of soluble proteins for durum wheat glutenin in pasta as a function of time during successive resting phase at 30°C (0 to 120 min) and HT drying (120 to 420 min). *Error bars feature the relative standard deviation.*

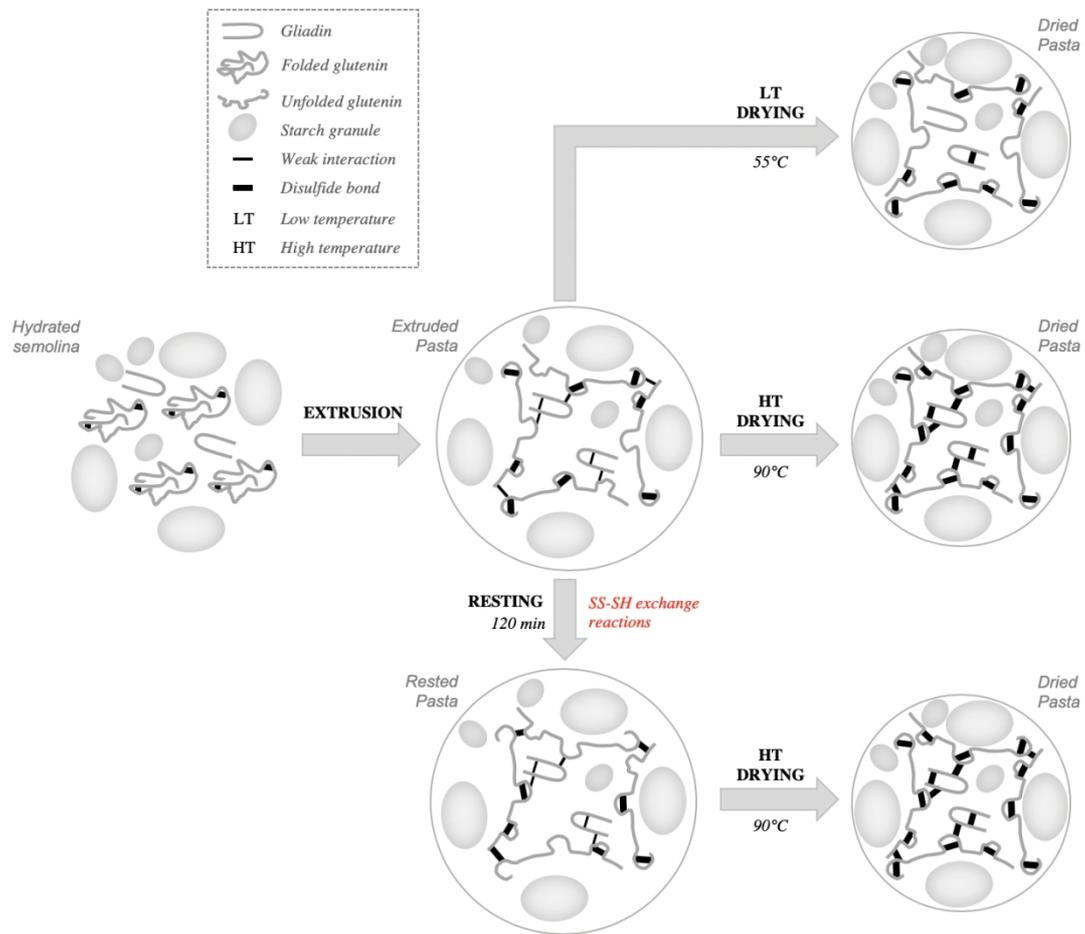


Figure 5: Mechanistic model of insolubilisation of wheat gluten proteins upon resting and upon high or low temperature drying in pasta.

Highlights

- Gluten protein insolubilisation upon resting follows a first order kinetics.
- High resting temperatures lead to higher levels of protein insolubilisation.
- Resting enables covalent bonds creation through SS-SH exchange reactions.
- High temperature drying has a higher impact on pasta qualities compared to resting.
- Proteins insolubilisation has no significant impact on cooked pasta properties.

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