

Title	Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread
Authors	Hoehnel, Andrea;Axel, Claudia;Bez, Jrgen;Arendt, Elke K.;Zannini, Emanuele
Publication date	2019-08-01
Original Citation	Hoehnel, A., Axel, C., Bez, J., Arendt, E. K. and Zannini, E. (2019) 'Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread', Journal of Cereal Science, 89, pp. 102816 (8 pp). doi: 10.1016/j.jcs.2019.102816
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0733521019301377 - 10.1016/j.jcs.2019.102816
Rights	© 2019, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC BY-NC-ND 4.0 licence. - https://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2026-09-22 17:35:37
Item downloaded from	https://hdl.handle.net/10468/8345

Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread

Andrea Hoehnel^a, Claudia Axel^a, Jürgen Bez^b, Elke K. Arendt^{a,c,*},
Emanuele Zannini^a

^aUniversity College Cork, School of Food and Nutritional Sciences, College Road, Ireland

^bFraunhofer Institute for Process Engineering and Packaging, 85354 Freising, Germany

^cAPC Microbiome Ireland, Cork, Ireland

doi: <https://doi.org/10.1016/j.jcs.2019.102816>

Keywords:

wheat bread, plant-protein, legumes, carob, lupin, faba bean, potato,
GlutoPeak

Abbreviations: HPI, high-protein ingredient; TDF, total dietary fibre; %DM, percentage based on dry matter; FWA, Farinograph water absorption; TM, Torque maximum; PMT, peak maximum time; PV, peak viscosity; FV, final viscosity; DF, damping factor; SV, specific volume; CA, correlation analysis; cc, correlation coefficient; PCA, principal component analysis; proteinE, percentage of calories provided by protein

*Corresponding author. Tel: +353 21 490 2064; Fax: +353 21 427 0213; Email address: e.arendt@ucc.ie (E. K. Arendt)

Abstract

The orientation of consumers and industry towards plant-based foods on one hand and high-protein products on the other is persistently increasing. Bread, as a staple food, is a promising matrix for the incorporation of plant-based high-protein ingredients to combine both trends. This study aims to provide a better understanding of techno-functional changes and impacts of plant-proteins during bread production, which could advance the development of high-quality products with high levels of plant-protein. A selection of high-protein ingredients from wheat, maize, potato, carob, pea, lupin and faba bean were subjected to compositional analysis and applied in wheat bread formulations, replacing 15 % of wheat flour. Their impact on dough properties (gluten-aggregation, pasting behaviour, rheology) as well as bread quality (volume, crumb structure, crumb hardness) was analysed. The high-protein ingredients were found to affect gluten-aggregation, pasting and bread characteristics. Results indicated a weakened gluten-network in doughs containing potato and pea protein. Also pasting behaviour was mostly affected by the potato protein suggesting a heat induced improvement of its baking performance. Good bread quality, represented by high specific volumes and low crumb hardness, was observed for gluten, zein and carob. Breads with pea, lupin and faba bean showed only slightly inferior quality characteristics.

1 1. Introduction

2 Plant proteins are a growing market in the food sector and represent an
3 opportunity to meet nutritional needs of the growing world population, while
4 at the same time realising the transition to a more sustainable food produc-
5 tion. Thus, the demand from consumers, industry and governmental institu-
6 tions for high-quality food with high contents of plant-protein is substantial.
7 Bread plays an important role in the human diet and relatively large amounts
8 are consumed worldwide (Henchion et al., 2017). It is therefore a promising
9 matrix for the incorporation of plant-protein ingredients in order to supply
10 a broad range of customers with plant-based high-protein food. Definitions
11 and requirements for the attribute 'high-protein' can be found in many reg-
12 ulations all over the world and they vary greatly. According to regulation
13 (EC) No 1924/2006, a product qualifies for the claim 'high in protein' if 20 %
14 of its calories are provided by proteins. In order to reach this protein content
15 while maintaining adequate product quality, plant-based ingredients with
16 high protein contents can be utilised. Protein ingredients from plant sources
17 are usually classified by their protein content: as flours (protein < 65 %DM),
18 concentrates (protein > 65 %DM) and isolates (protein > 90 %DM) (Boye
19 et al., 2010). However, the terminology is sometimes misleading since es-
20 pecially isolated legume protein products often do not reach a protein level
21 of 90 %DM but are still considered isolates regarding their production pro-
22 cedure (Arntfield and Maskus, 2011). Hence, this study will focus on the
23 generalised term high protein ingredients (HPIs). The partial substitution of
24 wheat flour for bread production by HPIs from numerous sources has been
25 previously investigated, including dairy proteins (Kenny et al., 2000); cereals

(Bugusu et al., 2002), pseudo-cereals (Sanz-Penella et al., 2013) and legumes (Villarino et al., 2015; Turfani et al., 2017; Marchais et al., 2011). However, these studies are usually focused on one type of plant-protein. This study aims for a comparison of HPIs from different plant sources and investigates their impact on wheat bread formulations and their suitability to produce high-protein quality breads. The selected HPIs are the cereal proteins gluten (wheat) and zein (maize), potato protein, and proteins from the following legumes: carob (*Ceratonia siliqua*), pea (*Pisum sativum*), lupin (*Lupinus angustifolius*) and faba bean (*Vicia faba*). While some of these ingredients are commercially available and well characterised, others are produced from emerging protein sources and their properties and potential for bakery applications need to be explored. Wheat-gluten can be described as a group of proteins, which are mainly responsible for the structure-forming ability of wheat flour during dough production and baking. Gluten has unique viscoelastic properties, protein-protein interactions, water holding capacity and thermosetting characteristics (Day, 2011). It is widely used as bread improver in the baking industry. Zein is the prolamine fraction originating from maize-proteins. Amongst other cereal-prolamines, it exhibits the highest content of hydrophobic amino acids and thus a very high protein hydrophobicity and low water solubility (Belitz et al., 1986). Potato proteins can be obtained from the side-product potato juice from potato starch production and therefore represent an economically promising ingredient for plant-based high-protein foods. Previous studies report high water solubility and indicate outstanding techno-functional properties including heat induced gelling (Alting et al., 2011). Legumes are generally accepted to be beneficial

51 with regard to environmental aspects, due to their ability to fix nitrogen,
52 and from a nutritional perspective (Henchion et al., 2017). While cereal pro-
53 teins exhibit high contents of sulphur-containing amino acids and are low
54 in other essential amino acids (e.g., lysine), legumes represent the opposite
55 and are therefore favourable for incorporation in cereal products to balance
56 the amino acid profile and increase protein quality (Henchion et al., 2017).
57 Important protein ingredients from legumes are protein-rich flours (obtained
58 by dry-processing) and isolates (produced by wet-processing). Dry-processed
59 (i.e., air-classified) products exhibit native protein characteristics and lower
60 protein contents, whereas protein isolates have a higher protein-purity and
61 altered properties conditioned by the isolation procedure (Schutyser et al.,
62 2015). Furthermore, isolates mainly consist of the protein fractions albumins
63 and globulins. An exceptional legume protein is carob germ protein, which
64 contains a high glutelin-fraction (about 68 %) called caroubin. This protein
65 has been reported to have gluten-like properties (Smith et al., 2010; Feillet
66 and Roulland, 1998). The objective of this study is to characterise protein-
67 rich fractions of a wide range of plant sources and to evaluate their impact
68 on dough and bread quality characteristics.

69 **2. Experimental**

70 *2.1. Material*

71 Five commercially available high-protein ingredients (HPIs) were used in
72 this study. Potato protein isolate (Patissonate 306 P) was obtained from
73 Avebe, the Netherlands; pea protein isolate (NUTRALYS PEA BF) from
74 Roquette, France; carob germ flour (GRINDSTED VEG PRO S1) from

75 Danisco, UK; vital gluten (NUTRALYS W) from Roquette, France; and corn
76 protein (Zein) from Flo Chemical Corporation, Massachusetts, US. Addition-
77 ally, two HPIs: blue lupin protein isolate and faba bean flour (fine fraction,
78 protein-rich) were experimentally produced and provided by Fraunhofer In-
79 stitute IVV, Freising, Germany. Wheat flour was supplied by Whitworth
80 Bros Ltd, UK; dry yeast (4 % moisture, 50 % protein, 5 % fat, 40 % car-
81 bohydrates) by Puratos, Belgium; salt by Glacia British Salt Ltd, UK; and
82 vegetable oil by Musgrave, Ireland. Chemicals were purchased from Sigma-
83 Aldrich (Missouri, USA) unless stated otherwise.

84 *2.2. Compositional analysis*

85 HPIs and wheat flour were subjected to compositional analysis including
86 determination of protein (nitrogen-to-protein conversion factor 6.25, based
87 on MEBAK 1.5.2.1), dry matter/moisture (air-oven method at 130 °C until
88 constant mass reached), ash (incineration in muffle furnace at 550 °C for
89 5 h, charred prior to muffling using open flame, based on AOAC 923.03)
90 and fat (Soxhlet method using SoxCap and Soxtec (Foss UK Ltd, UK)),
91 digestion with 4 M HCl prior to extraction, based on AACC 30-25.01). The
92 analysis of total dietary fibre (TDF) was performed by Concept Life Science
93 Ltd in accordance with AOAC 991.43. The carbohydrate value was obtained
94 by subtraction (i.e., $100\% - [\text{protein}\% + \text{moisture}\% + \text{ash}\% + \text{fat}\% + \text{TDF}\%]$).
95 Total starch content was analysed using the enzyme kit K-TSTA supplied by
96 Megazyme, Ireland. All values, except the moisture content, are expressed
97 as percentage based on dry matter (%DM).

98 *2.3. Empirical dough analysis*

99 Properties of wheat flour and mixtures of wheat flour and HPIs (HPI/flour
100 blends) in a ratio of 85 % to 15 %, respectively, were analysed. Moisture con-
101 tents of blends were calculated considering the determined moisture contents
102 of wheat flour and HPIs according to their ratios.

103 *2.3.1. Farinograph*

104 Water absorption (FWA) was determined by Farinograph (Brabender
105 GmbH and Co KG, Duisburg, Germany) water absorption (FWA) was deter-
106 mined following AACC 54-21.02. Titration trials were performed adjusting
107 doughs from wheat flour and HPI/flour blends to a consistency of (500 ± 20)
108 Farinograph units (FU).

109 *2.3.2. GlutoPeak test*

110 Gluten-aggregation properties of wheat flour and the HPI/flour blends
111 were investigated using the GlutoPeak (Brabender GmbH and Co KG, Duis-
112 burg, Germany). This device applies high shear to a flour/water slurry. 9 g
113 flour (based on 14 % moisture; adjustments according to AACC 82-23.01)
114 were added to 9 g of deionised water (36 °C) weighed into the sample cup
115 (adjusted to reach a slurry weight of 18 g). The measurement was started im-
116 mediately after a brief premixing step. The paddle speed was set to 2750 rpm
117 and water circulating through the jacketed sample cup maintained the sam-
118 ple temperature at 36 °C. The torque reading was recorded over time. The
119 maximum test time was set to 10 min and measurements were stopped ap-
120 prox. 30 to 50 s after detection of the major peak. The curve was evaluated
121 by the software provided with the instrument regarding Torque Maximum

122 (TM, expressed in Brabender units BU) and Peak Maximum Time (PMT,
123 expressed in s).

124 2.3.3. *Rapid visco analysis*

125 The pasting behaviour was examined using Rapid Visco Analysis (RVA
126 Super 3, Newport Scientific, Warriewood, Australia) following AACC 76-
127 21.02. A heating profile was applied: equilibration at 50 °C for 1 min, heating
128 to 95 °C at 0.2 °C/s, holding at 95 °C for 162 s, cooling to 50 °C at 0.2 °C/s,
129 maintaining at 50 °C for 120 s. The pasting variables peak viscosity (PV),
130 setback and final viscosity (FV) were determined from the viscogram using
131 manufacturer-supplied software.

132 2.4. *Recipe adaptation and bread production*

133 Bread samples were prepared according to the formulation in Table 1.
134 For incorporation of HPIs, 15 % of wheat flour were replaced. Water levels
135 were applied as determined by Farinograph trials - FWAs (Table 3). Baking
136 properties of the HPIs were evaluated by preparation and examination of
137 micro-scale bread loafs (based on 65 g of dough). The straight dough method
138 was applied. After activating the yeast by dissolving in 30 °C tap water for
139 10 min, the yeast suspension was added to the remaining, previously weighed
140 ingredients. A total dough amount of 630 g was prepared by mixing with a
141 Kenwood Chef (Kenwood Manufacturing Co. Ltd., UK) kitchen machine at
142 speed 1 and 2 for 1 and 7 min, respectively. After dividing the dough into
143 nine pieces of 65 g $\pm$ 1 g, the pieces were moulded (very sticky doughs were
144 shaped with a dough scraper), put into baking tins and proofed for 75 min at
145 85 % humidity and 30 °C (KOMA BV Sunriser, Reormond, the Netherlands).

146 Baking was performed in a deck oven (MIWE Condo, Arnstein, Germany)
147 at 210 °C top and bottom temperature for 14 min with steaming the baking
148 chamber prior to loading with 700 ml and open draft throughout the whole
149 baking process. Breads were removed from tins and left on a grid at ambient
150 temperature for 1 h to cool down. The presented values represent the mean
151 of three independently performed baking trials.

152 2.5. Dough rheology

153 Dough was produced according to the procedure reported in section 2.4,
154 but yeast was omitted. Dough was allowed to rest for about 10 min before
155 measurements. Viscoelastic properties of doughs were examined by using a
156 stress/strain-controlled rotational rheometer (MCR 301 Anton Paar, GmbH,
157 Germany) equipped with a PP50 parallel-plate measuring system (serrated
158 surface to avoid slippage). The lower plate was set to 30 °C. After positioning
159 10 g of dough between the plates, the upper plate was lowered to 1.025 mm,
160 the sample trimmed and a layer of mineral oil applied to prevent desiccation.
161 The final gap was set to 1 mm prior to starting the test. The rheological
162 variables storage modulus (G'), loss modulus (G'') and damping factor ($DF =$
163 $\tan\delta = G''/G'$), describing viscoelastic dough properties, were obtained from
164 frequency sweeps ($\omega = 100$ -0.1 Hz; data obtained at angular frequency of
165 2.54 Hz for control and 2.58 Hz for HPI doughs, respectively) A constant
166 strain of 0.01 % (linear viscoelastic range previously determined by amplitude
167 sweeps) was applied.

168 2.6. Bread characteristics

169 Specific volume (SV) was measured with a Volscan Profiler (Stable Micro
170 Systems, Surrey, UK). To analyse crumb structure and hardness, three slices
171 (25 mm) were cut out of the middle of each of 3 loaves. A C-Cell Imaging
172 System (Calibre Control International Ltd, UK) was used to capture images
173 of the slices and to determine the variables: number of cells and area of cells.
174 Crumb hardness was analysed with a TA-XT2i Texture Analyser (Stable
175 Micro Systems, Surrey, UK) equipped with a 25 kg load cell. A 20 mm
176 cylindrical probe was used to compress the centre of the slice to 40 % of its
177 height as part of a texture profile analysis (TPA): test speed 5 mm/s, post-
178 test speed 10 mm/s, trigger force 0.05 N, waiting time between compressions
179 5 s. Lightness of crust (L^*) was measured by a Colorimeter CR-400 (Konica
180 Minolta, Japan) using the CIE $L^*a^*b^*$ colour space.

181 2.7. Statistical analysis

182 All measurements were performed in triplicate. Data analysis was carried
183 out using R (version 3.5.1). One-way ANOVA with post-hoc pairwise Tukey
184 test was used to show significant differences ($p < 0.05$). A correlation analy-
185 sis (CA, significance level $p < 0.05$) determining correlation coefficients (cc)
186 as well as a principal component analysis (PCA) was conducted using the
187 main technological properties of doughs and breads to evaluate and visualise
188 correlations and identify groups among control and HPI formulations.

189 3. Results

190 3.1. Composition

191 The protein content determined for wheat flour is 14.09 %DM (Table 2).
192 High-protein ingredients show values at least 3.5 times higher. The lowest
193 levels were obtained for carob (55.04 %DM) and faba bean (61.25 %DM). Pea
194 and gluten contain 80.19 %DM and 83.11 %DM protein, respectively, repre-
195 senting the medium-protein-level HPis. Zein, potato and lupin show protein
196 values over 90 %DM ranging from 91.79 %DM for zein to 94.51 %DM for
197 lupin. In spite of different protein levels, the authors decided for a constant
198 replacement of 15% wheat flour in order to reach a comparable dilution of
199 wheat gluten and starch. The determined fat content of gluten, potato and
200 carob was < 1 %DM. Wheat flour (1.97 %DM), zein (2.66 %DM), lupin
201 (2.94 %DM), faba bean (3.81 %DM) and pea (6.45 %DM) are slightly higher
202 in fat. Potato, gluten and zein show low ash contents between 0.05 %DM
203 (potato) and 1.16 %DM (zein), similar to wheat flour with 1.09 %DM. In
204 contrast to this, ash levels of faba bean, lupin, pea and carob range from
205 5.43 %DM (faba bean) up to 7.04 %DM (carob). Total dietary fibre ac-
206 counts for 2.88 %DM in zein, 2.30 %DM in wheat flour and less in the other
207 HPis. Carob however contains 17.67 %DM of fibre. A total starch content of
208 72.38 %DM was found for wheat flour. HPis contain less than 0.2 %DM of
209 starch; except gluten and faba bean, which show starch values of 4.95 %DM
210 and 7.77 %DM, respectively. In order to interpret the protein levels of control
211 and HPI formulations, percentages of calories provided by protein (proteinE)
212 were calculated. This was achieved by taking into consideration the compo-
213 sitional data of all ingredients (Table 2) as well as the ingredient ratios in the

formulations (Table 1). Protein accounts only for 14.48 % of calories in the control formulation. All HPIs formulations show values higher than 20 % ranging from 21.23 % (carob) to 26.64 % (lupin).

3.2. Empirical dough analysis

3.2.1. Farinograph

In wheat dough production, the amount of water added and the resulting dough consistency are important parameters to allow sufficient distribution and hydration of dough materials and gluten-network development. The obtained FWAs (Table 3) show significant differences and indicate different water absorptions of HPIs and different abilities to compete for water with wheat flour. For wheat flour a FWA of 63.0 % was determined. Except for zein (60.8 %) and faba bean (62.2 %), all HPI/flour blends absorb significantly more water than wheat flour. A moderate increase was observed for potato (65.2 %) and lupin (66.2 %). Carob, gluten and pea caused the highest FWAs accounting for 69.8 %, 70.2 % and 71.7 %, respectively.

3.2.2. GlutoPeak test

During GlutoPeak measurements, a high speed rotating element subjects the sample slurry to an intense mechanical action. This allows gluten-aggregation and network-formation. The result is an increase in recorded torque, followed by a decline due to a destruction of the network by further mixing. Considering the small sample sizes and short measuring times, the GlutoPeak test is ideal to provide valuable information about rheological properties of HPI/flour blends. Measurements for the gluten formulation could not be performed with the reported method due to exceeded maxi-

238 mum torque of the device. Figure 1B schematically shows a typical Gluto-
239 Peak curve of wheat flour for comparison and explanation of variables. The
240 torque curve observed for wheat flour (Figure 1A) slightly rises and reaches
241 the equilibrium plateau in an initial phase. The following strong increase in
242 torque reaches the TM of 74 BU at a PMT of 53 s. The replacement of 15 %
243 of wheat flour by HPis leads to aggregation profiles entirely different to the
244 typical curve shape. Significantly different PMTs and TMs were obtained.
245 Pea shows an immediate increase in torque within the first few seconds of
246 the test and is lacking the equilibrium plateau. The TM of 83 BU is reached
247 after 16 s in form of a sharp peak. After the maximum, a small decrease of
248 torque was observed. In contrast to this, zein exhibits a curve with a small
249 equilibrium plateau at the start and two broad peaks. A TM of 48 BU was
250 detected after 105 s. The overall curve shape of carob with two broad peaks
251 and a slightly pronounced equilibrium plateau is similar to zein. However,
252 the curve obtained for carob appears much more condensed, TM is higher
253 (58 BU) and PMT lower (41 s). The aggregation profiles of lupin and faba
254 bean both exhibit a slightly pronounced equilibrium plateau and a sharp
255 peak, followed by a slight decrease in torque and a small shoulder. The TM
256 determined for lupin is with 59 BU higher than for faba bean (51 BU). Peak
257 maximum times accounting for 34 s (lupin) and 33 s (faba bean) and are
258 not significantly different from each other. The GlutoPeak curve of potato is
259 lacking the equilibrium plateau. Torque increases immediately and reaches
260 the maximum of 54 BU after 17 s which is pronounced as a steady state
261 rather than a sharp peak. It is followed by a very strong decrease in torque.

262 3.2.3. *Rapid visco analysis*

263 Rapid visco analysis of wheat flour and HPI/flour blends was performed
264 to investigate the impact of HPIS on pasting properties and their behaviour
265 during heating. Significant differences were observed amongst the HPI/flour
266 blends and compared to wheat flour (Table 3). Except for potato, all HPIS
267 lead to a decrease in PV compared to wheat flour with 2390 cP. The lowest
268 PVs from 1415 cP (zein) to 1540 cP (lupin) were obtained for zein, lupin,
269 pea and carob. Slightly higher PVs (but smaller than wheat flour) were
270 measured for gluten (1683 cP) and faba bean (1852 cP). Only the HPI/flour
271 blend containing potato showed no significant difference in PV compared
272 to wheat flour. Setbacks of all HPI/flour blends are lower than for wheat
273 flour (1282 cP). However, potato (1058 cP), faba bean (1158 cP) and lupin
274 (1161 cP) show setbacks in the same range as wheat flour. The other HPIS
275 exhibit much smaller values between 680 cP (pea) and 877 cP (zein). De-
276 termined FVs range from 1589 cP (pea) up to 2544 cP (wheat flour). Final
277 viscosities of all HPI/flour blends were lower than for wheat flour (2544 cP).
278 While potato has a PV similar to wheat flour, it shows a significantly lower
279 FV.

280 3.3. *Dough rheology*

281 Control and HPI doughs (full recipe, yeast omitted) were subjected to
282 oscillatory tests in order to obtain information about their viscoelastic prop-
283 erties. A DF of 0.401 (Table 3) was obtained for the control. All doughs
284 containing legume HPIS have a significantly lower DF. The decline is more
285 pronounced for pea (0.325), carob (0.327) and faba bean (0.329) than for
286 lupin (0.349). The lower DFs represent a more elastic behaviour of these

287 doughs compared to the control. The DFs of zein (0.387) and gluten (0.385)
288 are not significantly different from the control. The highest DF was observed
289 for potato, representing the sample with the most viscous behaviour.

290 3.4. *Quality characteristics of breads*

291 A visual evaluation of the breads (Figure 2) reveals striking differences
292 in colour, size and crumb structure. This observation was confirmed by
293 determination of bread quality characteristics. The SV of the control bread
294 is 2.55 ml/g (Table 3). The gluten bread reaches a SV of 3.81 ml/g, which is
295 higher than the SV of all other breads. Breads from lupin (1.98 ml/g) and
296 pea (2.00 ml/g) are smaller than the control; breads containing faba bean
297 (2.26 ml/g), zein (2.63 ml/g), carob (2.73 ml/g) and potato (2.81 ml/g) are
298 not significantly different from the control. However, significant differences
299 were observed within the latter group and between this group and the smaller
300 breads with lupin and pea. The results for crumb structure variables show
301 few significant differences. The number of cells ranges from 1543 (faba bean)
302 to 1902 (gluten). The cell areas lie between 44.5 % (pea) and 49.0 % (gluten).
303 Potato, faba bean and gluten exhibit larger cells than the pea bread. With a
304 hardness of 4.12 N, the gluten bread is softer than the control (11.81 N). In
305 contrast to this, potato (19.02 N) and lupin (20.11 N) lead to a harder crumb.
306 Carob (7.84 N), zein (15.10 N), faba bean (20.11 N) and pea (16.68 N) breads
307 are not significantly different in hardness compared to the control. The
308 darkest crust was observed for faba bean (57.07) and the lightest for zein
309 (72.80). Crusts of the control (71.84) and the potato (70.58) bread exhibit
310 a lightness similar to the zein bread. Carob, lupin, gluten and pea breads
311 represent moderate L^* values ranging from 62.33 (carob) to 66.83 (pea).

312 3.5. *Principal component analysis*

313 A PCA was performed based on selected dough and bread characteristics.
314 The first Dimension mainly represents hardness, cell area, number of cells and
315 SV, whereas Dimension 2 models a measure of DF, PV and FV. Hierarchical
316 classification was used to divide the formulations into 5 groups. Cluster A in
317 the upper half of the diagram represents the control and potato formulation
318 and is characterised by high PV and FV. Clusters B and C in the bottom left
319 comprise formulations containing legumes which exhibit low DFs, SVs and
320 higher hardness. Zein and carob formulations represent cluster D with low
321 PV and FV but medium SV and hardness. The gluten formulation forms
322 cluster E and is clearly separated from the other groups by its very high SV
323 and area of cells as well as low hardness.

324 4. Discussion

325 The objective of this study is to evaluate and compare the performance
326 of different HPIs in wheat bread formulations and their potential for the
327 production of high-protein breads. The proteinE was calculated based on
328 compositional results and exceeds 20 % for all HPI formulations in this study
329 (Table 2). Compositional changes occurring during proofing and baking (i.e.,
330 starch degradation, sugar consumption by yeast, denaturation and degrada-
331 tion of proteins (Rosell, 2011)) will slightly change the proteinE of breads
332 compared to formulations. Nevertheless, the values suggest a great poten-
333 tial for these formulations to produce high-protein breads in accordance with
334 regulatory requirements in Europe. Next to legal compliance, an under-
335 standing of the effects leading to different qualities of high-protein breads is

important for further product prototyping. The proteins present in the HPIs are likely to affect the characteristics of the formulations depending on their structural and functional properties and by potential interactions with wheat flour components (protein impact). Additionally, the replacement of 15 % of wheat flour leads to a dilution of the technologically most important flour components gluten and starch (dilution effect); and the presence of minor components in the HPIs can also influence dough and baking characteristics.

4.1. *Water absorption*

One major impact of the HPIs on the formulations is a change in their water absorption to reach a certain consistency. In spite of the dilution effect on gluten and starch, an overall trend towards increased FWAs was observed because HPIs mainly contain proteins, which absorb water themselves. The partial substitution of wheat flour by legume protein isolates or flours, namely carob, pea and lupin, has been reported to increase FWAs (Turfani et al., 2017; Marchais et al., 2011; López, 2014). An increase of protein content in wheat flour by 1 % is expected to cause an average FWA increase of 1 % (Sluimer, 2005). This is in accordance with the FWA increase of 7 % for the gluten/flour blend, whose protein content is 9.6 % higher than for the wheat flour. However, a general correlation between total protein contents of HPI/flour blends and FWAs could not be observed (cc: 0.19). In contrast to the general trend, zein caused a FWA decrease. This can be explained by its high insolubility combined with a very high number of hydrophobic amino acids and overall protein hydrophobicity (Belitz et al., 1986) leading to a low water absorption. No significant change in FWA was observed for faba bean. This difference, especially compared to the other legume HPIs, might

361 be due to its relatively low protein content and its production procedure.
362 Air-classification of legumes, compared to wet protein isolation procedures,
363 usually produces ingredients with higher solubility (Schutyser et al., 2015),
364 which could also lead to lower water absorption.

365 4.2. Impact of HPIs on gluten-aggregation

366 While GlutoPeak measurements have been utilised to investigate wheat
367 flour quality and differences in wheat protein properties, they, to the best
368 of our knowledge, have not been used to determine aggregation behaviour
369 of wheat flour blends containing non-wheat HPIs. High-quality wheat flours
370 with higher protein contents (i.e., higher gluten contents) usually exhibit ear-
371 lier and higher peaks than weak flours in GlutoPeak curves due to a stronger
372 gluten-network (Amoriello et al., 2016). Based on this, measurements with
373 HPI/flour blends are expected to show lower and later peaks compared to the
374 wheat flour (dilution effect). Even though the results in this study do show
375 an overall tendency towards lower TMs, the majority of the peaks exhibit
376 earlier instead of later PMTs (except zein). This suggests that there are other
377 factors affecting the gluten-aggregation in HPI/flour blends. According to
378 Bouachra et al. (2017), who reported an impact of lactic acid on GlutoPeak
379 curves, the presence of (positive) charges in the sample influences gluten-
380 aggregation. An increased number of charges leads to stronger intramolecu-
381 lar repulsive forces which promotes faster unfolding of gluten molecules and
382 therefore accelerates gluten-aggregation. At the same time, the increased
383 presence of charges in the sample causes a more rapid breakdown of gluten
384 because of higher intermolecular repulsive forces and the hindrance of the
385 formation of new bonds. The applied HPIs differ in their solubility and con-

386 tent of charged amino acids, which are both factors to increase the number
387 of available charges in the sample slurry. Legumes and potato exhibit high
388 contents of charged amino acids; additionally, potato proteins are highly wa-
389 ter soluble (Boye et al., 2010; Arntfield and Maskus, 2011; van Gelder and
390 Vonk, 1980; Alting et al., 2011). Zein on the other hand, contains only few
391 charged amino acids and has a very high overall hydrophobicity (Belitz et al.,
392 1986). In accordance with the present results, the content of charged amino
393 acids in combination with protein solubility seems to be the major influence
394 of HPIs on PMTs. A flour with a fast build-up of gluten-network, sharp
395 gluten-peak and rapid breakdown is considered weak, whereas a longer PMT
396 seems to allow for a fully developed and more stable gluten-network (Gold-
397 stein et al., 2010). This implies a relatively weak gluten-network with pea and
398 potato; medium strength with faba bean, lupin and carob; and high strength
399 with zein. The TM appears to be mainly related to the water absorption of
400 HPIs. A good correlation between FWA and TM was found (cc: 0.86) when
401 comparing HPI/flour blends (wheat flour exempt because of different gluten
402 content). Furthermore, interactions between non-wheat proteins and gluten
403 can occur and affect gluten-aggregation. Curves of all HPI/flour blends (ex-
404 cept potato) exhibit a more or less pronounced second peak. This could
405 be caused by interactions between gluten and HPI proteins subsequent to
406 gluten-aggregation and during breakdown, delaying a rapid torque decrease.
407 In general, synergistic interactions between different food proteins forming
408 a stronger coaggregated network have been previously reported (Lin et al.,
409 2017). Bugusu et al. (2002) showed interactions between zein proteins and
410 gluten in wheat dough systems using confocal microscopy after fluorescence

labelling of the proteins. Feillet and Roulland (1998) described caroubin, the main protein fraction in carob, as a gluten-like protein with similar rheological properties and similar protein interactions. Since zein and carob are the HPIs which lead to the most pronounced second peak in the GlutoPeak curve, a synergistic network-formation of these proteins with gluten can be hypothesised.

4.3. Impact of heat treatment on HPI/flour blends

The behaviour of a dough and its constituents during and after heat treatment is of major importance with regard to the baking process. Rapid visco analysis provides information on heat induced changes in viscosity of flours suspended in excess water. In wheat flour samples, it is mainly a tool to investigate starch gelatinisation properties. In HPI/flour blends, the starch content is substantially reduced (dilution effect). Less available starch in the samples leads to lower viscosities. This is in accordance with the generally lower PVs observed for HPI/flour blends compared to wheat flour. Only for gluten and faba bean, which contain considerable amounts of starch (4.95 % and 7.77 %, respectively), the decrease in PV is less pronounced. A positive correlation between the calculated sample starch content and PV was detected (cc: 0.950; potato exempt). Also López (2014) reported a decreased PV in RVA when partially replacing wheat flour by plant-protein isolates. Marco and Rosell (2008) observed the same trend in rice-flour systems. The results of the present study suggest a unique pasting behaviour of potato protein in mixture with wheat flour. In spite of a much lower starch content, a PV as high as for wheat flour and a relatively low Setback and FV were measured. Marco and Rosell (2008) proposed an impact of heat induced

436 gelation of non-wheat proteins on PV and setback of rice flour/plant-protein
437 isolate mixtures. Although literature on gelling behaviour of potato proteins
438 is scarce, some of the protein fractions (e.g., patatin) have been reported to
439 form strong gels with properties similar to ovalbumin and beta-lactoglobulin
440 (Alting et al., 2011).

441 4.4. *Viscoelastic dough properties as affected by HPIs*

442 The DFs reveal a clear grouping of the samples by botanical source of the
443 proteins. All legume proteins lead to lower DF, doughs with cereal HPIs ex-
444 hibit DFs similar to the control, and the potato protein increases dough DF
445 representing a larger viscous proportion than the control. However, small-
446 deformation properties, as measured by oscillatory tests, do not necessarily
447 represent large-deformation properties and performance in baking (Sliwinski
448 et al., 2004). The fact that the potato dough shows the highest DF can
449 be related to its rapid gluten-breakdown observed in GlutoPeak measure-
450 ments. The constant mixing time applied for all recipes might have exceeded
451 the mixing tolerance of gluten in the potato formulation causing a lack of
452 elasticity and a higher viscous proportion.

453 4.5. *Quality characteristics of high-protein breads*

454 Gluten is known to improve SV and used as an additional ingredient in
455 wheat bread recipes (Arendt and Zannini, 2013). The replacement of 15%
456 of wheat flour by gluten leads to breads expectedly high in quality charac-
457 terised by the highest SV and the lowest hardness in comparison to all other
458 formulations. The legume breads from pea, lupin and faba bean show ten-
459 dencies towards lower SV and higher hardness compared to the control. The

460 partial replacement of wheat flour by pea protein isolate (Marchais et al.,
461 2011) and lupin flour (Villarino et al., 2015) has been previously reported
462 to decrease SVs and increase crumb hardness. Specific volume and crumb
463 hardness of faba bean were not significantly different from the control, which
464 suggests an adequate quality. Carob and zein breads show better overall
465 quality characteristics than the control. This is consistent with the findings
466 from Turfani et al. (2017) who reported higher bread volumes with composite
467 wheat/carob flour; Bugusu et al. (2002) observed the same trend for breads
468 from composite wheat/zein flours. Interactions and potentially synergistic
469 network-formation have been suggested by GlutoPeak results for carob and
470 zein. Due to the rapid gluten-breakdown observed in the GlutoPeak test and
471 the high viscous proportion of the dough (high DF), potato was expected to
472 show poor bread quality. However, a high SV but also a high hardness were
473 measured. As suggested by RVA, the potato protein is substantially affected
474 by heat treatment. This seems to have a positive impact on expansion prop-
475 erties of the dough during baking. The high hardness might be caused by a
476 competition of gelling protein and gelatinising starch for water during heat-
477 ing. Thus, less starch would be gelatinised and a higher initial hardness of the
478 product would be observed. A hydration depletion of starch has been previ-
479 ously reported to increase crumb hardness by Martínez et al. (2018). Crust
480 browning in breads is the result of caramelisation of sugars and the Maillard
481 reaction. Components involved in those reactions are carbohydrates, proteins
482 and water (Purlis, 2010). No correlation between L^* and protein content of
483 the formulations was detected (cc: 0.04). This might be related to the fact
484 that the HPIs contain varying amounts of carbohydrates and lysine, which

485 is an important source of primary amines for Maillard reaction in proteins
486 (Purlis, 2010) and therefore substantially supports browning.

487 4.6. *Effects of minor components*

488 The values for carbohydrate contents of HPIs in this study were obtained
489 by calculation. Considering the determined contents of total starch and pre-
490 liminary results for Fructose, Glucose, Maltose and Glucose (data not shown),
491 the values suggest considerable amounts of non-starch carbohydrates and fer-
492 mentable sugars present in some of the HPIs; faba bean, carob, gluten and
493 pea in particular. Additional to its effect on crust browning, this can have
494 an impact on yeast activity and SV of the breads. Carob leads to breads
495 with high quality characterised by a high SV and low crumb hardness. Next
496 to its protein properties, also its high fibre content can be partly responsible
497 for this result. Carob seeds are the raw material for the production of locust
498 bean gum. This hydrocolloid has been reported to improve baking properties
499 and loaf volume (Azizi and Rao, 2004). Even though germ and endosperm,
500 which is the source of locust bean gum, are separated during the production
501 procedure, small amounts of locust bean gum hydrocolloids might be present
502 in the fibre fraction of the investigated carob HPI contributing to its high
503 bread quality. Also lipids are known to play an important role in wheat flour
504 doughs. Depending on their nature, they are believed to have a positive ef-
505 fect on crumb softness and to support a finer crumb structure (Pareyt et al.,
506 2011). The fine crumb structure (characterised by a low cell area) observed
507 for the pea bread could therefore be related to the high fat content of this
508 HPI.

509 5. Conclusion

510 This study provides a comparative evaluation of HPIs from different plant
511 sources applied in wheat bread formulations. The baking performance of a
512 formulation mainly depends on the rheological properties of the dough and
513 its behaviour during heat treatment and subsequent cooling. In plant-based
514 HPI formulations, the proteins were found to affect both. An accelerated and
515 weakened gluten-aggregation was observed for pea and potato. GlutoPeak
516 results further suggest secondary network-formation between non-wheat pro-
517 teins, especially carob and zein, and gluten. Due to heat induced gelation of
518 non-wheat proteins, dough characteristics can undergo major changes during
519 the baking process and potentially improve baking performance as observed
520 for the potato formulation. The overall quality of the potato formulation is
521 very similar to the control as visualised by PCA. Zein and carob, with dough
522 characteristics very different to the control, lead to improved bread quality.
523 Amongst the remaining legume formulations (pea, lupin, faba bean), espe-
524 cially the final quality of the faba bean bread is remarkable, since this HPI
525 was produced without a wet protein isolation step and therefore promises
526 better sustainability. The establishment of a generic rule as to how HPIs
527 influence final bread quality is difficult. Many factors play an important role
528 and might even oppose each other's impact on the bread formulation. Key
529 properties of HPIs were found to be gelling behaviour upon heat treatment,
530 the ability of co-networking with gluten, the degree of gluten-network impair-
531 ment and the extent of the presence of other, potentially techno-functional,
532 minor components. Particularly the legume formulations, which promise
533 balanced amino acid profiles for human nutrition, should be in the focus of

534 future studies.

535 **Acknowledgements**

536 The authors want to thank Tom Hannon for technical support, Concept
537 Life Science Ltd. for performing fibre analysis and Mareile Heitmann for
538 proofreading and constructive criticism. The work for this study has been un-
539 dertaken as part of the project PROTEIN2FOOD. This project has received
540 funding from the European Union's Horizon 2020 research and innovation
541 programme (grant agreement No 635727).

542 **References**

- 543 Alting, A.C., Pouvreau, L., Giuseppin, M.L.F., van Nieuwenhuijzen, N.H., 2011. Potato
544 proteins, in: Phillips, G.O., Williams, P.A. (Eds.), Handbook of Food Proteins. Wood-
545 head Publishing Limited, Cambridge, pp. 316–334.
- 546 Amoriello, T., Turfani, V., Galli, V., Mellara, F., Carcea, M., 2016. Evaluation of a
547 new viscometer performance in predicting the technological quality of soft wheat flour.
548 Cereal Chemistry 93, 364–368.
- 549 Arendt, E.K., Zannini, E., 2013. Cereal grains for the food and beverage industries.
550 Woodhead Publishing Limited, Cambridge.
- 551 Arntfield, S.D., Maskus, H.D., 2011. Peas and other legume proteins, in: Phillips, G.O.,
552 Williams, P.A. (Eds.), Handbook of Food Proteins. Woodhead Publishing Limited,
553 Cambridge, pp. 233–266.
- 554 Azizi, M.H., Rao, G.V., 2004. Effect of surfactant gel and gum combinations on dough
555 rheological characteristics and quality of bread. Journal of Food Quality 27, 320–336.
- 556 Belitz, H.D., Kieffer, R., Seilmeier, W., Wieser, H., 1986. Structure and function of gluten
557 proteins. Cereal Chemistry 63, 336–341.

558 Bouachra, S., Begemann, J., Aarab, L., Hüskén, A., 2017. Prediction of bread wheat
 559 baking quality using an optimized GlutoPeak®-Test method. *Journal of Cereal Science*
 560 76, 8–16.

561 Boye, J., Zare, F., Pletch, A., 2010. Pulse proteins : Processing , characterization ,
 562 functional properties and applications in food and feed. *Food Research International*
 563 43, 414–431.

564 Bugusu, B.A., Hamaker, B.R., Rajwa, B., 2002. Interaction of maize zein with wheat
 565 gluten in composite dough and bread as determined by confocal laser scanning mi-
 566 croscopy. *Scanning* 24, 1–5.

567 Day, L., 2011. Wheat gluten: Production, properties and application, in: Phillips, G.O.,
 568 Williams, P.A. (Eds.), *Handbook of Food Proteins*. Woodhead Publishing Limited,
 569 Cambridge, pp. 267–288.

570 Feillet, P., Roulland, T.M., 1998. Caroubin: A gluten-like protein isolated from carob
 571 bean germ. *Cereal Chemistry* 75, 488–492.

572 van Gelder, W.M.J., Vonk, C.R., 1980. Amino acid composition of coagulable protein
 573 from tubers of 34 potato varieties and its relationship with protein content. *Potato*
 574 *Research* 23, 427–434.

575 Goldstein, A., Ashrafi, L., Seetharaman, K., 2010. Effects of cellulosic fibre on physical
 576 and rheological properties of starch, gluten and wheat flour. *International Journal of*
 577 *Food Science and Technology* 45, 1641–1646.

578 Henchion, M., Hayes, M., Mullen, A., Fenelon, M., Tiwari, B., 2017. Future Protein Supply
 579 and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods* 6,
 580 53.

581 Kenny, S., Wehrle, K., Stanton, C., Arendt, E.K., 2000. Incorporation of dairy ingredi-
 582 ents into wheat bread: Effects on dough rheology and bread quality. *European Food*
 583 *Research and Technology* 210, 391–396.

- 584 Lin, D., Lu, W., Kelly, A.L., Zhang, L., Zheng, B., Miao, S., 2017. Interactions of vegetable
585 proteins with other polymers: Structure-function relationships and applications in the
586 food industry. *Trends in Food Science & Technology* 68, 130–144.
- 587 López, E., 2014. Influence of the addition of lupine protein isolate on the protein and
588 technological characteristics of dough and fresh bread with added Brea Gum. *Food*
589 *Science and Technology (Campinas)* 2014, 195–203.
- 590 Marchais, L.P.D., Foisy, M., Mercier, S., Villeneuve, S., Mondor, M., 2011. Bread-making
591 potential of pea protein isolate produced by a novel ultrafiltration/diafiltration process.
592 *Procedia Food Science* 1, 1425–1430.
- 593 Marco, C., Rosell, C.M., 2008. Effect of different protein isolates and transglutaminase on
594 rice flour properties. *Journal of Food Engineering* 84, 132–139.
- 595 Martínez, M.M., Román, L., Gómez, M., 2018. Implications of hydration depletion in
596 the in vitro starch digestibility of white bread crumb and crust. *Food Chemistry* 239,
597 295–303.
- 598 Pareyt, B., Finnie, S.M., Putseys, J.A., Delcour, J.A., 2011. Lipids in bread making:
599 Sources, interactions, and impact on bread quality. *Journal of Cereal Science* 54, 266–
600 279.
- 601 Purlis, E., 2010. Browning development in bakery products - A review. *Journal of Food*
602 *Engineering* 99, 239–249.
- 603 Rosell, C.M., 2011. The Science of Doughs and Bread Quality, in: Preedy, V.R., Watson,
604 R.R., Patel, V.B. (Eds.), *Flour and Breads and their Fortification in Health and Disease*
605 *Prevention*. Academic Press, London, pp. 3–14.
- 606 Sanz-Penella, J.M., Wronkowska, M., Soral-Smietana, M., Haros, M., 2013. LWT - Food
607 Science and Technology Effect of whole amaranth flour on bread properties and nutri-
608 tive value. *LWT - Food Science and Technology* 50, 679–685.

- 609 Schutyser, M.A., Pelgrom, P.J., van der Goot, A.J., Boom, R.M., 2015. Dry fractionation
610 for sustainable production of functional legume protein concentrates. *Trends in Food*
611 *Science and Technology* 45, 327–335.
- 612 Sliwinski, E.L., Kolster, P., van Vliet, T., 2004. Large-deformation properties of wheat
613 dough in uni- and biaxial extension. Part I. Flour dough. *Rheologica Acta* 43, 306–320.
- 614 Sluimer, P., 2005. *Principles of Breadmaking: Functionality of Raw Materials and Process*
615 *Steps*. American Association of Cereal Chemists Inc., St. Paul, Minnesota.
- 616 Smith, B.M., Bean, S.R., Schober, T.J., Michael Tilley, Herald, T.J., Aramouni, F., 2010.
617 Composition and molecular weight distribution of carob germ protein fractions. *Journal*
618 *of Agricultural and Food Chemistry* 58, 7794–7800.
- 619 Turfani, V., Narducci, V., Durazzo, A., Galli, V., Carcea, M., 2017. Technological, nu-
620 tritional and functional properties of wheat bread enriched with lentil or carob flours.
621 *LWT - Food Science and Technology* 78, 361–366.
- 622 Villarino, C.B., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., Johnson, S.K., 2015. The
623 effects of Australian sweet lupin (ASL) variety on physical properties of flours and
624 breads. *LWT - Food Science and Technology* 60, 435–443.

Tables

Table 1: Recipe for control and HPI formulations

Ingredient	% based on flour	% based on recipe
Wheat flour	100.0 (85.0**)	59.70
HPI	0.0 (15.0**)	0.00
Baker's yeast	2.0	1.19
NaCl	2.0	37.31
Oil	1.0	1.19
Water	62.5 (FWA*/**)	0.60
Total	167.5	100.00

* FWA - Farinograph water absorption according to Table 3

** for HPI formulations

Table 2: Composition of wheat flour and HPIs

Component	Wheat flour/control	Gluten	Zein	Potato	Carob	Pea	Lupin	Faba bean
Moisture [%]	13.01 ± 0.05 ^f	8.20 ± 0.02 ^c	6.44 ± 0.08 ^b	10.87 ± 0.02 ^e	6.06 ± 0.02 ^a	9.73 ± 0.02 ^d	6.45 ± 0.06 ^b	13.07 ± 0.12 ^f
Protein [%DM]	14.09 ± 0.00 ^a	83.11 ± 0.42 ^e	91.79 ± 0.38 ^f	94.06 ± 2.13 ^{fg}	55.04 ± 0.28 ^b	80.19 ± 1.43 ^d	94.51 ± 1.50 ^g	61.25 ± 0.66 ^c
Fat [%DM]	1.97 ± 0.10 ^{bcd}	0.72 ± 0.02 ^{ab}	2.66 ± 0.12 ^{cde}	0.12 ± 0.07 ^a	0.20 ± 0.02 ^a	6.45 ± 0.52 ^f	2.94 ± 0.13 ^{de}	3.81 ± 0.13 ^e
Ash [%DM]	1.09 ± 0.01 ^c	0.87 ± 0.01 ^b	1.16 ± 0.09 ^d	0.05 ± 0.04 ^a	7.04 ± 0.03 ^h	5.90 ± 0.01 ^g	5.62 ± 0.01 ^f	5.43 ± 0.03 ^e
Total dietary fibre (TDF) [%DM]	2.30	<0.1	0.75	<0.1	17.67	2.88	<0.1	0.35
Carbohydrates* [%DM]	80.56	15.31	3.19	5.76	20.05	4.58	0.00	29.17
Total starch [%DM]	72.38 ± 0.10 ^c	4.95 ± 0.27 ^a	<0.2	<0.2	<0.2	<0.2	<0.2	7.77 ± 0.02 ^b
proteinE (formulation)** [%]	14.48	24.78	26.23	26.13	21.23	24.19	26.64	21.24

Means ± standard deviation with different letters in the same row were significantly different ($p < 0.05$).

* calculated by subtraction, required for calculation of proteinE of formulation

** calculated based on compositional analysis of ingredients

Table 3: Properties of wheat flour and HPI/flour blends and quality characteristics of control and HPI bread formulations

Variable	Wheat flour/control	Gluten	Zein	Potato	Carob	Pea	Lupin	Faba bean
Farinograph								
Farinograph water absorption (FWA) [%]	63.0 ± 0.5 ^b	70.2 ± 0.2 ^d	60.8 ± 0.2 ^a	65.2 ± 0.3 ^c	69.8 ± 0.6 ^d	71.7 ± 0.6 ^e	66.2 ± 0.3 ^c	62.2 ± 0.3 ^b
GlutoPeak								
Peak maximum time (PMT) [s]	53 ± 2 ^e	n.d.	105 ± 4 ^f	17 ± 3 ^a	41 ± 1 ^d	25 ± 1 ^b	33 ± 1 ^c	34 ± 1 ^c
Torque maximum (TM) [BE]	74 ± 1 ^e	n.d.	48 ± 1 ^a	54 ± 0 ^b	58 ± 1 ^c	72 ± 3 ^e	59 ± 1 ^c	51 ± 2 ^{ab}
Rapid Visco Analyser								
Peak viscosity (PV) [cP]	2390 ± 13 ^e	1683 ± 12 ^c	1415 ± 39 ^a	2365 ± 44 ^e	1540 ± 14 ^b	1518 ± 12 ^b	1500 ± 15 ^b	1852 ± 28 ^d
Setback [cP]	1282 ± 7 ^f	848 ± 9 ^c	877 ± 30 ^c	1058 ± 10 ^d	791 ± 17 ^b	680 ± 6 ^a	1161 ± 4 ^e	1158 ± 3 ^e
Final viscosity (FV) [cP]	2544 ± 10 ^e	1783 ± 4 ^c	1689 ± 52 ^b	2192 ± 22 ^d	1665 ± 35 ^b	1589 ± 19 ^a	2162 ± 15 ^d	2200 ± 17 ^d
Rheometer								
Damping factor (DF)	0.401 ± 0.004 ^c	0.385 ± 0.005 ^c	0.387 ± 0.004 ^c	0.437 ± 0.016 ^d	0.327 ± 0.005 ^a	0.325 ± 0.002 ^a	0.349 ± 0.001 ^b	0.329 ± 0.002 ^a
Bread analysis								
Specific volume (SV) [ml/g]	2.55 ± 0.20 ^{bc}	3.81 ± 0.05 ^d	2.63 ± 0.06 ^{bc}	2.81 ± 0.23 ^c	2.73 ± 0.34 ^{bc}	2.00 ± 0.08 ^a	1.98 ± 0.03 ^a	2.26 ± 0.08 ^{ab}
Number of cells	1671 ± 47 ^{ab}	1902 ± 57 ^b	1835 ± 73 ^{ab}	1811 ± 240 ^{ab}	1709 ± 105 ^{ab}	1708 ± 92 ^{ab}	1596 ± 91 ^{ab}	1543 ± 115 ^a
Cell area [%]	45.5 ± 0.8 ^{ab}	49.0 ± 0.2 ^c	46.3 ± 0.3 ^{ab}	46.6 ± 0.2 ^b	46.0 ± 1.5 ^{ab}	44.5 ± 0.7 ^a	45.0 ± 0.6 ^{ab}	46.6 ± 0.5 ^b
Hardness [N]	11.81 ± 1.62 ^{bc}	4.12 ± 0.45 ^a	15.10 ± 1.47 ^{cd}	19.02 ± 4.80 ^d	7.84 ± 1.08 ^{ab}	16.68 ± 1.24 ^{cd}	20.11 ± 1.14 ^d	16.30 ± 3.08 ^{cd}
Lightness of crust (L*)	71.84 ± 3.48 ^{de}	66.48 ± 0.23 ^{bc}	72.80 ± 1.26 ^e	70.58 ± 0.84 ^{cde}	62.33 ± 2.52 ^{ab}	66.83 ± 0.64 ^{bcd}	64.18 ± 0.79 ^b	57.06 ± 2.62 ^a

Means ± standard deviation with different letters in the same row were significantly different at (p < 0.05).

n.d. - not detectable (analysis could not be performed due to exceeded maximum torque of the device)

Figures

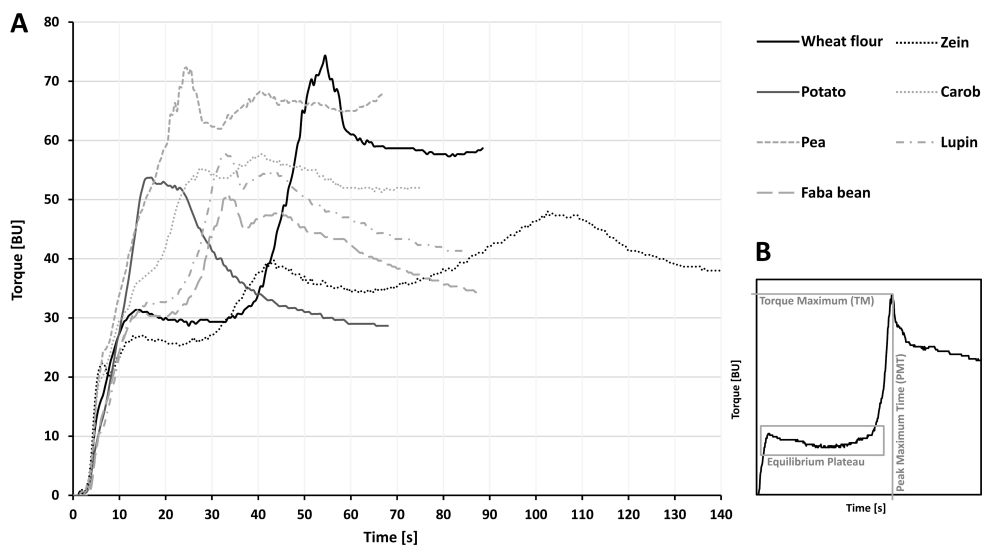


Figure 1: A - GlutoPeak curves of wheat flour and HPI/flour blends; B - schematic GlutoPeak curve of wheat flour with explanation of associated variables and terms

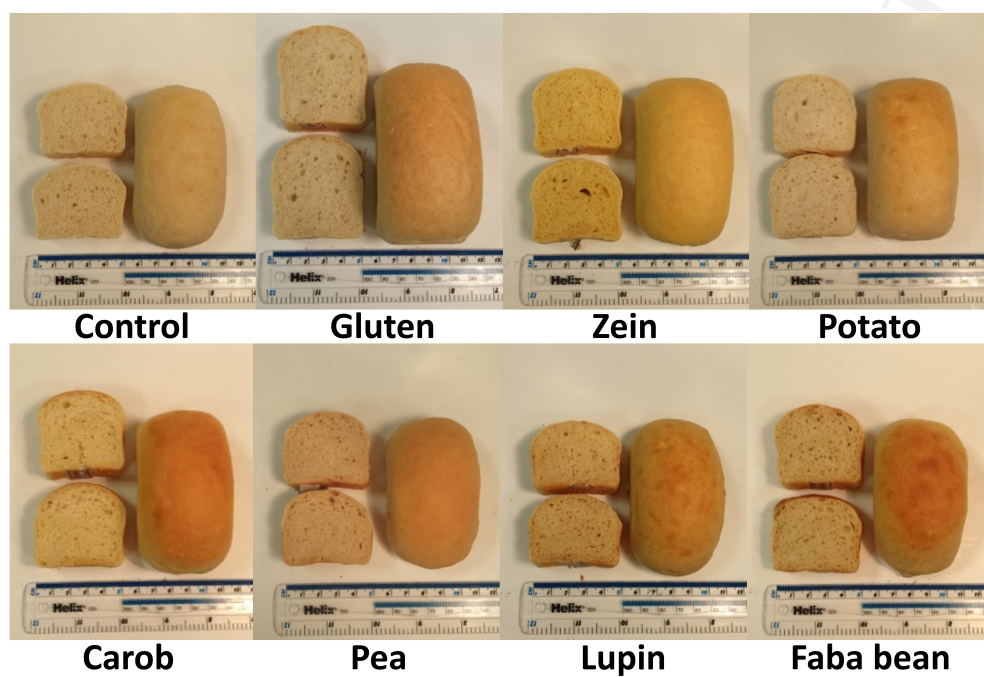


Figure 2: Appearance of loaves and slices of control and HPI breads

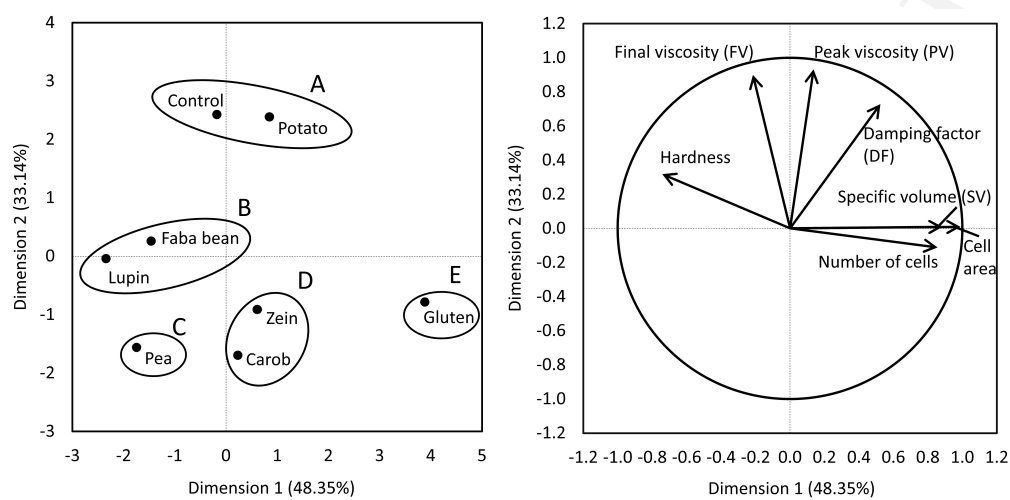


Figure 3: Principal component analysis of dough and bread characteristics: left - analysed observations with hierarchical classification, right - analysed variables