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1 **The use of red lentil flour in bakery products: how do particle size and substitution level affect**  
2 **rheological properties of wheat bread dough?**

3

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#### 34 **Abstract**

35 Inclusion of pulses flour in bread formulation has important nutritional effects but its successful  
36 implementation is challenging and requires a good understanding of the effect of flour functionality,  
37 granulometry and substitution level on bread quality. Accordingly, this work studied red lentil flour and its  
38 dimensional fractions (coarse, medium, fine, extra-fine), considering compositional, morphological,  
39 functional, and thermal properties. Additionally, the effect of substituting wheat flour with lentil flour and its  
40 fractions at different levels (0, 10, 15, 20, 25 and 30% [w/w] flour basis) on dough rheology was studied using  
41 a Mixolab device, to predict bread quality. Although flour's properties were significantly affected by particle  
42 size, multivariate statistics suggested that the substitution level was the major factor affecting rheological  
43 properties of doughs made with blends of wheat and lentil flours. A 10% substitution level of wheat flour by  
44 lentil flour provides optimum rheological properties regardless of lentil flour particle size, while at higher  
45 substitution level (15-30%), a coarse fraction can provide higher performance compared to unfractionated flour  
46 and finer fractions. The results of this study pose an important base to intelligently develop wheat-lentil bread  
47 applications in the future.

48

49 **Keywords:** bread dough, red lentil flour, particle size, Mixolab, physico-chemical properties.

50

#### 51 **Abbreviations**

52 PS, particle size; SL, substitution level; red lentil flours – L, unfractionated; EFL, extra-fine; FL, fine;  
53 ML, medium; CL, coarse; STD, common wheat flour Type 00; d. b., dry basis; w. b., wet basis;  
54 alveographic parameters - W (J 10-4), baking strength; P/L ratio, curve configuration ratio; P (mm),  
55 dough tenacity; L (mm), dough extensibility; R/T, room temperature; WHC, water holding capacity;  
56 OHC, oil holding capacity; S<sub>p</sub>, swelling power; DSC, differential scanning calorimeter; ΔH, enthalpy;

57  $T_{on}$ , onset temperature;  $T_p$ , peak temperature;  $T_{off}$ , offset temperature; WA, water absorption;  
58 ANOVA, analysis of variance.

59

## 60 1. Introduction

61 Pulses are common to culinary traditions worldwide. As a source of carbohydrate, protein, dietary fiber,  
62 vitamins, minerals, and phytochemicals, they are important for human nutrition and health, especially among  
63 low-income populations (Foschia, Horstmann, Arendt, & Zannini, 2017; Boukid, Zannini, Carini, & Vittadini,  
64 2019b, Bresciani & Marti, 2019). Beside their environmental sustainability, interest in adding pulses to food  
65 products is rising, since consumers are increasingly health- and environment-conscious (Malcolmson, Boux,  
66 Bellido, & Frohlich, 2013; FAO 2019).

67 Pulse flour has been used frequently to nutritionally enhance food products, including bread, as a functional  
68 ingredient, to partially substitute wheat flour (Borsuk, Arntfield, Lukow, Swallow, & Malcolmson, 2012;  
69 Foschia et al., 2017; Melini, Melini, Luziatelli, & Ruzzi, 2017; Sozer, Holopainen-Mantila, & Poutanen, 2017;  
70 Bresciani & Marti, 2019). Among pulses, lentils (*Lens culinaris* Medik.) are widely used in baking because of  
71 their mild taste and protein functionality (Joshi, Timilsena, & Adhikari, 2017). Notwithstanding its nutritional  
72 benefits, use of pulse flour in breadmaking is hampered by unavoidably poorer finished products' quality  
73 (Monnet, Laleg, Michon, & Micard, 2019; Bresciani & Marti, 2019), which may depend on the level of  
74 inclusion in the product formulation as well as its functional characteristics, e.g., granulometry.

75 Flour granulometry has recently gained much attention as a mean to modulate flour functionality and control  
76 nutrients bioaccessibility, in respect to the relationship between degree of grinding and preservation of cell  
77 structural integrity. Fine particle size is generally associated with more cell rupture and release of cell  
78 components, while larger flour granulometry assures better preservation of cell integrity that hinders the action  
79 of digestive enzymes (Rovalino-Córdova, Fogliano, & Capuano, 2019; Boukid et al., 2019a, Pellegrini,  
80 Vittadini, & Fogliano, 2020; Lin et al., 2020). More extensive milling (500  $\mu\text{m}$  flour granulometry) was  
81 associated to greater starch damage, lower water absorption capacity, and higher peak and final viscosities in  
82 lentil flour compared to coarser fractions (790, 1000, 1270  $\mu\text{m}$ ; Bourré et al., 2019). A general increase in total  
83 starch and a decrease in protein content, bulk density and oil holding capacity with the decrease in particle size  
84 (210, 149, 105 and 74  $\mu\text{m}$ ) were found by comparing two lentil flours (Indian cv. L-4076 and Turkish cv.

85 Çiftçi), while the pasting and thermal properties were dependent on flour particle size and cultivar (Ahmed,  
86 Taher, Mulla, Al-Hazza, & Luciano, 2016). In bakery applications, the use of 500 µm lentil flour (20% wheat  
87 flour substitution) was found to produce a firmer bread compared to the one made with coarser fractions (790,  
88 1000, 1270 µm; Bourré et al., 2019), while fine lentil flour (~17 µm, 75% wheat flour substitution) was  
89 reported to yield to a softer wheat-based pita bread if compared to a coarser flour (~190 µm; Borsuk et al.,  
90 2012). Furthermore, from a nutritional perspective, a positive association between the use of rich-in-intact-  
91 cells lentil flour fractions (>200 µm) and reduced *in vitro* starch digestibility of derivatives has been reported  
92 (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019).

93 To the authors' best knowledge, no reports are available in the literature on the combined effect of particle size  
94 and substitution level on wheat bread dough rheology, a basic knowledge that can greatly help predicting,  
95 improving, and understanding the bread making process. Consequently, the objective of the present study was  
96 to evaluate the effect of particle size (PS), obtained with sieving fractionation after a conventional roller  
97 milling process, on compositional, functional, and thermal properties of red lentil flour compared to common  
98 wheat flour, and to investigate the impact of PS and substitution level (SL) on wheat dough rheology using a  
99 Mixolab device to predict the product quality in the baking process.

100

## 101 **2. Materials and methods**

### 102 **2.1 Raw materials**

103 Unfractionated red lentil flour (L) was kindly provided by Molino Martino Rossi SpA (Gadesco Pieve  
104 Delmona, Italy), and was produced by subjecting dehulled red lentils to roller milling.

105 Common wheat flour Type 00 [ashes ≤ 0.55 dry basis (d. b.); protein ≥ 9% d.b., moisture ≤ 14.5% wet basis  
106 (w. b.); W= 376 10-4 J and P/L = 0.62 (Molino Agugiaro & Figna, Collecchio, PR, Italy)] was used as a control  
107 (STD).

### 108 **2.2 Flour fractionation**

109 Flours were fractionated using a Giuliani Technologie Sieve (IG-GLOBE 300 rpm). 100 g flour was sieved for  
110 40min through certified 22-mesh (200 µm), 23-mesh (160 µm), and 25-mesh (100 µm) test sieves (Giuliani  
111 Technologie, Italy). Lentil flour fractions were named as extra-fine (EFL, <100 µm), fine (FL, 100-160 µm),  
112 medium (ML, 160-200 µm), and coarse (CL, >200 µm).

113 **2.3 Physicochemical characterization of flours**

114 **2.3.1 Proximate composition**

115 Flour samples were analyzed for total protein (%N x 5.70, AACCI method 46-12.01), lipid (% AACCI  
116 Method 30-25.01), and ash (% AACCI method 08-01.01) contents. Dry matter was determined by oven drying  
117 for 1 h to constant weight at 130 °C (adapted from AACCI method 44-15.02), and carbohydrates were  
118 determined by difference, and compositional data expressed as % (g /100 g) of dry matter. Analyses were  
119 performed in duplicate and results were expressed as mean ± standard deviation.

120 **2.3.2 Water holding capacity (WHC) and oil holding capacity (OHC)**

121 WHC and OHC were determined following Nguyen, Mounir, & Allaf (2015), with modifications. Briefly,  
122 100.0 ± 0.5 mg flour were mixed with 1.0 mL distilled water (WHC) or sunflower oil (OHC), vortexed for 30  
123 s, then left for 30 min at R/T. Mixtures were centrifuged at 2061 g (4000 rpm) for 20 min (Eppendorf 5810 R,  
124 Germany), and the supernatant decanted. WHC and OHC were calculated as the ratio between grams of water  
125 or oil retained per gram of solid. Results were expressed as mean ± standard deviation of three replicates.

126 **2.3.3 Swelling power (S<sub>p</sub>)**

127 S<sub>p</sub> was measured following Yadav, Yadav, & Dhull's method (2012), with modifications. Suspensions (2%  
128 w/v) were heated 60, 70, 80 and 90 °C for 1 h, cooled at 30 °C for 30 min and were then centrifuged at 8243  
129 g (8000 rpm) for 20 min. The weight of the resulting pellet was determined. S<sub>p</sub> was calculated as the ratio  
130 between sediment and fresh sample weights. Values were reported as mean ± standard deviation of three  
131 replicates.

132 **2.4 Thermal properties**

133 Thermal properties were measured using a differential scanning calorimeter (DSC, Q100 TA Instruments,  
134 USA), calibrated with indium and mercury. Distilled water was added to flour in a 3:1 ratio and equilibrated  
135 overnight at R/T. Samples were prepared placing 5-10 mg of water-flour suspension in stainless steel pans  
136 (Perkin Elmer, USA) hermetically sealed, quench-cooled to 30 °C, then heated to 100 °C at 5 °C/min, using  
137 an empty pan as reference. Enthalpy (ΔH, J/g), onset (T<sub>on</sub>, °C), peak (T<sub>p</sub>), and offset (T<sub>off</sub>, °C) transition  
138 temperatures were obtained from heat flow curves using Universal Analysis Software, Version 4.5A (TA  
139 Instruments, USA). Data were expressed as three replicate averages for each flour sample.

140 **2.5 Optical microscopy**

141 Size and distribution of single or grouped cells in lentils fraction were examined by optical microscopy (DM  
142 4000B, Leica, Germany). Flour particles on a slide under a coverslip were stained with toluidine blue (0.1%).  
143 Three slides were analyzed for each flour. Multiple images of cells (5) and cell agglomerates (15) were taken  
144 (Leica DMC2900, Germany) at magnification 20× and 5× respectively. Cell aggregate areas were measured  
145 using Leica Imaging software (IM50 Version 4.1).

## 146 **2.6 Rheology**

147 The impact of lentil flour PS and SL on the rheological properties of wheat-flour-based dough was studied  
148 using a Mixolab (Chopin, Tripette et Renaud, France) and AACC 54-60.01 and Chopin+ protocol (Table 1;  
149 75 g dough samples). STD was enriched with L or its fractions (CL, ML, FL, EFL) at 0, 10, 15, 20, 25 and  
150 30% (w/w).

151 Mixolab software was used to measure Water absorption (WA, %); initial target consistency C1 (Nm); torque  
152 at the end of the holding time at 30 °C (C1.2, Nm); minimum torque C2 (Nm); peak torque C3 (Nm); stability  
153 of hot-formed gel C4 (Nm); final torque C5 (Nm) measured after cooling at 50 °C. Temperatures (Tp, °C) and  
154 time (min) upon the appearance of different types of torque were also recorded. In addition, stability (resistance  
155 to kneading) and amplitude (elasticity) were measured as software outputs. Analyses were run in duplicate.

## 156 **2.7 Statistical analyses**

157 One-way ANOVA and Duncan's post-hoc test were performed to determine the effect of particle size on  
158 physicochemical and rheological properties. Two-way ANOVA was used to determine the impact of PS and  
159 SL on dough rheology. All statistical analyses were performed at 0.05 significance level using SPSS Statistical  
160 Software (Version 25.0, IBM SPSS Inc., USA).

## 161 **3. Results and discussion**

### 162 **3.1 Characterization of lentil flour and its fractions**

163 Particle mass distribution (%) of STD, L and L fractions are reported in Table 2, and indicate that lentil flour  
164 contained a higher amount of larger particles as compared to STD. STD had significantly higher carbohydrates  
165 ( $74.72 \pm 0.32\%$ ) and moisture content ( $10.78 \pm 0.03\%$ ), but lower protein ( $12.62 \pm 0.37\%$ ) and ash ( $0.33 \pm$   
166  $0.01\%$ ) than L and its fractions (Table 2), as expected (Boukid et al, 2019b). STD fat content ( $1.54 \pm 0.01\%$ )  
167 was similar to CL. Proximate composition of all lentil flours are in concordance with the findings of Hall,  
168 Hillen, & Garden Robinson (2017). Among the different fractions, CL showed significantly lower protein and

169 higher carbohydrate content. Fat and protein content were inversely related to PS, while carbohydrate and  
170 moisture content decreased slightly with PS decrease. Ash content decreased with PS reduction, conceivably  
171 due to mineral association with starch granules of CL fractions, as postulated by Shafi, Baba, & Masoodi  
172 (2017).

### 173 **3.2 Optical microscopy**

174 Morphology of lentil flour fractions components (cell aggregates, cells, starch granules) was observed under  
175 optical microscopy (Fig. 1). Lentil starch granules were elliptical to round, with a central elongated or starred  
176 hilum (Fig. 1) as previously reported (Joshi et al., 2017). Average cell aggregate areas decreased significantly  
177 with decreasing flour PS, as previously reported (Boukid et al., 2019a). Specifically, cell aggregate areas  
178 decreased as follows: CL ( $\approx 144,000 \mu\text{m}^2$ ) > ML ( $\approx 90,000 \mu\text{m}^2$ ) > FL ( $\approx 50,000 \mu\text{m}^2$ ) > EFL ( $\approx 7,000 \mu\text{m}^2$ ).  
179 Cell aggregates prevalently consisted of intact rather than fractured cells in CL (Figs. 1a and 1b), both intact  
180 and fractured cells in ML (Figs. 1c and 1d), free starch granules and cell wall fragments in FL (Figs. 1e and  
181 1f), prevalently free starch granules and fragmented cell walls in EFL (Figs. 1g and 1h). This is particularly  
182 significant because of the relationship between flour structural attributes and the response of its constituents  
183 to processing (shear, temperature, and time) and their functional and nutritional properties in dough and final  
184 product (Boukid et al., 2019a, Pellegrini et al., 2020).

### 185 **3.3 Water holding capacity, oil holding capacity, and swelling power**

186 WHC defines ability to hold water against gravity and it is an important parameter for breadmaking  
187 functionality, as a high water incorporation in dough (high WHC) improves bread's properties (Jarpa-Parra,  
188 2018; Ma et al., 2011).

189 STD showed WHC (1.02 g/g, Table 3) within the range previously identified for wheat gluten (Wang, Zhao,  
190 Yang, Jiang, 2006), while WHC for L flours ranged between 1.18 g/g and 1.85 g/g (Table 3), concordantly  
191 with previous studies (L'Hocine, Boye, & Arcand, 2006; Lee, Htoon, Uthayakumaran, & Paterson, 2007;  
192 Boye, Zare, & Pletch, 2010). For L samples, the highest WHC were in L, CL, and ML, while FL was  
193 significantly lower (1.50 g/g) as was EFL (1.18 g/g). The WHC decrease for finest particles could be attributed  
194 to the lower carbohydrates, higher protein and fat contents and potentially higher starch damage compared to  
195 the coarser fractions, in agreement with literature (Robertson et al., 2000; Aguilera, Esteban, Benitez, Molla,  
196 & Martin-Cabrejas, 2009; Luhovyy, Hamilton, Kathirvel, & Mustafaalsaafin, 2017; Lin et al., 2020).



197 OHC is an important property in bakery products when fat absorption is desirable for flavor retention,  
198 palatability, and shelf-life extension (Adebowale & Lawal, 2004). Regarding OHC (Table 3), no significant  
199 differences were found between wheat and lentil flours, except for CL which had a lower OHC. This may be  
200 explained by its protein content and, therefore, lower lipophilic tendency (Walde, Tummala, Lakshminarayan,  
201 & Balaraman, 2005; Bolade, Adeyemi, & Ogunsua, 2009).

202  $S_p$  defines the water absorbed and trapped in the gel network created by starch granule hydrogen bonds during  
203 heating and stirring in excess of water (Li et al., 2014). At low temperatures, thermal energy swells starch  
204 granules without disruptions; greater thermal energy with temperature increases induces crystalline structure  
205 breakdown and increased  $S_p$  (Li et al., 2014). In all samples,  $S_p$  increased with rising temperature until 80 °C,  
206 and then remained constant as previously reported (Chung, Liu, Donner, Hoover, Warkentin, & Vandenberg,  
207 2008; Boukid et al., 2019a).

208 Among samples, STD showed a greater  $S_p$  increase with rising temperatures, reaching values notably higher  
209 than those of L and its fractions at 90 °C. Overall, despite higher free amylose content and lower lipid-amylose  
210 complexes in pulses compared to cereals,  $S_p$  is lower in pulses than in cereals. Wani, Sogi, Hamdani, Gani,  
211 Bhat, & Shah, (2016) related this behavior to a greater degree of amylose and amylopectin interactions which,  
212 in turn, prevent starch molecules from releasing amylose during gelatinization. Overall,  $S_p$  depends on several  
213 factors, e.g., starch and cultivar sources, amylose/amylopectin ratio, size, morphology and ultrastructure of  
214 starch granules and cell wall intactness, temperature, and pH (Wani et al., 2016; Boukid et al., 2019a).

215 Considering PS,  $S_p$  of lower PS fractions (ML, FL, EFL) was significantly higher than the whole and coarser  
216 fractions. The presence of fractured cells and free starch granules in ML, FL, and EFL, as discussed in the  
217 optical microscopy section, may explain the higher  $S_p$ .

### 218 **3.4 Thermal properties**

219 DSC thermograms and thermal properties of the studied flours are reported in Fig. 2 and Table 4, respectively.  
220 Wheat flour showed a unique thermal transition at 53 – 75 °C related to starch gelatinization. Instead, two  
221 endothermic peaks were evident for L flour and its fractions (Fig. 2). The first peak (55 – 80 °C) was attributed  
222 to starch gelatinization, while the 80 – 96 °C transition was previously related to amylose-lipid complexes  
223 melting or protein denaturation (Chung et al., 2008; Barbana, & Boye, 2013; Zeng, Gao, & Li, 2014; Ahmed  
224 et al., 2016). The starch gelatinization peak shifted to higher temperatures in L than in STD, suggesting higher

225 energy to initiate starch gelatinization in lentil flours. The different gelatinization properties of cereals vs.  
226 pulses are likely attributable to several factors such as crystallinity, starch granule size, intermolecular bonding,  
227 and others (Ai & Jane, 2018). Moreover, DSC thermograms showed the gelatinization event starting with a  
228 minor peak in L samples, indicating that, although the majority of lentil flour starch gelatinizes at higher  
229 temperature than STD, a small fraction of starch has a tendency to gelatinize at a lower temperature.  
230 Considering gelatinization peaks in L samples, CL showed the lowest  $T_{on}$  ( $\approx 55$  °C) among all the samples  
231 which were comparable ( $\approx 57$  °C), whereas  $T_p$  was lowest in L ( $\approx 69$  °C) and highest in EFL ( $\approx 70$  °C).  $T_{off}$   
232 occurred at 79-81 °C in all L flours. Gelatinization enthalpy of STD ( $\approx 2.00$  J g<sup>-1</sup>) and lentil flours was  
233 significantly different only in L ( $\approx 1.50$  J g<sup>-1</sup>) and FL ( $\approx 1.40$  J g<sup>-1</sup>). Thermal parameters of the second  
234 endothermic peak ( $T_{on}$ ,  $T_p$ ,  $T_{off}$  and  $\Delta H$ ) were not significantly different as a function of lentil flour PS (Table  
235 4). Overall, PS did not affect lentil flour endothermic events, as observed by Boukid et al. (2019a).

### 236 **3.5 Rheology**

237 To deem lentil flours suitable for breadmaking, composite wheat/lentil flour blends at different SLs were  
238 formulated, and dough rheology measured. The Mixolab protocol used (Table 1) simulated the breadmaking  
239 process and explored dough's thermo-mechanical behavior under mixing and temperature stress. Additionally,  
240 Mixolab data provide information on protein quality (strength), starch behavior (gelatinization, stability and  
241 retrogradation) during heating and cooling, enzymatic activity, and their combined effects (Dubat, 2010;  
242 AACC 54-60.01).

243 Table 5 shows the effect of PS, SL, and their interactions (PS x SL) on each Mixolab parameter using 2-way  
244 ANOVA. Based on statistical analyses (F significance level and sum square percent of factors studied), PS did  
245 not significantly affect C1\_t (maximum torque at 30 °C) nor the time to attain C2, C3, C4 and C5. In contrast,  
246 PS significantly ( $P \leq 0.05$ ) affected most torque [C1.2 (Nm, 5.07%); C2 (Nm, 8.84%); C3 (Nm, 10.97%); C5  
247 (Nm, 5.62%)], but showed no significant effect on torque temperature and amplitude. Moreover, PS effects on  
248 stability (4.47%) and WA (0.95%) were low.

249 Investigating further using 2-way ANOVA, the results showed that almost all Mixolab parameters were  
250 controlled by SL, which had the highest influence on torque times [C1\_t (96.71%); C2\_t (96.58%); C3\_t  
251 (53.86%); C4\_t (52.28%); C5\_t (28.40%)], torque [C1.2 (91.28%); C2 (83.29%); C3 (53.28%); C4 (72.58%);  
252 C5 (68.62%)], and above all torque temperature [C1 (34.14%); C2 (89.12%); C5 (44.44%)]. Similarly, SL

253 greatly influenced the doughs' elasticity (77.66%), stability, (93.93%) and water absorption (97.99%) of the  
254 doughs. Considering PS and SL simultaneously, a smaller synergic contribution was found in the Mixolab  
255 data, compared to the two factors taken independently. Multivariate analyses confirmed PS and SL interactions  
256 which significantly ( $P \leq 0.05$ ) affected C3\_t (37.29%), torque values except for C4 [C1.2 (Nm, 3.65%); C2  
257 (Nm, 7.87%); C3 (Nm, 35.75%); C5 (Nm, 26.76%)], stability (1.60%) and WA (1.06%), with a modest effect  
258 on C3, C3\_t and C5.

259 Such findings suggest that SL was the predominant factor affecting the dough's entire rheological and thermo-  
260 mechanical behavior when analyzed with the Mixolab to predict baking quality. These results can also be  
261 observed in Mixolab curves of L samples (Fig. 3a): the higher the SL, the greater the variance from the STD  
262 curve, especially in the part referring to protein characteristics (i.e. stability during kneading and the protein  
263 weakening illustrated in Table 1). In fact, as per Table S1, increasing L level addition caused a significant ( $P$   
264  $\leq 0.001$ ) increase in WA, reduction in C1.2 and C2 torques and dough stability, and delayed protein weakening  
265 (C2\_t increases with SL increase). Since this curve concerns a protein weakening due to kneading and  
266 temperature effects, reduction in these parameters with an SL increase indicates worsening of wheat protein  
267 functionality in breadmaking. Additionally, an increased SL significantly ( $P \leq 0.001$ ) worsened the pasting  
268 consistency of the dough (C3 decrease with SL increase), which may be related to the lower  $S_p$  of pulses than  
269 cereals, as above.

270 Flour samples at 10% SL (Figs. 3b and 3c) were more aligned to the STD curve than those at 30%. Addition  
271 of lentil flour at 10% SL significantly ( $P \leq 0.05$ ) influenced C1\_t, C1.2 and C5 parameters (Table 1) and WA,  
272 while none of the remaining parameters were significantly different from those of STD (Table S2). These  
273 observations indicated that STD dough enriched with 10% lentil flour can provide a nutritional benefit (e.g.  
274 the use of L flour results in a 9% and 64% increase in protein and ash contents, respectively) without altering  
275 the rheological profile of the dough at any PS.

276 Predictably, the effect of adding lentil flour (whole or fractionated) became more significant with increased  
277 SLs. Indeed, besides the aforementioned parameters, a progressive significant ( $P \leq 0.05$ ) reduction in C2, C3  
278 and stability was observed with 15% SL (Tables S3-S6). At the highest SL, the Mixolab curves were virtually  
279 halved compared to STD (Figure 3c), with almost all torques, times and temperatures significantly ( $P \leq 0.05$ )  
280 affected by SL.

281 As reported previously (Erukainure et al., 2016; Dabija, Codină, & Fradinho, 2017), increasing lentil flour SL  
282 causes dough weakening, disruption of protein-starch complexes, and alteration of starch gelatinization,  
283 amylase activity, and retrogradation processes, implying worse dough handling and baking properties. Indeed,  
284 dough weakening as a consequence of pulse flour content is attributable to a decrease in wheat gluten proteins  
285 and various components vying for water such as non-gluten proteins and fiber (Hallén İbanoğlu, & Ainsworth  
286 2004; Rosell, Marco, García-Alvárez, & Salazar, 2011).

287 Interestingly, at SL  $\geq$  15%, the effect on the dough's rheology was dependent on PS. The use of CL caused a  
288 significantly ( $P \leq 0.001$ ) lower deterioration in dough rheology than that caused by the finest particles (FL and  
289 EFL). Indeed, at any SL, almost all Mixolab parameters for CL doughs resulted closer to the STD curve than  
290 those recorded with FL and EFL flours, especially those related to the flours' protein quality (Table 1).

291 Moreover, focusing on the three stages governed by modification of the physicochemical properties of starch  
292 (Table 1), it can be seen that, at any SL, lentil flour addition significantly ( $P \leq 0.001$ ) affected gelatinization  
293 and retrogradation (decrease in C3 and C5 compared to STD) without showing a trend as a function of PS.

294 Considering the contribution of starch retrogradation on bread staling phenomena, a reduction in C5 and its  
295 variability as a function of SL x PS may suggest potential shelf-life improvements in finished bakery products  
296 compared to STD, due to lower staling rates during storage (Erukainure, Okafor, Ogunji, Ukazu, Okafor, &  
297 Eboagwu, 2016; Dabija, Codină, & Fradinho, 2017).

#### 298 **4. Conclusions**

299 This study explored the effect of PS on the compositional, functional, morphological, and thermal properties  
300 of whole red lentil flour. In addition, the impact of incorporating lentil flour PS and SL on the rheological  
301 properties of wheat-flour-based dough was investigated to predict dough quality in baking.

302 Fractionation significantly affected the WHC, OHC and  $S_p$  of whole red lentil flour, while microscopy  
303 confirmed associations between PS and cell intactness. However, multivariate statistics suggest that these  
304 factors only slightly affect the rheology of wheat-based dough enriched with lentil flour of different PS,  
305 demonstrating that the major factor affecting the rheology is SL.

306 Besides the nutritional benefit derived by the enrichment in protein and ash contents at any SL, lentil/wheat-  
307 flour blends up to 10% SL provide the best properties in baking at any PS, while at higher SLs, a general  
308 worsening effect on dough rheology may occur, which resulted also dependent upon flour PS. Indeed, with a

309 rheological profile closer to STD, especially in stages governed by protein characteristics, coarser fractions  
310 (>200 µm) can yield higher performance than unfractionated flour and finer fractions.

311 These findings advocate the use of lentil flour with a PS ~200 µm for breadmaking, although further studies  
312 are needed to confirm the effect of PS and SL on the quality of bread made from lentil/wheat flour blends,  
313 especially in the case of high substitution level.

314

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318

#### 319 **Declaration of Competing Interest**

320 The authors declared that there is no conflict of interest.

321

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325

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- 457

Table 1: Settings used in Mixolab Chopin + protocol and Mixolab recorded curve.

Chopin + protocol		Mixolab Output
Parameter	Value	
Mixing speed	80 rpm	
Target torque (for C1)	1.10 Nm	
Dough weight	75 g	
Tank temperature	30 °C	
Temperature 1 <sup>st</sup> step	30 °C	
Duration 1 <sup>st</sup> step	8 min	
Temperature 2 <sup>nd</sup> step	90 °C	
1 <sup>st</sup> temperature gradient	4 °C/min	
Duration 2 <sup>nd</sup> step	7 min	
2 <sup>nd</sup> step gradient	-4 °C/min	
Temperature 3 <sup>rd</sup> step	50 °C	
Duration 3 <sup>rd</sup> step	5 min	
Total analysis time	45 min	

Table 2: Particle size distribution (%) and proximate composition (g/100 g) of different flour samples. L, whole lentil flour; LC, coarse lentil flour; LM, medium lentil flour; LF, fine lentil flour; LEF, extra-fine lentil flour; STD, common wheat flour. Proximate composition values are expressed as mean  $\pm$  SD (n=2). Values followed by different letters in each column are significantly different ( $P < 0.05$ ).

	Particle mass distribution (%)				Protein	Fat	Moisture	Ash	Carbohydrates
	<100 $\mu$ m	160-100 $\mu$ m	200-160 $\mu$ m	>200 $\mu$ m					
L	$\approx 19\%$	$\approx 18.5\%$	$\approx 20\%$	$\approx 42\%$	$24.13 \pm 0.38a$	$1.10 \pm 0.02e$	$9.96 \pm 0.12c$	$2.39 \pm 0.01c$	$62.43 \pm 0.27c$
LC				$\approx 42\%$	$21.21 \pm 0.23b$	$1.54 \pm 0.01d$	$10.18 \pm 0.03b$	$2.44 \pm 0.00b$	$64.53 \pm 0.24b$
LM			$\approx 20\%$		$23.64 \pm 0.25a$	$1.83 \pm 0.01c$	$10.01 \pm 0.00c$	$2.46 \pm 0.00a$	$62.07 \pm 0.24cd$
LF		$\approx 18.5\%$			$24.03 \pm 0.01a$	$1.87 \pm 0.01b$	$9.71 \pm 0.11d$	$2.44 \pm 0.00b$	$61.95 \pm 0.08d$
LEF	$\approx 19\%$				$24.06 \pm 0.00a$	$2.09 \pm 0.03a$	$9.57 \pm 0.02e$	$2.33 \pm 0.00d$	$61.91 \pm 0.02d$
STD	$\approx 72\%$	$\approx 22\%$	$\approx 5\%$	$\approx 0.5\%$	$12.62 \pm 0.37c$	$1.54 \pm 0.01d$	$10.78 \pm 0.03a$	$0.33 \pm 0.01e$	$74.72 \pm 0.32a$

Table 3: Effects of PS on WHC, OHC and Swelling power of L flour, its fractions and STD. WHC, water holding capacity; OHC, oil holding capacity;  $S_p$ , Swelling Power; L, whole lentil flour; LC, coarse lentil flour; LM, medium lentil flour; LF, fine lentil flour; LEF, extra-fine lentil flour; STD, common wheat flour. Values are expressed as mean  $\pm$  SD (n=3). Values followed by different lowercase letters in each column are significantly different ( $P < 0.05$ ). Values followed by different capital letter in each row are significantly different ( $P < 0.05$ ).

	WHC (g/g)	OHC (g/g)	$S_p$ (g/g)			
	25°C	25°C	60°C	70°C	80°C	90°C
<b>L</b>	$1.68 \pm 0.04a$	$0.71 \pm 0.04a$	$5.87 \pm 0.17abC$	$6.87 \pm 0.59bcB$	$8.56 \pm 0.38abA$	$8.13 \pm 0.29dA$
<b>LC</b>	$1.73 \pm 0.12a$	$0.63 \pm 0.04b$	$5.17 \pm 0.3bC$	$6.73 \pm 0.72cB$	$8.39 \pm 0.41abA$	$8.29 \pm 0.27cdA$
<b>LM</b>	$1.85 \pm 0.07a$	$0.71 \pm 0.07a$	$5.23 \pm 0.08abC$	$7.24 \pm 0.43abcB$	$8.93 \pm 0.23abA$	$9.08 \pm 0.14bcA$
<b>LF</b>	$1.50 \pm 0.18b$	$0.77 \pm 0.04a$	$6.01 \pm 0.41aC$	$7.69 \pm 0.43abcB$	$9.09 \pm 0.38aA$	$9.38 \pm 0.57bA$
<b>LEF</b>	$1.18 \pm 0.03c$	$0.76 \pm 0.04a$	$5.40 \pm 0.43abC$	$7.85 \pm 0.32abB$	$8.77 \pm 0.64abAB$	$9.65 \pm 0.29bA$
<b>STD</b>	$1.02 \pm 0.02c$	$0.79 \pm 0.03a$	$5.89 \pm 0.63abC$	$8.19 \pm 0.57aB$	$8.32 \pm 0.27bB$	$10.80 \pm 0.63aA$

Table 4: Thermal properties of L flour and fractions compared to STD. L, whole lentil flour; LC, coarse lentil flour; LM, medium lentil flour; LF, fine lentil flour; LEF, extra-fine lentil flour; STD, common wheat flour. Values are expressed as mean  $\pm$  SD (n=3). Values followed by different lowercase letters in each column are significantly different ( $P < 0.05$ ).

	Starch gelatinization				Amylose – lipid complexes or protein denaturation			
	T <sub>on</sub> (C°)	T <sub>p</sub> (C°)	T <sub>off</sub> (C°)	$\Delta H$ (J g <sup>-1</sup> )	T <sub>on</sub> (C°)	T <sub>p</sub> (C°)	T <sub>off</sub> (C°)	$\Delta H$ (j g <sup>-1</sup> )
L	57.76 $\pm$ 0.39a	69.22 $\pm$ 0.12b	79.12 $\pm$ 0.67b	1.51 $\pm$ 0.32b	80.35 $\pm$ 0.47b	87.02 $\pm$ 1.14a	95.70 $\pm$ 0.82a	0.38 $\pm$ 0.14a
LC	55.49 $\pm$ 0.41b	69.75 $\pm$ 0.17ab	80.01 $\pm$ 0.74ab	1.77 $\pm$ 0.38ab	80.80 $\pm$ 0.47b	86.32 $\pm$ 0.39a	95.10 $\pm$ 0.62a	0.25 $\pm$ 0.04a
LM	57.07 $\pm$ 0.16a	69.47 $\pm$ 0.2ab	81.01 $\pm$ 0.74a	2.19 $\pm$ 0.12a	82.22 $\pm$ 1.06a	86.71 $\pm$ 1.94a	95.89 $\pm$ 0.91a	0.23 $\pm$ 0.04a
LF	57.3 $\pm$ 0.79a	69.58 $\pm$ 0.22ab	79.30 $\pm$ 1.88ab	1.4 $\pm$ 0.19b	80.70 $\pm$ 0.26b	87.01 $\pm$ 0.98a	94.81 $\pm$ 2.27a	0.28 $\pm$ 12a
LEF	57.41 $\pm$ 0.57a	70.04 $\pm$ 0.71a	79.64 $\pm$ 0.37ab	1.75 $\pm$ 0.29ab	81.57 $\pm$ 0.67ab	86.83 $\pm$ 0.64a	93.71 $\pm$ 0.54a	0.23 $\pm$ 0.04a
STD	52.99 $\pm$ 1.07c	65.83 $\pm$ 0.28c	75.05 $\pm$ 0.54c	2.02 $\pm$ 0.14a	-	-	-	-

Table 5: F significance level and sum square percent of the studied factor and their combinations on Mixolab parameters. ns not significant, SS sum of square. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ .

Factors	Particle size (PS)		Substitution level (SL)		PS x SL	
	SS%	Significance	SS%	Significance	SS%	Significance
C1_t (min)	1,56	ns	96,71	***	1,73	ns
C2_t (min)	0,50	ns	96,58	***	2,92	ns
C3_t (min)	8,85	ns	53,86	***	37,29	*
C4_t (min)	4,66	ns	52,28	***	43,06	ns
C5_t (min)	11,11	ns	28,40	*	60,49	ns
C1.2 (Nm)	5,07	***	91,28	***	3,65	***
C2 (Nm)	8,84	***	83,29	***	7,87	***
C3 (Nm)	10,97	*	53,28	***	35,75	*
C4 (Nm)	9,34	ns	72,58	*	18,08	ns
C5 (Nm)	5,62	***	68,62	***	25,76	***
C1_tp (°C)	8,49	ns	34,17	*	57,34	ns
C2_tp (°C)	1,22	ns	89,12	***	9,66	ns
C3_tp (°C)	5,87	ns	24,47	ns	69,66	ns
C4_tp (°C)	11,87	ns	15,28	ns	72,85	ns
C5_tp (°C)	5,78	ns	44,44	*	49,78	ns
Amplitude (Nm)	3,11	ns	77,66	***	19,23	ns
Stability (min)	4,47	***	93,93	***	1,60	***
WA (%)	0,95	***	97,99	***	1,06	***

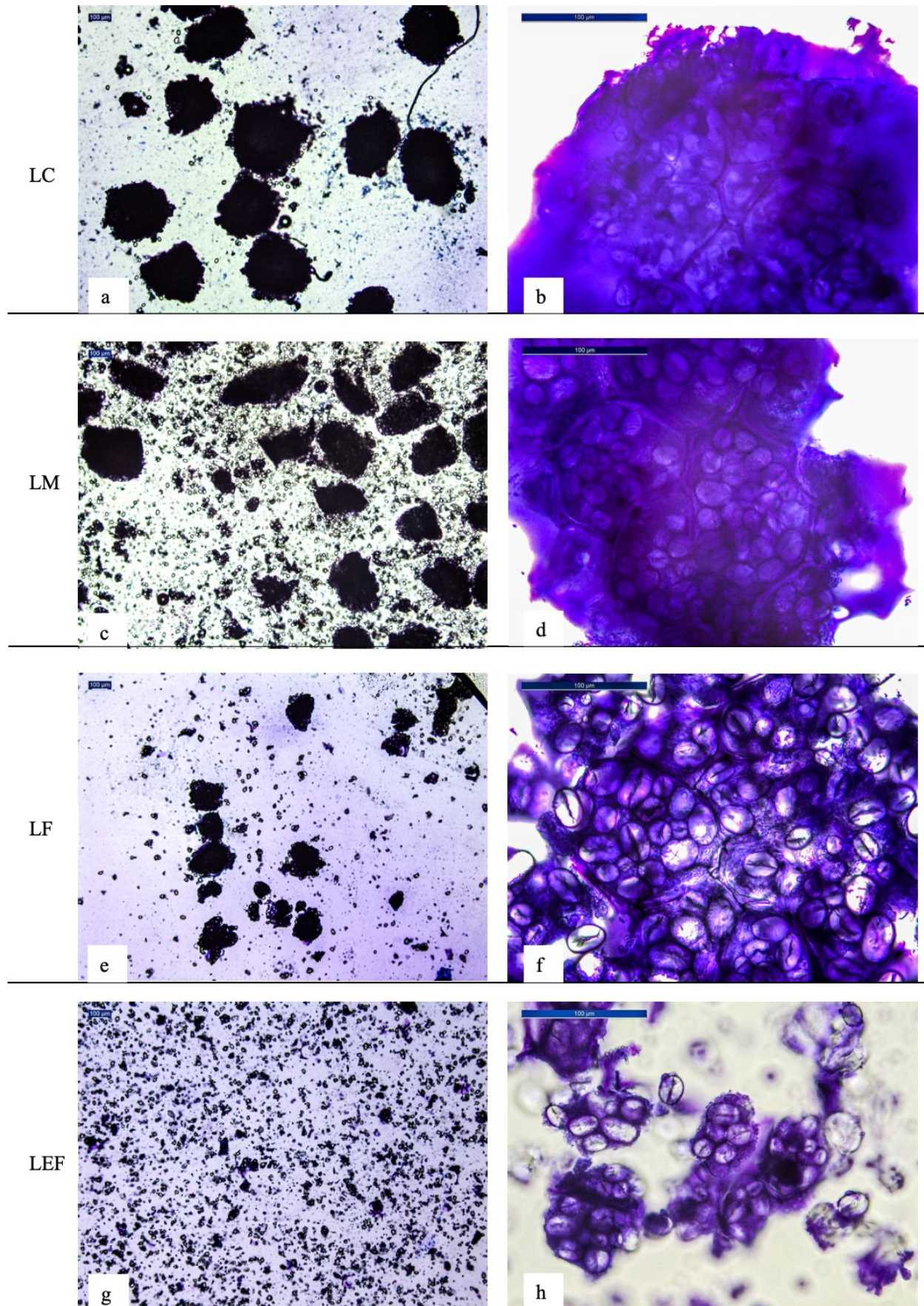


Fig. 1. Cell aggregates morphology (a, c, e, g; magnified 5x) and cells morphology (b, d, f, h; magnified 20x) in L fractions using optical microscope.

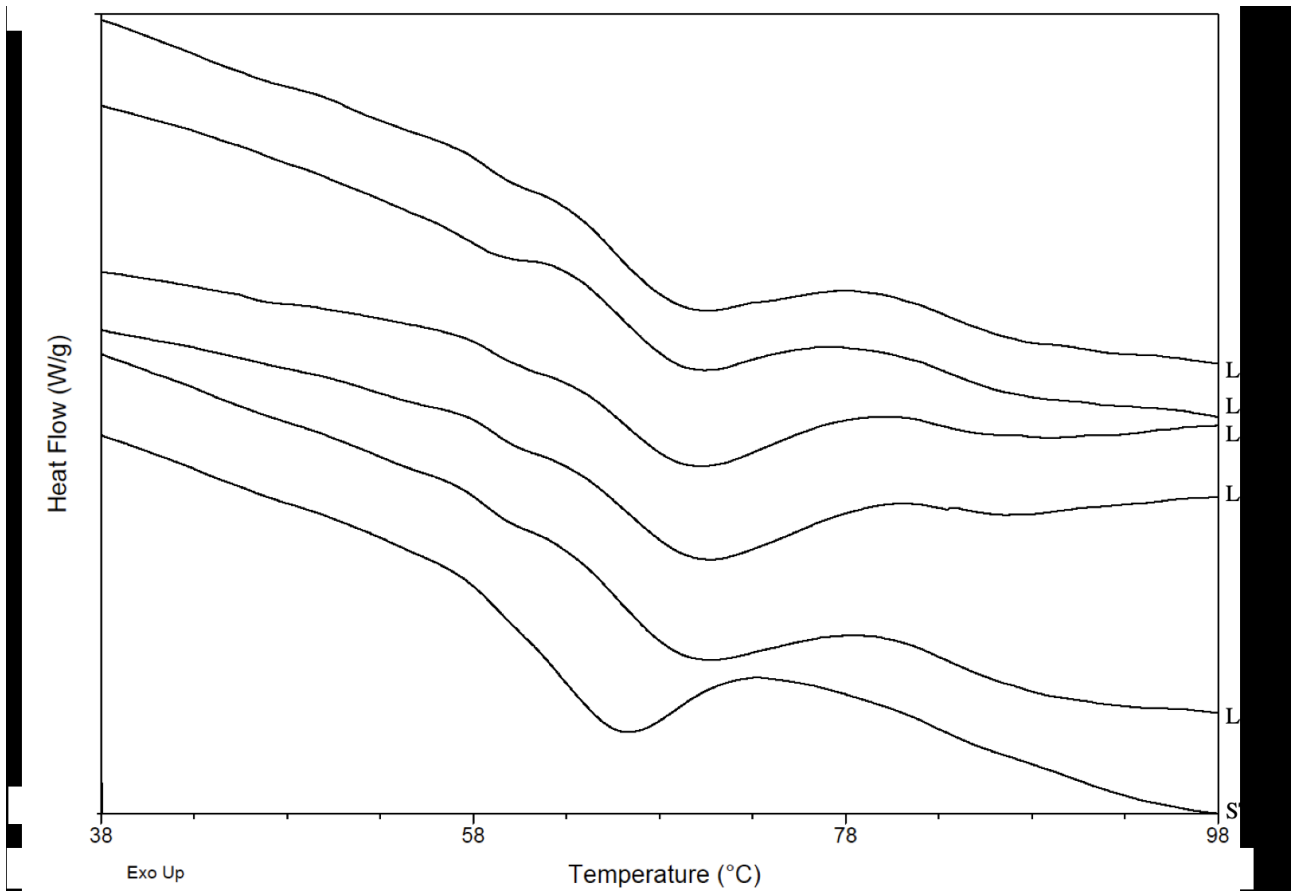
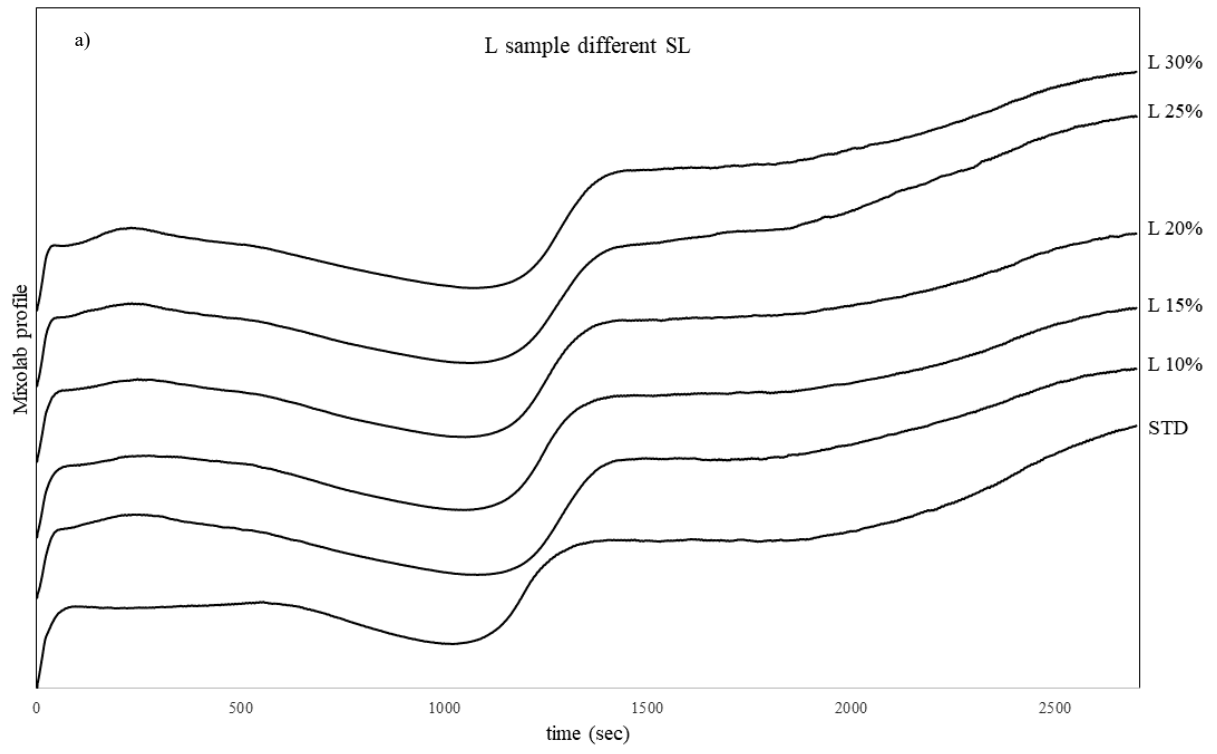


Fig. 1. DSC thermograms of STD, L sample and its fractions in the range 38-98°C.





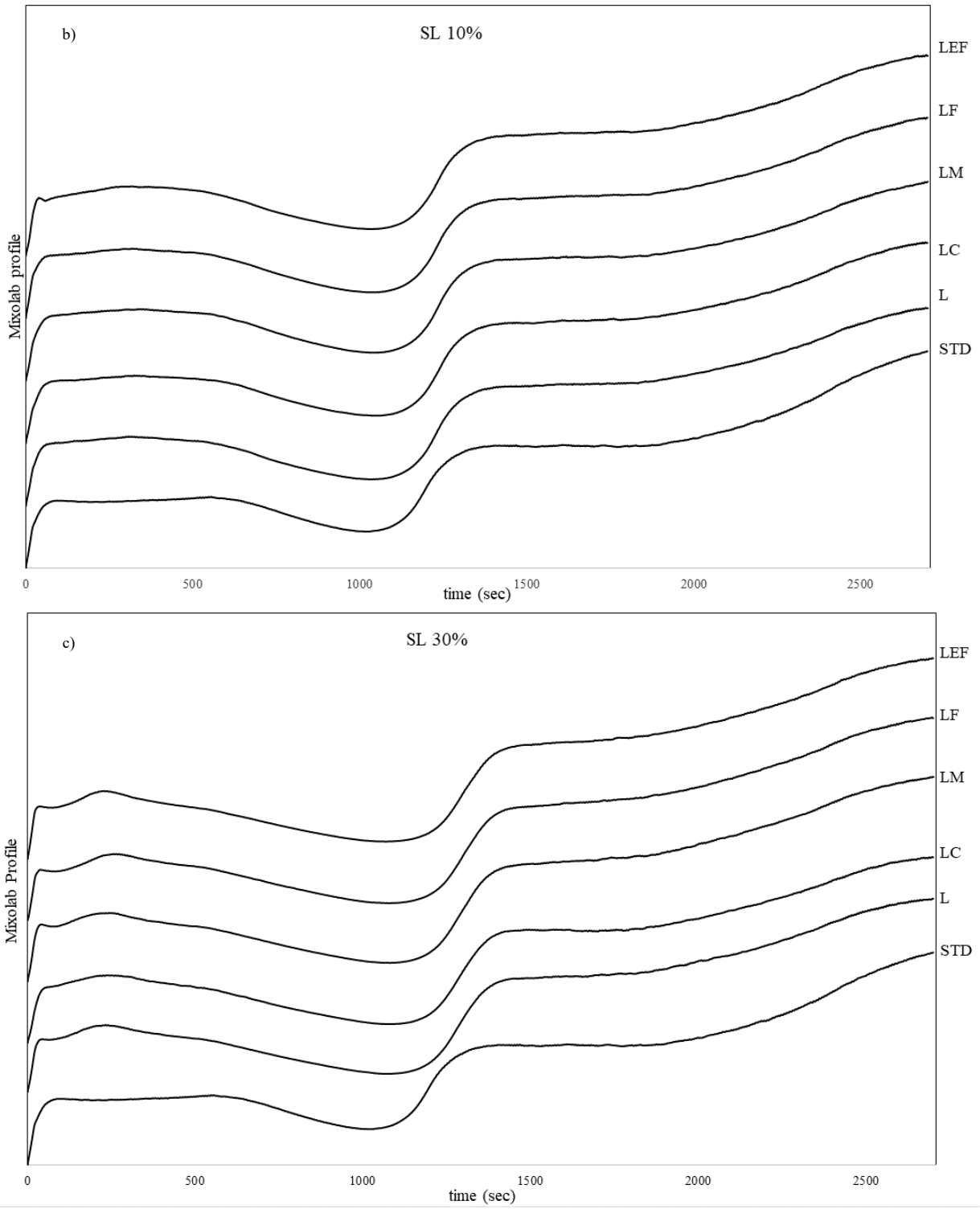


Fig. 3. Mixolab profile wheat-based dough of (a) L samples at all the SL tested; (b) flours at 10% SL; (c) flours at 30% SL.