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

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Abstract

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

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

Abbreviations

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

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

1. **Introduction**

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

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

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

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

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

2. Materials and methods

2.1 Raw materials

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

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

2.2 Flour fractionation

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

2.3 Physicochemical characterization of flours

2.3.1 Proximate composition

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

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

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

2.3.3 Swelling power (S_p)

S_p was measured following Yadav, Yadav, & Dhull's method (2012), with modifications. Suspensions (2% 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 g (8000 rpm) for 20 min. The weight of the resulting pellet was determined. S_p was calculated as the ratio between sediment and fresh sample weights. Values were reported as mean ± standard deviation of three replicates.

2.4 Thermal properties

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

2.5 Optical microscopy

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

2.6 Rheology

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

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

2.7 Statistical analyses

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

3. Results and discussion

3.1 Characterization of lentil flour and its fractions

Particle mass distribution (%) of STD, L and L fractions are reported in Table 2, and indicate that lentil flour contained a higher amount of larger particles as compared to STD. STD had significantly higher carbohydrates (74.72 ± 0.32%) and moisture content (10.78 ± 0.03%), but lower protein (12.62 ± 0.37%) and ash (0.33 ± 0.01%) than L and its fractions (Table 2), as expected (Boukid et al, 2019b). STD fat content (1.54 ± 0.01%) was similar to CL. Proximate composition of all lentil flours are in concordance with the findings of Hall, Hillen, & Garden Robinson (2017). Among the different fractions, CL showed significantly lower protein and higher carbohydrate content. Fat and protein content were inversely related to PS, while carbohydrate and moisture content decreased slightly with PS decrease. Ash content decreased with PS reduction, conceivably due to mineral association with starch granules of CL fractions, as postulated by Shafi, Baba, & Masoodi (2017).

3.2 Optical microscopy

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

3.3 Water holding capacity, oil holding capacity, and swelling power

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

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

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

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

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

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

3.4 Thermal properties

DSC thermograms and thermal properties of the studied flours are reported in Fig. 2 and Table 4, respectively. Wheat flour showed a unique thermal transition at 53 – 75 °C related to starch gelatinization. Instead, two endothermic peaks were evident for L flour and its fractions (Fig. 2). The first peak (55 – 80 °C) was attributed to starch gelatinization, while the 80 – 96 °C transition was previously related to amylose-lipid complexes melting or protein denaturation (Chung et al., 2008; Barbana, & Boye, 2013; Zeng, Gao, & Li, 2014; Ahmed et al., 2016). The starch gelatinization peak shifted to higher temperatures in L than in STD, suggesting higher energy to initiate starch gelatinization in lentil flours. The different gelatinization properties of cereals vs. pulses are likely attributable to several factors such as crystallinity, starch granule size, intermolecular bonding, and others (Ai & Jane, 2018). Moreover, DSC thermograms showed the gelatinization event starting with a minor peak in L samples, indicating that, although the majority of lentil flour starch gelatinizes at higher temperature than STD, a small fraction of starch has a tendency to gelatinize at a lower temperature.

Considering gelatinization peaks in L samples, CL showed the lowest T_{on} (≈55 °C) among all the samples which were comparable (≈57 °C), whereas T_p was lowest in L (≈69 °C) and highest in EFL (≈70 °C). T_{off} occurred at 79-81 °C in all L flours. Gelatinization enthalpy of STD (≈2.00 J g-1) and lentil flours was significantly different only in L (≈1.50 J g-1) and FL (≈1.40 J g-1). Thermal parameters of the second endothermic peak (T_{on}, T_p, T_{off} and ΔH) were not significantly different as a function of lentil flour PS (Table 4). Overall, PS did not affect lentil flour endothermic events, as observed by Boukid et al. (2019a).

3.5 Rheology

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

Table 5 shows the effect of PS, SL, and their interactions (PS x SL) on each Mixolab parameter using 2-way ANOVA. Based on statistical analyses (F significance level and sum square percent of factors studied), PS did not significantly affect C1_t (maximum torque at 30 °C) nor the time to attain C2, C3, C4 and C5. In contrast, PS significantly ($P \leq 0.05$) affected most torque [C1.2 (Nm, 5.07%), C2 (Nm, 8.84%); C3 (Nm, 10.97%); C5 (Nm, 5.62%)], but showed no significant effect on torque temperature and amplitude. Moreover, PS effects on stability (4.47%) and WA (0.95%) were low.

Investigating further using 2-way ANOVA, the results showed that almost all Mixolab parameters were controlled by SL, which had the highest influence on torque times [C1_t (96.71%); C2_t (96.58%); C3_t (53.86%); C4_t (52.28%); C5_t (28.40%)], torque [C1.2 (91.28%); C2 (83.29%); C3 (53.28%); C4 (72.58%); C5 (68.62%)], and above all torque temperature [C1 (34.14%); C2 (89.12%); C5 (44.44%)]. Similarly, SL greatly influenced the doughs' elasticity (77.66%), stability, (93.93%) and water absorption (97.99%) of the doughs. Considering PS and SL simultaneously, a smaller synergic contribution was found in the Mixolab data, compared to the two factors taken independently. Multivariate analyses confirmed PS and SL interactions which significantly ($P \leq 0.05$) affected C3_t (37.29%), torque values except for C4 [C1.2 (Nm, 3.65%); C2 (Nm, 7.87%); C3 (Nm, 35.75%); C5 (Nm, 26.76%)], stability (1.60%) and WA (1.06%), with a modest effect on C3, C3_t and C5.

Such findings suggest that SL was the predominant factor affecting the dough's entire rheological and thermo-mechanical behavior when analyzed with the Mixolab to predict baking quality. These results can also be observed in Mixolab curves of L samples (Fig. 3a): the higher the SL, the greater the variance from the STD curve, especially in the part referring to protein characteristics (i.e. stability during kneading and the protein weakening illustrated in Table 1). In fact, as per Table S1, increasing L level addition caused a significant ($P \leq 0.001$) increase in WA, reduction in C1.2 and C2 torques and dough stability, and delayed protein weakening (C2_t increases with SL increase). Since this curve concerns a protein weakening due to kneading and temperature effects, reduction in these parameters with an SL increase indicates worsening of wheat protein functionality in breadmaking. Additionally, an increased SL significantly ($P \leq 0.001$) worsened the pasting consistency of the dough (C3 decrease with SL increase), which may be related to the lower S_p of pulses than cereals, as above.

Flour samples at 10% SL (Figs. 3b and 3c) were more aligned to the STD curve than those at 30%. Addition of lentil flour at 10% SL significantly ($P \leq 0.05$) influenced C1_t, C1.2 and C5 parameters (Table 1) and WA, while none of the remaining parameters were significantly different from those of STD (Table S2). These observations indicated that STD dough enriched with 10% lentil flour can provide a nutritional benefit (e.g. the use of L flour results in a 9% and 64% increase in protein and ash contents, respectively) without altering the rheological profile of the dough at any PS.

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

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

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

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

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

4. Conclusions

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

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

Besides the nutritional benefit derived by the enrichment in protein and ash contents at any SL, lentil/wheat-flour blends up to 10% SL provide the best properties in baking at any PS, while at higher SLs, a general worsening effect on dough rheology may occur, which resulted also dependent upon flour PS. Indeed, with a rheological profile closer to STD, especially in stages governed by protein characteristics, coarser fractions (>200 µm) can yield higher performance than unfractionated flour and finer fractions.

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

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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

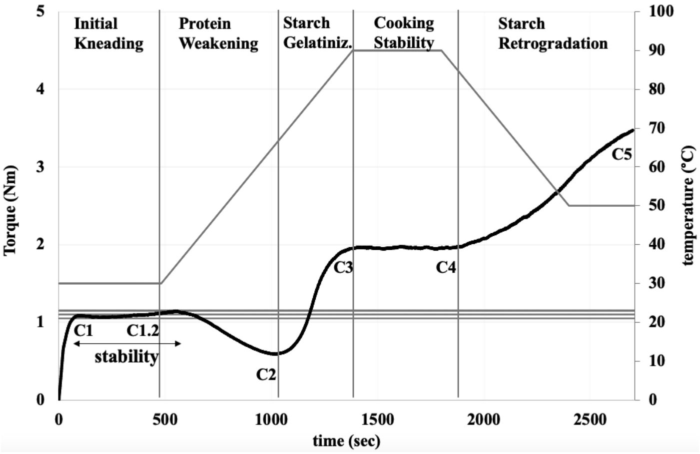
<table>
<tr><th colspan="2">Chopin + protocol</th><th>Mixolab Output</th></tr>
<tr><th>Parameter</th><th>Value</th><th></th></tr>
<tr><td>Mixing speed</td><td>80 rpm</td><td rowspan="13">
</td></tr>
<tr><td>Target torque (for C1)</td><td>1.10 Nm</td></tr>
<tr><td>Dough weight</td><td>75 g</td></tr>
<tr><td>Tank temperature</td><td>30 °C</td></tr>
<tr><td>Temperature 1st step</td><td>30 °C</td></tr>
<tr><td>Duration 1st step</td><td>8 min</td></tr>
<tr><td>Temperature 2nd step</td><td>90 °C</td></tr>
<tr><td>1st temperature gradient</td><td>4 °C/min</td></tr>
<tr><td>Duration 2nd step</td><td>7 min</td></tr>
<tr><td>2nd step gradient</td><td>-4 °C/min</td></tr>
<tr><td>Temperature 3rd step</td><td>50 °C</td></tr>
<tr><td>Duration 3rd step</td><td>5 min</td></tr>
<tr><td>Total analysis time</td><td>45 min</td></tr>
</table>

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 ± SD (n=2). Values followed by different letters in each column are significantly different ($P < 0.05$).

	Particle mass distribution (%)								
	<100 µm	160-100 µm	200-160 µm	>200 µm	Protein	Fat	Moisture	Ash	Carbohydrates
L	≈ 19%	≈ 18.5%	≈ 20%	≈ 42%	24.13 ± 0.38a	1.10 ± 0.02e	9.96 ± 0.12c	2.39 ± 0.01c	62.43 ± 0.27c
LC				≈ 42%	21.21 ± 0.23b	1.54 ± 0.01d	10.18 ± 0.03b	2.44 ± 0.00b	64.53± 0.24b
LM			≈ 20%		23.64 ± 0.25a	1.83 ± 0.01c	10.01 ± 0.00c	2.46 ± 0.00a	62.07 ± 0.24cd
LF		≈ 18.5%			24.03 ± 0.01a	1.87 ± 0.01b	9.71 ± 0.11d	2.44 ± 0.00b	61.95 ± 0.08d
LEF	≈ 19%				24.06 ± 0.00a	2.09 ± 0.03a	9.57 ± 0.02e	2.33 ± 0.00d	61.91 ± 0.02d
STD	≈72%	≈22%	≈5%	≈0.5%	12.62 ± 0.37c	1.54 ± 0.01d	10.78 ± 0.03a	0.33 ± 0.01e	74.72 ±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 ± 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
L	1.68 ± 0.04a	0.71 ± 0.04a	5.87 ± 0.17abC	6.87 ± 0.59bcB	8.56 ± 0.38abA	8.13 ± 0.29dA
LC	1.73 ± 0.12a	0.63 ± 0.04b	5.17 ± 0.3bC	6.73 ± 0.72cB	8.39 ± 0.41abA	8.29 ± 0.27cdA
LM	1.85 ± 0.07a	0.71 ± 0.07a	5.23 ± 0.08abC	7.24± 0.43abcB	8.93 ± 0.23abA	9.08 ± 0.14bcA
LF	1.50 ± 0.18b	0.77 ± 0.04a	6.01 ± 0.41aC	7.69 ± 0.43abcB	9.09 ± 0.38aA	9.38 ± 0.57bA
LEF	1.18 ± 0.03c	0.76 ± 0.04a	5.40 ± 0.43abC	7.85 ± 0.32abB	8.77 ± 0.64abAB	9.65 ± 0.29bA
STD	1.02 ± 0.02c	0.79 ± 0.03a	5.89 ± 0.63abC	8.19 ± 0.57aB	8.32 ± 0.27bB	10.80 ± 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 ± 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_{on} (C°)	T_p (C°)	T_{off} (C°)	ΔH (J g^{-1})	$T_{o\,n}$(C°)	T_p (C°)	T_{off} (C°)	ΔH (j g^{-1})
L	57.76 ± 0.39a	69.22 ± 0.12b	79.12 ± 0.67b	1.51 ± 0.32b	80.35 ± 0.47b	87.02 ± 1.14a	95.70 ± 0.82a	0.38 ± 0.14a
LC	55.49 ± 0.41b	69.75 ± 0.17ab	80.01 ± 0.74ab	1.77 ± 0.38ab	80.80 ± 0.47b	86.32 ± 0.39a	95.10 ± 0.62a	0.25 ± 0.04a
LM	57.07 ± 0.16a	69.47 ± 0.2ab	81.01 ± 0.74a	2.19 ± 0.12a	82.22 ± 1.06a	86.71 ± 1.94a	95.89 ± 0.91a	0.23 ± 0.04a
LF	57.3 ± 0.79a	69.58 ± 0.22ab	79.30 ± 1.88ab	1.4 ± 0.19b	80.70 ± 0.26b	87.01 ± 0.98a	94.81 ± 2.27a	0.28 ± 12a
LEF	57.41 ± 0.57a	70.04 ± 0.71a	79.64 ± 0.37ab	1.75 ± 0.29ab	81.57 ± 0.67ab	86.83 ± 0.64a	93.71 ± 0.54a	0.23 ± 0.04a
STD	52.99 ± 1.07c	65.83 ± 0.28c	75.05 ± 0.54c	2.02 ± 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 ≤ 0.05, **p ≤ 0.01, *** p ≤ 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	***

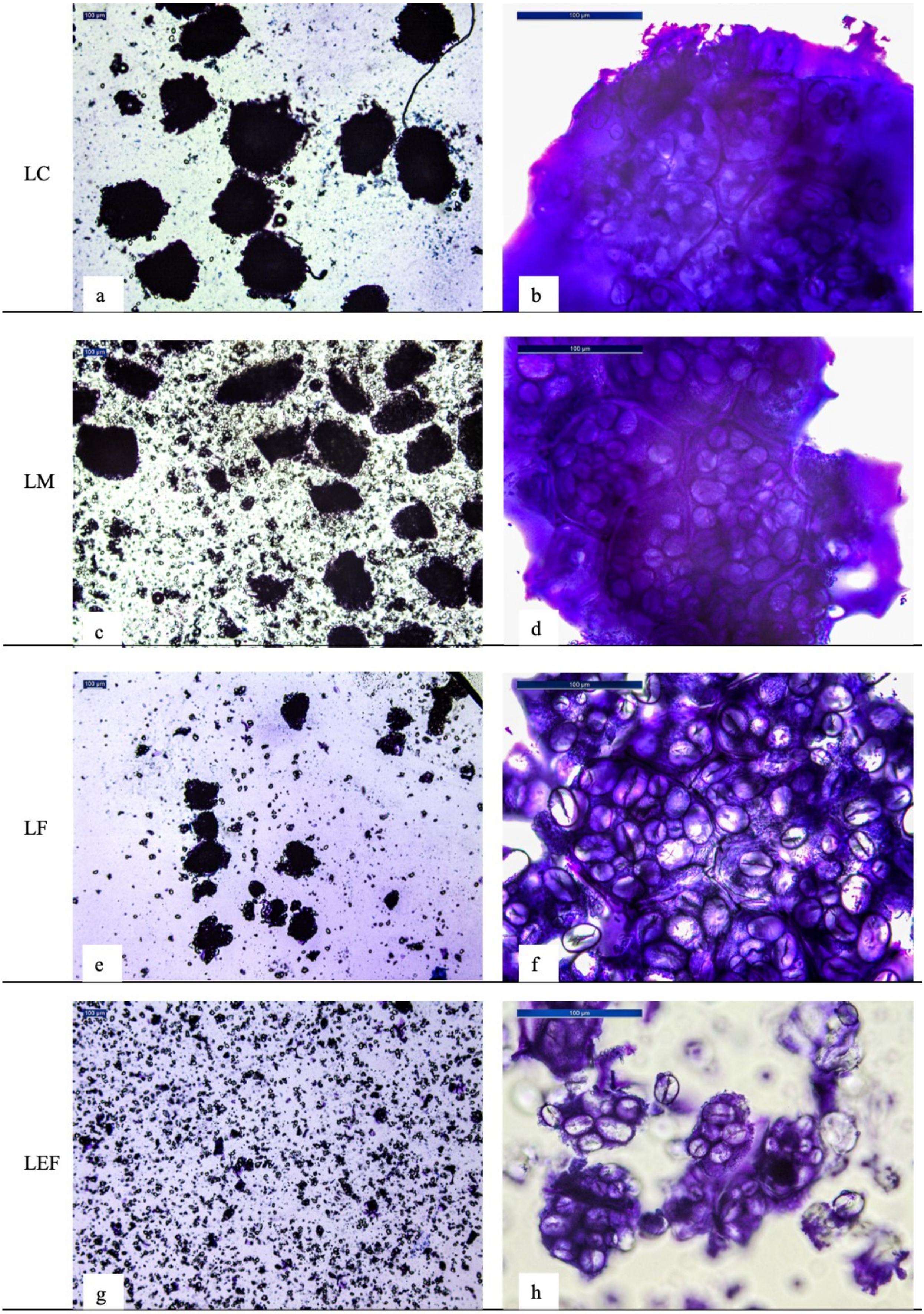


Fig. 1. Cell aggregates morphology (a, c, e, g; magnified 5x) and cells morphology (b, d, f, h; magnified 20x) in L fractions using optical microscope.

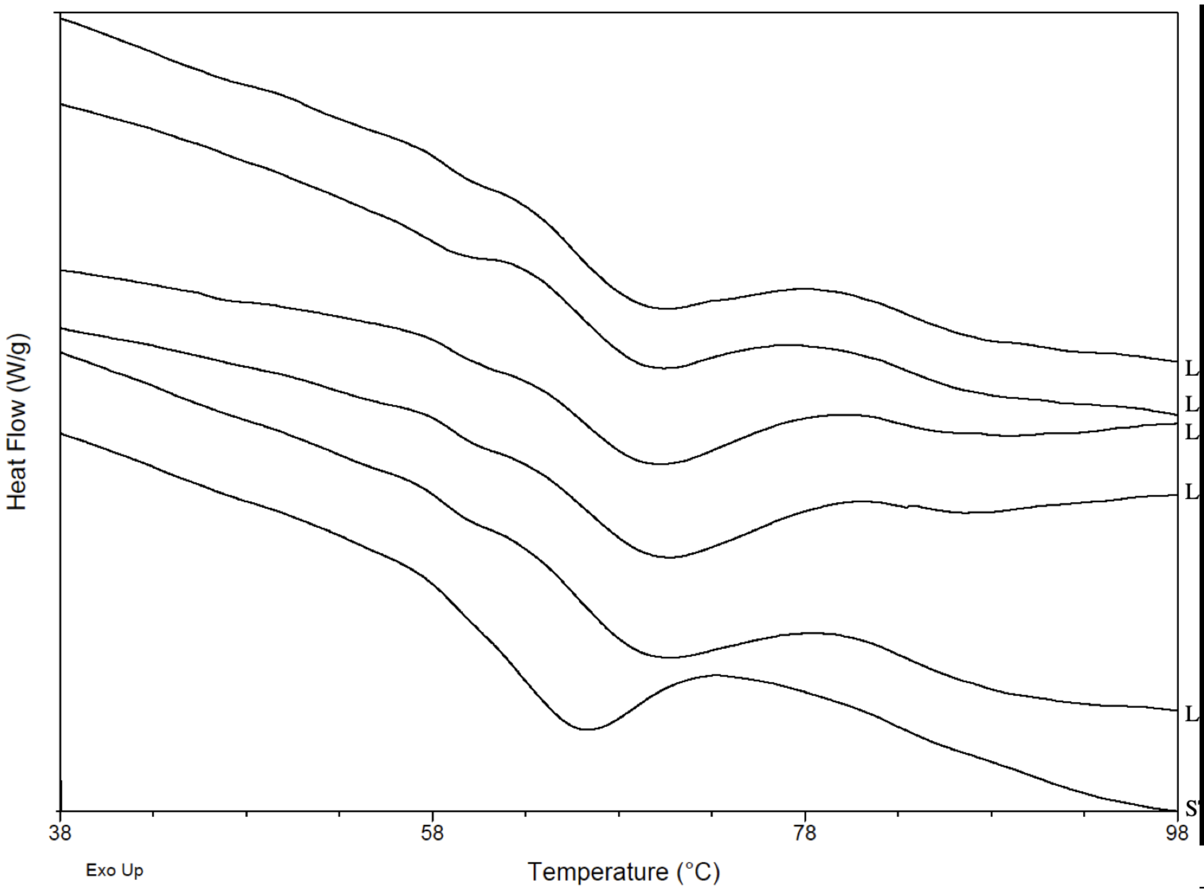
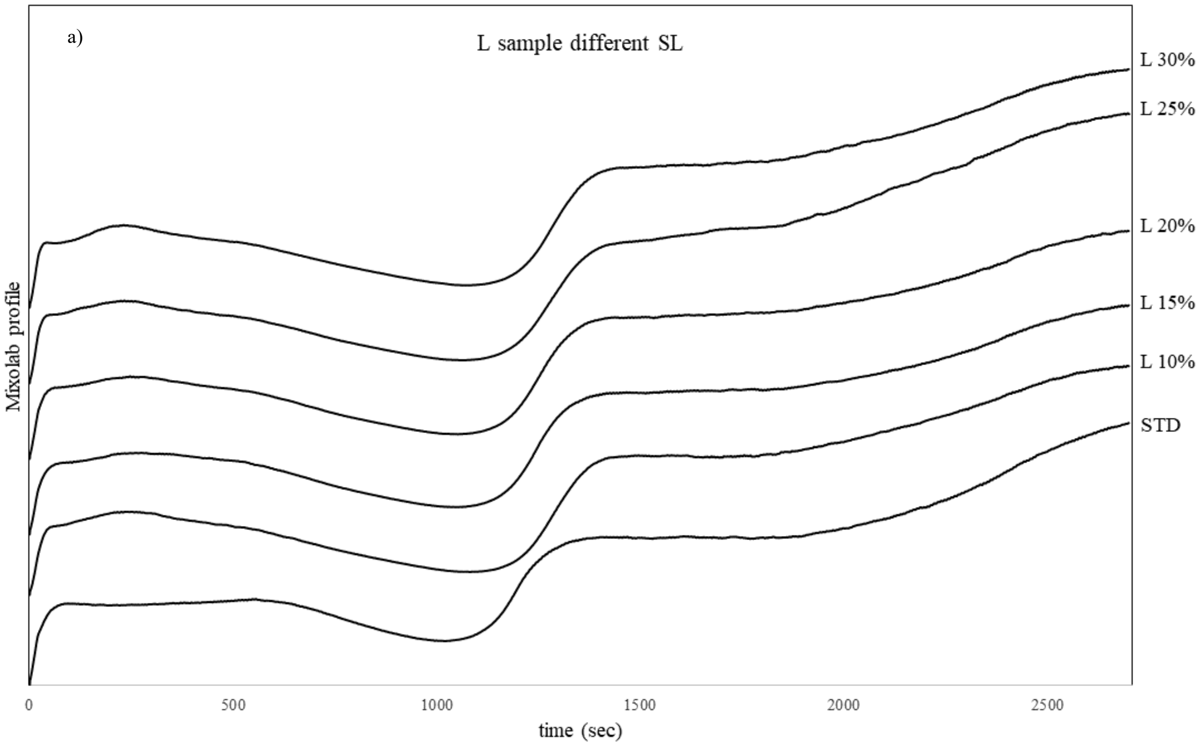


Fig. 1. DSC thermograms of STD, L sample and its fractions in the range 38-98°C.


a)
L sample different SL
L 30%
L 25%
L 20%
L 15%
L 10%
STD
Mixolab profile
0
500
1000
1500
2000
2500
time (sec)

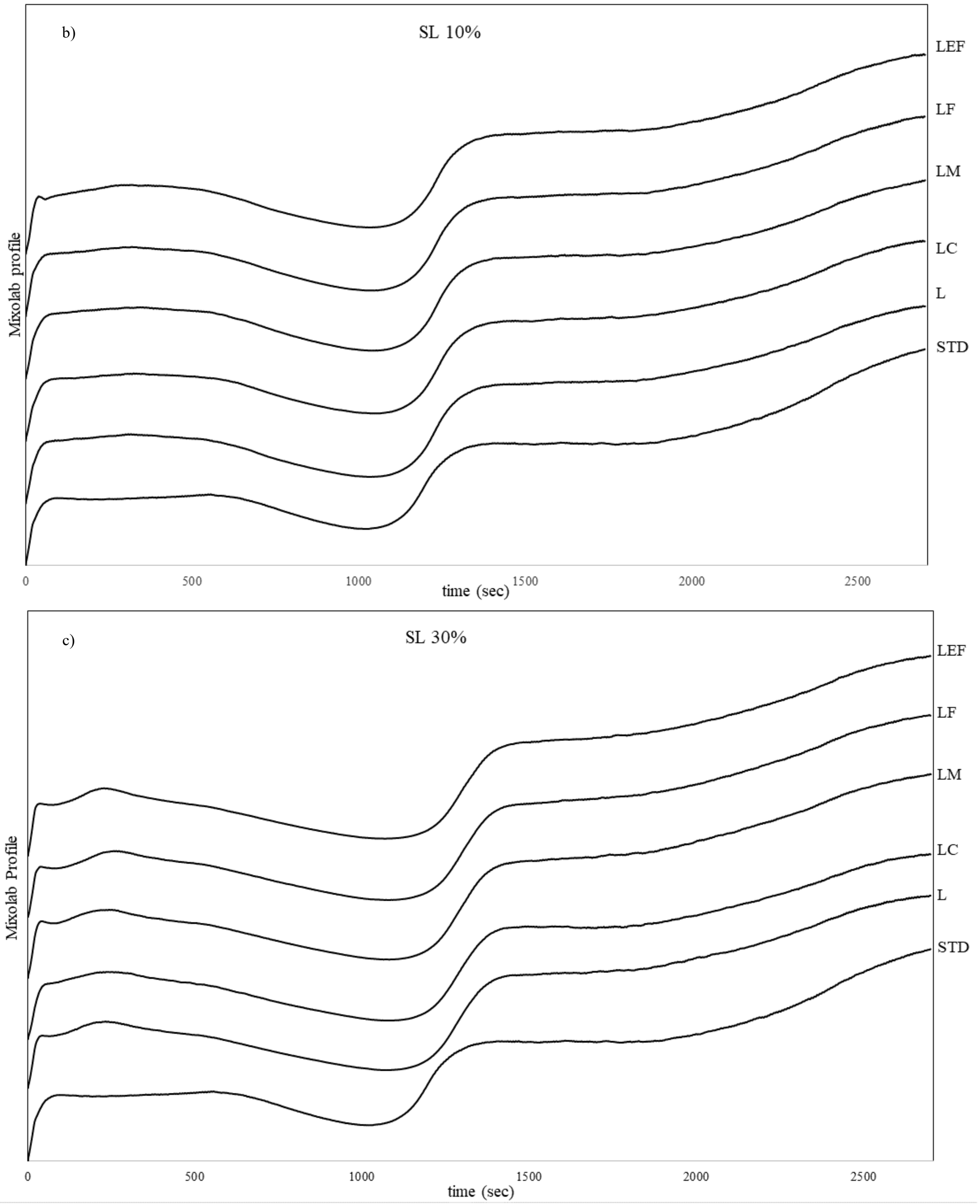


Fig. 3. Mixolab profile wheat-based dough of (a) L samples at all the SL tested; (b) flours at 10% SL; (c) flours at 30% SL.