Empirical rheology and pasting properties of soft-textured durum wheat (*Triticum turgidum* ssp. *durum*) and hard-textured common wheat (*T. aestivum*)

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Keywords

Puroindolines; kernel hardness; gluten aggregation; dough rheology

Abstract

Puroindoline (PIN[1]) proteins are the molecular basis for wheat kernel texture classification and affect flour milling performance. This study investigated the effect of PINs on empirical rheology and pasting properties in *T. turgidum* ssp. *durum* and *T. aestivum*. Soft wheat (cv. Alpowa), durum wheat (cv. Svevo) and their derivatives in which PINs were deleted (Hard Alpowa) or expressed (cv. Soft Svevo). Presence of PINs affected flour particle size and damaged starch. PINs increased the pasting temperature and breakdown viscosity, while the effect on peak viscosity and setback were not consistent. Presence of PINs was negatively associated with GlutoPeak gluten aggregation energy and farinograph dough stability, suggesting a weakening of the gluten matrix. As regards dough extensibility, the role of PINs was evident only in common wheat: 5DS distal end deletion increased the resistance to extension, without affecting the dough extensibility. This study showed PINs to have different impact on pasting and rheological properties of *T. aestivum* and *T. turgidum* ssp. *durum* flours.

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[1] **List of abbreviation**

AU, arbitrary unit; BE, Brabender equivalent; BU, Brabender unit; FU, farinograph unit; GPU, GlutoPeak unit; LT30, Loss of Torque 30 s after maximum torque; PIN, puroindoline protein; SKCS, Single Kernel Characterization System.

1. Introduction

Puroindolines (PINs) are wheat endosperm proteins associated with starch granules. They are considered minor components due to their low level (about 0.1%) in wheat (Dubreil et al., 1998). Despite the low level of occurrence, PINs play a key role in determining the kernel hardness of wheat (Morris, 2002; Bhave and Morris, 2008), which is defined as the force required to crush the kernels. The expression of PINs is controlled by *Puroindoline a* (*Pin a*) and *Puroindoline b* (*Pin b*) genes (Morris, 2002; Bhave and Morris, 2008) located on the distal end of the short arm of chromosome 5D (5DS). Functional expression of both genes results in soft kernel texture while the presence of only one functional gene or mutation in either of the genes results in hard kernel texture.

Common wheat (*Triticum aestivum* L) endosperm texture ranges from soft to very hard, while durum (*T. turgidum* ssp. *durum*) – which does not contain the 5D chromosome and therefore no PIN genes - has harder kernel texture. Kernel texture has been an important index in wheat commercialization, with hard kernel wheat attracting higher purchase value (Turnbull and Rahman, 2002), due principally to the higher protein content compared to soft wheat (Pauly et al., 2013a). The different kernel texture of the grains influences milling and end-use quality characteristics that have been extensively reported in recent reviews on this topic (Pauly et al., 2013a, b). Soft wheat requires less energy to mill, has higher break flour yield, smaller flour particle size and less damaged starch compared to hard wheat (Martin et al., 2007). The comparatively higher proportion of intact starch granules in soft wheat flours – together with the lower protein content - result in lower water absorption compared to hard wheat flour. Flours from soft wheat are used in making pastries and cookies while flours from hard wheat are used for bread and other leavened products. On the other hand, durum wheat is considered the best raw material for producing pasta and cous-cous.

The probable effect of PINs on dough rheology and product end-use quality has elicited considerable interest over the last decade. Most of the studies were carried out using fractionation/reconstitution experiments. Addition of purified PINs at 0.1% level produced opposite effects on dough strength and extensibility for flours with good or poor bread making performance (Dubreil et al., 1998). In particular, addition of PINs to good and poor bread quality flours increased and decreased dough strength and extensibility, respectively. Rouille et al. (2005) reported that adding 0.2% PINs to bread flour resulted in increased crumb grain fineness without affecting the bread specific volume, suggesting that PINs affect gas cell stabilization in bread dough (Pauly et al., 2013b). Similarly, Pauly et al. (2013c) recently reported PINs as exerting a softening effect when present above 0.07% in biscuit flour, highlighting how the level of PINs is critical to product quality. Although these studies expanded our knowledge about the role of PINs in dough and product characteristics, they present some limitations. First, PINs were usually added at levels higher than those naturally present in flour. Second, PINs isolation could have altered their functional properties, likely affecting protein interactions and, thus, dough rheology. Finally, Triton X-114 – which is generally used to isolate PINs – is very difficult to remove from protein samples. Thus, the presence of this detergent could impact the outcome of experiments (Pauly et al., 2013b).

About a decade ago, some authors investigated the effects of PINs on bread quality using transgenic lines in which PINs were over-expressed. Hogg et al. (2005) demonstrated that transgenic over-expression of PINs in common wheat decreased loaf volume and crumb grain scores. Cytological processes (homoeologous recombination) have also been used to transfer PIN genes to durum wheat producing soft durum lines (Gazza et al., 2011; Morris et al., 2011). The driving force behind producing soft-textured durum varieties is the potential increase in durum wheat production and end-use product applications. In theory, a broader, more diverse range of end-use for durum wheat should drive consumer demand and, hence, production (Morris et al., 2015). On the effect of durum kernel modification on product, pasta cooking quality was unaffected by the kernel hardness, whereas bread from durum wheat exhibited an increase in loaf volume associated with kernel softening (Gazza et al., 2011).

Similarly, back-cross seven (BC_7) of common soft wheat cultivar (Alpowa) was used to produce near-isogenic hard kernel lines lacking puroindolines (Morris and King, 2008). No information on the rheological properties of these near-isogenic wheat lines with modified kernel texture (hard-textured and soft-textured) is available.

This study investigated the effects of PINs expression or deletion on pasting properties, gluten aggregation, dough mixing and extensibility of soft-textured durum and hard-textured wheat. It will contribute to improve our understanding of the role of PINs in wheat quality and utilization.

2. Materials and methods

2.1 Wheat samples

Wheat cultivars (cvs.) Alpowa (soft wheat, *T. aestivum* L.), hard kernel Alpowa (Hard Alpowa), durum wheat (*T. turgidum* L. ssp. *durum*) cv. Svevo, and soft kernel durum wheat cv. Soft Svevo were used in the study. Hard Alpowa is a back-cross seven near-isogenic line of the soft wheat cv. Alpowa that lacks the distal portion of short arm of chromosome 5D (Morris and King, 2008). It involved crossing donor parents possessing *Pin a* and *Pin b* halotype genes with white soft spring cv. Alpowa. F1 and F2 seeds were harvested, planted and allowed to self. F3 seeds from individual F2 plants were subjected to progeny phenotypic screening. A homozygous hard plant was selected for backcrossing using Alpowa as recurrent male parent. The process was repeated to identify plants homozygous for hardness trait (Hard Alpowa). Soft Svevo was developed from recurrent back-crossing durum wheat cv. Svevo with Langdon durum that had *Pin a* and *Pin b* which were translocated from chromosome 5D of soft wheat cv. Chinese Spring (Morris et al., 2011). Alpowa and Hard Alpowa were grown in St. Paul (MN, US) and harvested in 2014. Svevo and Soft Svevo were grown in Pullman (WA, US) in 2013.

Wheat grains were conditioned (14.5 g/100 g moisture for Alpowa and Soft Svevo; 15.5 g/100g for Hard Alpowa; 16.5 g/100 g moisture for Svevo) and subsequently milled with a Quadrumat Junior (C.W. Brabender Inc., South Hackensack, NJ, USA) according to Approved Method 26-50.01 (AACCI, 1999).

2.2 Single Kernel Characterization System

Single Kernel Characterization System (SKCS) hardness values of the wheat cultivars were determined according to Approved Method 55-31.01 (AACCI, 1999).

2.3 Physicochemical characterization of flours

Moisture content was measured by drying the sample at 180 °C for 4 min in an infrared balance (MB 45, OHAUS, Parsippany, NJ). Damaged starch levels were measured according to Approved Methods 76-31.01 (AACCI, 1999). Flour particle size distribution was analyzed according to the Approved Method 55-60.01 (AACCI, 1999).

2.4 Pasting Properties

The pasting properties of the wheat flours were determined using a Micro-Visco Amylograph device (C. W. Brabender Instruments, South Hackensack, NJ). Fifteen grams of flour (14% moisture basis) were dispersed in 100 mL distilled water and stirred at 250 rpm. The following temperature profile was applied: mixing at 30°C for 3 min, heating from 30 °C to 95 °C at a rate of 7.5 °C/min, holding at 95 °C for 5 min, cooling from 95 °C to 30 °C at a rate of -7.5 °C/min, and holding at 30°C for 2 min. The following indices were considered: *(i)* Pasting temperature (temperature at which an initial increase in viscosity occurs); *(ii)* Peak viscosity (maximum viscosity achieved during the heating cycle); *(iii)* Peak temperature (temperature at the maximum viscosity); *(iv)* Breakdown viscosity (index of viscosity decrease during the holding period, corresponding to viscosity difference between peak and after holding at 95 °C); *(v)* Setback viscosity (index of the viscosity increase during, corresponding to the difference between the final viscosity at 30 °C and the viscosity reached after the holding period at 95 °C). Peak viscosity, breakdown, and setback viscosities were expressed in Brabender Units (BU). Pasting temperature and peak temperature were expressed in °C. For each sample the test was run in triplicate.

2.5 GlutoPeak Test

Gluten aggregation properties of flour samples were evaluated using the GlutoPeak device (C.W. Brabender Inc., South Hackensack, NJ, USA), as reported by Chandi and Seetharaman (2012). An aliquot of 8.5 g of flour (14% moisture basis) was dispersed in 9.5 g of 0.5M $CaCl_2$. Sample temperature was maintained at 34 °C by circulating water through the jacketed sample cup. The paddle was set to rotate at 1,900 rpm and the test was carried out for 7 minutes. The main indices automatically evaluated by the software provided with the instrument (GlutoPeak v. 2.1.0) were: *(i)* Peak maximum time (expressed in seconds), corresponding to the time before torque decreased due to gluten break down; *(ii)* Maximum torque (expressed in Brabender Equivalents - BE), corresponding to the peak occurring as gluten aggregates; *(iii)*

Energy to peak (expressed in GlutoPeak Unit - GPU), corresponding to the area under the curve until the maximum torque. In addition, the loss of torque 30 s after maximum torque (%) - corresponding to the decrease in torque 30 s after peak (LT30s) – was calculated. For each sample the test was run in triplicate.

2.6 Mixing Properties

The behavior of the dough during mixing was measured using a Farinograph - AT (C.W. Brabender Inc., South Hackensack, NJ, USA) equipped with a 50 g mixing bowl and according to Approved Method AACCI 54 -21.02 (AACCI 2000). The following indices were considered: *(i)* Water absorption (expressed in per cent), corresponding to the amount of water needed to reach the optimal consistency (500±20 Farinograph Unit, FU); *(ii)* Dough development time, corresponding to the time from first addition of water to the point of maximum consistency range; *(iii)* Stability, corresponding to the time difference between when the curve reaches (arrival time) and leaves (departure time) the 500 FU line. Each dough sample was analyzed in duplicate.

2.7 Dough Extensibility

Dough extensibility was measured with a micro-Extensograph instrument (C.W. Brabender Inc., South Hackensack, NJ, USA) on a 20 g dough piece, according to the manufacturer's manual. Dough was prepared according to AACCI Approved Method 54-10.01 in the 50 g test bowl of the farinograph, with addition of 2% NaCl, on a flour weight basis. The following parameters were considered: *(i)* Resistance to extension (expressed in BU), measured 50 mm after the curve has started and is related to the elastic properties of dough; *(ii)* Maximal resistance to extension (expressed in BU); *(iii)* Extensibility (expressed in mm) corresponding to distance at sample rupture; *(iv)* Energy (expressed in arbitrary units, AU) corresponding to the area under the curve; *(v)* Ratio, corresponding to the ratio between extensibility and resistance; *(vi)* Ratio Max, corresponding to the ratio between extensibility and maximal resistance to extension. Measurements for each sample were performed in duplicate and from each dough two subsamples were tested.

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed utilizing Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Puroindolines presence was used as a factor. When the factor effect was found to be significant ($p \leq 0.05$), significant differences among the respective means were determined using Fisher's Least Significant Difference (LSD) test.

3. Results and Discussion

3.1 Kernel and flour characterization

Physical characteristics of wheat samples are summarized in Table 1. Durum wheat Svevo and Hard Alpowa samples exhibited higher SKCS hardness values than Soft Svevo and Alpowa soft wheat, respectively. Kernel texture in wheat is controlled by *Pin a* and *Pin b* genes: soft wheat has both functional *Pin a* and *Pin b*, while hard wheat has either one or a mutation of either *Pin a* or *Pin b*. Durum wheat does not contain any of these endosperm-softening PIN genes, and therefore, it has very hard kernels. Similarly, the Hard Alpowa is missing the distal portion of chromosome 5DS and thus is also missing the PIN genes. The differences in PIN expression affected the flour protein concentration. Flours from grains without PINs (Svevo and Hard Alpowa) showed higher protein content than the corresponding samples with PINs (Table 1). The effect of PINs expression on protein content needs further investigation.

Kernel hardness affects various flour properties including particle size distribution and damaged starch (Table 1). As regards particle size, milling Svevo grain (using a mill for common wheat) resulted in two main fractions: one fraction with particle size ≥75 µm (55% of total) and another with particle size <75 µm (45% of total). PIN expression and the consequential soft kernel texture affected milling properties of the sample. Indeed, flour from Soft Svevo had a higher percentage of particles < 75 µm (75% of total) and lower percentage of ≥ 75 µm (25% of total). Moreover, differences in particle size contributed to differences in color between the two flours which is in agreement with Gazza et al. (2011). Color attributes - with particular regards to yellowness - are of great importance in durum wheat quality evaluation. Svevo exhibited a higher yellowness (b*) than Soft Svevo (20.0 vs 14.4, Fig. S1). Differences in color could also be attributed to differences in damaged starch granules, which do not reflect light as effectively as intact granules (Miskelly, 1984).

The deletion of the chromosome 5DS distal end where the *Pin a* and *Pin b* genes are located in Hard Alpowa resulted in an increase in kernel hardness and consequently larger flour particle size with a higher percentage of particles ≥ 75 µm compared to Alpowa (65 vs 48% of total for Hard Alpowa and Alpowa, respectively).

Differences in kernel texture also affected the level of damaged starch in the flours. As expected, in Alpowa and Soft Svevo, the percentage of damaged starch was significantly ($p \leq 0.05$) lower than in Hard Alpowa and Svevo, respectively (Table 1). The level of damaged starch in the flour contributes to the water absorption capacity of the flour during mixing. Damaged starch absorbs about twice its own weight of water, which is about 5 times greater than that of intact starch (Stauffer 2007), and depending on its level, makes a significant contribution to the overall water absorption capacity of flours (Cauvain, 2009).

3.2 Pasting properties

The effects of PINs on flour pasting properties are shown in Fig. 1. Soft Svevo showed higher pasting temperature, peak viscosity, breakdown viscosity and setback viscosity values than Svevo (Fig. 1Aand Table 2). The significantly ($p \leq 0.05$) higher pasting temperature in Soft Svevo compared to Svevo may be attributed to the presence of PINs, in agreement with a previous study on starch isolated from transgenic rice (Wada et al., 2010). PINs – which are localized at the starch surface (Feiz et al., 2009) - could inhibit the access of water to starch, which in turn would result in an extended time (and higher temperature) for starch to gelatinize and to reach peak viscosity. Interestingly, the detail in Fig. 1A showed for Soft Svevo a delay in granule swelling (related to increased viscosity) compared to Svevo, likely suggesting an effect of PINs on starch swelling at temperatures below 85 °C. As the temperature increased, Soft Svevo showed a higher peak viscosity than Svevo, indicating greater swelling capacity. However, Soft Svevo exhibited a slightly slower gelatinization rate, since it reached the peak viscosity at around 10 min, whereas Svevo reached the maximum value about 30 seconds earlier. PINs therefore seem to tolerate temperature, moderating temperature effect on starch properties. During the holding time at 95 °C for 5 min, Soft Svevo showed higher stability to high temperature and mixing as indicated by the lower breakdown value compared to Svevo. Finally, during the cooling step, starch in Soft Svevo showed a greater ability to reassociate in a new structure that exhibited higher viscosity compared to Svevo, therefore suggesting a higher retrogradation tendency.

As regards *T. aestivum*, Hard Alpowa showed a significant ($p \leq 0.05$) decrease in pasting temperature and breakdown viscosity than Alpowa (Fig. 1B and Table 2). These results are consistent with the results obtained for Svevo and Soft Svevo, which could be related to the impact of PINs expression (Svevo vs Soft Svevo) or 5DS distal end deletion (Alpowa vs Hard Alpowa) on pasting temperature and paste stability during the holding time at 95 °C. As regards the impact of PINs on viscosity during heating and cooling, Hard Alpowa showed higher peak viscosity and setback values than Alpowa. These results are in agreement with those obtained from reconstitution studies on common wheat flours which suggested that PINs affect pasting profiles by restricting starch water absorption and swelling in a diluted system, as in the case of the Micro-ViscoAmlograph test (Pauly et al., 2012; Debet and Gidley, 2006). Conversely, the impact of 5DS distal end deletion on peak viscosity and setback is not consistent with the trend observed for PINs expression (Fig. 1A).

Overall, the results on pasting properties suggest that PINs impact the temperature for onset of gelatinization (pasting temperature) and also the breakdown viscosity. However, the effect of PINs on starch swelling (peak viscosity) and retrogradation tendency (setback) remains unclear since it is apparently dependent on the type of wheat (i.e. *T. aestivum* or *T. turgidum* ssp. *durum*). Decreases in viscosity during heating and cooling have also been associated with an increase in damaged starch (Liu et al., 2014; Leon et al., 2006). This is consistent with our data on Svevo and Soft Svevo. On the contrary, since Hard Alpowa contained higher levels of damaged starch than Alpowa (Table 1), a lower maximum viscosity would have been expected for Hard Alpowa compared to Alpowa. This leads to the conclusion that PINS likely do affect flour pasting profiles.

3.3 Gluten Aggregation Properties

Fig. 2 presents the impact of PINs on gluten aggregation profile obtained by the GlutoPeak test. The parameters associated with the aggregation curves are reported in Table 2. During the test, the sample slurry is subjected to intense mechanical action, promoted by the speed (1,900 rpm) of the rotating element, which facilitates the formation of gluten, and a rapid increase of the torque curve is registered until the maximum torque is reached. Further mixing depolymerizes the network, with a concomitant decline in torque. The loss of torque 30s (LT30s) after maximum torque is an index of gluten strength during prolonged mixing.

In Svevo, PINs expression caused a significant ($p \leq 0.05$) decrease in maximum torque with no effect on the peak maximum time (Fig. 2A; Table 1). Consequently, GlutoPeak test energy, which is the area under the mixing curve to peak and takes into consideration the maximum torque and peak maximum time indices, decreased when PINs were expressed. This energy has been shown to correlate with gluten strength (measured as gluten index) and pasta cooking quality (Marti et al., 2014). Finally, 30s after maximum torque, Soft Svevo showed a significantly ($p \leq 0.05$) higher LT30s value than Svevo indicating a higher loss of torque and thus greater gluten breakdown due to over-mixing compared to Svevo.

The 5DS distal end deletion caused a significant ($p \leq 0.05$) decrease in peak maximum time and an increase in maximum torque and energy that suggest the presence of stronger gluten in Hard Alpowa compared to Alpowa (Fig. 2B), as supported by the energy value (Table 2). Among the GlutoPeak indices, the energy value is considered the most significant parameter for the prediction of the conventional parameters related to dough mixing such as stability, extensibility, and tenacity (Marti et al., 2015).

Since both PINs expression and 5DS distal end deletion did not affect the glutenin and gliadin genes, and therefore the gluten composition of the samples (data not shown), differences in gluten aggregation kinetics among the samples were likely related to PIN proteins. In flour, PINs are present at the starch granule surface and associate with polar lipids (Feiz et al., 2009). During dough mixing, they are removed from the granule surface and become incorporated in the gluten network, together with polar lipids (Finnie et al., 2010; Pauly et al., 2012). It may be hypothesized that PIN-polar lipid complexes interact with gluten proteins and delay and limit the extent of gluten aggregation.

3.4 Mixing Properties

The farinograph profiles of wheat samples are reported in Fig. 3. Soft Svevo showed lower water absorption capacity than Svevo, and similarly Alpowa showed lower water absorption capacity than Hard Alpowa (Table 2), reflecting the effect of high starch damage of the milling products from hard kernels compared with soft kernels (Table 1). Moreover as a consequence of PINs expression (Soft Svevo vs Svevo) dough development time and stability decreased (Fig. 3A). Indeed, differences in protein content between particular samples might account for the differences in dough strength. The protein contents of the samples of PINs expression and deletion (Table 1) are in agreement with previous reports that showed a decrease in flour protein when PINs were transgenically expressed (Hogg et al., 2005). Since in the present study each set of samples was grown under the same environmental conditions, results suggest that differences in protein content were solely related to presence of PINs that affected the mixing properties of the dough.

Our findings on mixing properties are in agreement with those reported by Hogg et al. (2005). On the contrary, studying the effects of grain texture on pasta-making and bread-making, Gazza et al. (2011) found that soft durum lines had higher stability than hard durum lines (cv. Langdon), likely due to the inability of damaged starch in hard durum lines flour to hold all the water absorbed initially. Moreover, soft-textured durum wheat lines did not differ from the hard durum lines in terms of dough mixing time (Gazza et al., 2011). These contrasting results confirm the observation made by Pauly et al. (2013c) that when puroindolines were added to biscuit flour at levels higher than 0.07%, it affected the dough texture. This suggests that for PINs to affect flour-dough quality parameters such as mixing time and stability, they will have to be present at a certain threshold level.

The 5DS distal end deletion (Alpowa vs. Hard Alpowa) increased both dough development time and stability (Table 2; Fig. 3B). The absence of PINs likely improved gluten protein interaction, resulting in increased dough development time and stability. The results agree with the typical farinograph profiles of strong and weak dough wheat flours. Usually, strong dough flours require higher amounts of water and longer mixing times to form a fully developed gluten network, which exhibits longer stability than flours with poor bread-making performance (Cauvain, 2009). Some of the differences in farinograph measurements (e.g. water absorption) could be attributed to protein content, damaged starch and flour particle size, whereas the differences in dough development time and stability are generally attributed to different types of gluten (Matsuo and Irvine, 1970).

3.5 Dough Extensibility

The tensile properties of dough were carried out on a “micro scale” using 20 g of dough and a micro-Extensograph which records the resistance of dough to stretching and the distance the stretched dough covers before breaking. The resistance of dough to the deformation forces is expressed as energy value and correlates well with the gas retention capacity of dough, volume of the end product after baking, handling properties, and is also taken as a guideline parameter for flour blending operations at milling facilities (Ktenioudaki et al., 2010). Hard wheat flours generally show high extensibility and a relatively high resistance to extension, a good balance of which is essential to hold gas bubbles during the fermentation of bread dough and other leavened products. On the other hand, doughs from soft wheat flours show high extensibility but low resistance to extension which makes them suitable for pastries and cakes.

Dough strength and extensibility of Soft Svevo were similar to Svevo (Table 2). Indeed, the PIN-possessing chromosome translocation does not alter any of the gluten proteins from the parent durum variety (Morris et al., 2011; Morris and King, 2008). For both Svevo and Soft Svevo, dough extensibility did not change for the different resting times (45, 90 and 135 min, data not shown). Gluten network in *T. turgidum* ssp.*durum* seems to be too tenacious for PINs to have a noticeable effect on dough extensibility. The results of the present work partially agree with previous studies. Gazza et al. (2011) reported no differences in dough extensibility (measured as Alveograph L value) between durum wheat with PINs and one with no PINs. On the other hand, dough strength (Alveograph W value, which is energy required to blow and break a bubble of dough) was significantly lower in soft durum lines compared with hard durum lines (Gazza et al., 2011). The Alveograph, however, is performed using a constant level of water absorption such that dough rheology is confounded with flour water absorption. Performing reconstitution experiments, Dubreil at al. (1998) showed that addition 0.1% of PINs drastically decreased the dough strength (Alveograph W) and increased the extensibility (measured as Alveograph L) in wheat flours with poor and medium bread-making performances. On the contrary, when PINs were added to a flour of good bread-making quality, an increase in W and a decrease in L were observed. Moreover, regardless of the bread baking quality of flour, tenacity (measured as Alveograph P) increased in the presence of PINs. It is important to keep in mind that contrasting results could be related to differences between the techniques. Firstly, the Extensograph stretches the dough in uniaxial mode while Alveograph expands the dough in all directions. Secondly, Extensograph works with doughs prepared to optimum hydration levels suited for different processing applications as in the real industrial world, whereas a constant amount of hydration is used in an Alveograph.

5DS distal end deletion did not affect dough extensibility (Table 2). On the other hand, Hard Alpowa showed a significantly (p≤0.05) higher resistance to extension and strength than Alpowa, suggesting that the presence of PINs improved the resistance to extension only in the case of weak flours. In addition, Hard Alpowa exhibited higher ratio values than Alpowa (Table 2). Since ratio indices are a measure of the balance between elasticity and extensibility, high values are generally indicative of tenacious/strong dough.

4. Conclusions

The study of the role of PINs on physical properties of doughs prepared from *T. aestivum* and *T. turgidum* ssp. *durum* - in which the genes for PINs were deleted or expressed, respectively – highlighted the following points: *(i)* wheat samples with PINs exhibited delayed starch gelatinization and less capacity to maintain the granule integrity at high temperature, *(ii)* wheat samples with PINs exhibited delayed gluten aggregation, likely due to the formation of PIN-lipid complexes that surround gluten proteins, *(iii)* wheat samples with PINs exhibited decreased dough stability, an indication that PINs interact with gluten, and *(iv)* the impact of PINs on starch swelling and dough extensibility is species- or variety-dependent.

The effects of PINs on dough rheological properties should be confirmed by investigating a larger number of varieties. Further studies should investigate the nature of PIN-gluten interactions and their potential role in product quality.

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Table 1. Kernel hardness, and flour particle size and damaged starch content of wheat samples possessing or lacking PINs

		Svevo	Soft Svevo	Alpowa	Hard Alpowa
SKCS		73	17	16	98
Particle size (%)	<75µm	44.1±1.3	73.9±0.9	51.5±1.2	34.8±1.5
	≥75µm	54.1±1.5	23.9±1.2	47.9±0.9	63.5±1.5
Protein g/100g		15.9±0.23	14.8±0.21	12.3±0.22	14.8±0.10
Damaged Starch (g/100g_{db})		12.1d	4.8b	4.5a	10.9c

SKCS - single kernel characterization system. Values in the same row with the same letters are not significantly different ($p \leq 0.05$)

Table 2. Gluten aggregation and dough mixing and extensibility properties of wheat samples possessing or lacking PINs

		Svevo	Soft Svevo	Alpowa	Hard Alpowa
Micro-Visco Amylograph test	Pasting temperature (°C)	58.3a	60.8b	60.8b	59.0a
	Peak viscosity (BU)	723c	849d	566a	638b
	Breakdown viscosity (BU)	123a	167b	323d	303c
	Setback viscosity (BU)	799c	988d	631a	746b
GlutoPeak test	Peak maximum time (s)	57.7b	58.3b	132.0c	50.3a
	Maximum torque (BE)	52c	34b	30a	54c
	Energy to peak (GPU)	2166c	762a	765a	1576b
	LT 30s (%)	18a	20b	30d	27c
Farinograph test	Water absorption (%)	76.6	60.6	57.2	68.8
	Development time (min:s)	04:51	01:50	01:35	05:31
	Stability (min:s)	03:20	02:35	02:13	08:30
Micro-Extensograph test	Extensibility (mm)	43a	43a	71b	72b
	Resistance (BU)	100b	95b	77a	101b
	Maximum resistance (BU)	101b	96ab	82a	108b
	Ratio	2.3a	2.2a	1.1b	1.4c
	Ratio Max	2.4a	2.2a	1.2b	1.5c
	Energy (AU)	3589a	3409a	4710b	6286c

Values in the same row with the same letters for each test are not significantly different ($p \leq 0.05$)

Figure Captions

Figure S1. Pictures showing colors of flours (a) Svevo and (b) Soft Svevo.

Figure 1. Pasting profile of (a) Svevo (black line) and Soft Svevo (grey line) flours and (b) Alpowa (grey line) and Hard Alpowa (black line). Dotted lines represent sample temperature.

Figure 2. Gluten aggregation profile of (a) Svevo (black line) and Soft Svevo (grey line) flours and (b) Alpowa (grey line) and Hard Alpowa (black line).

Figure 3. Mixing profile of (a) Svevo (black line) and Soft Svevo (grey line), (b) Alpowa (grey line) and Hard Alpowa (black line).

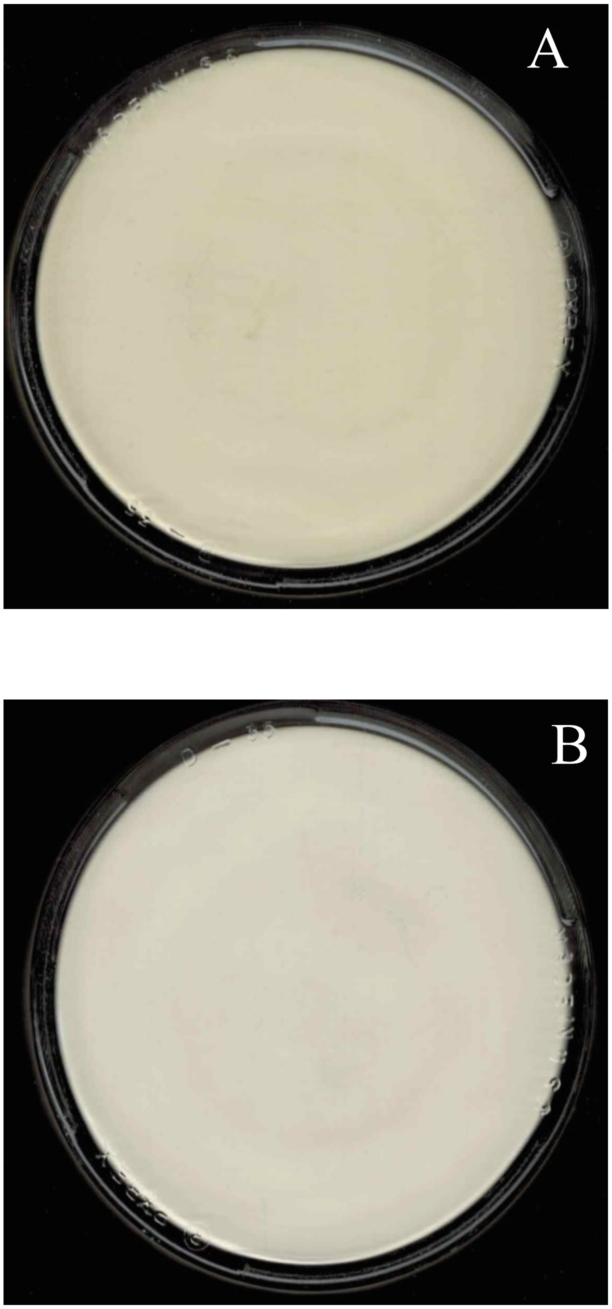
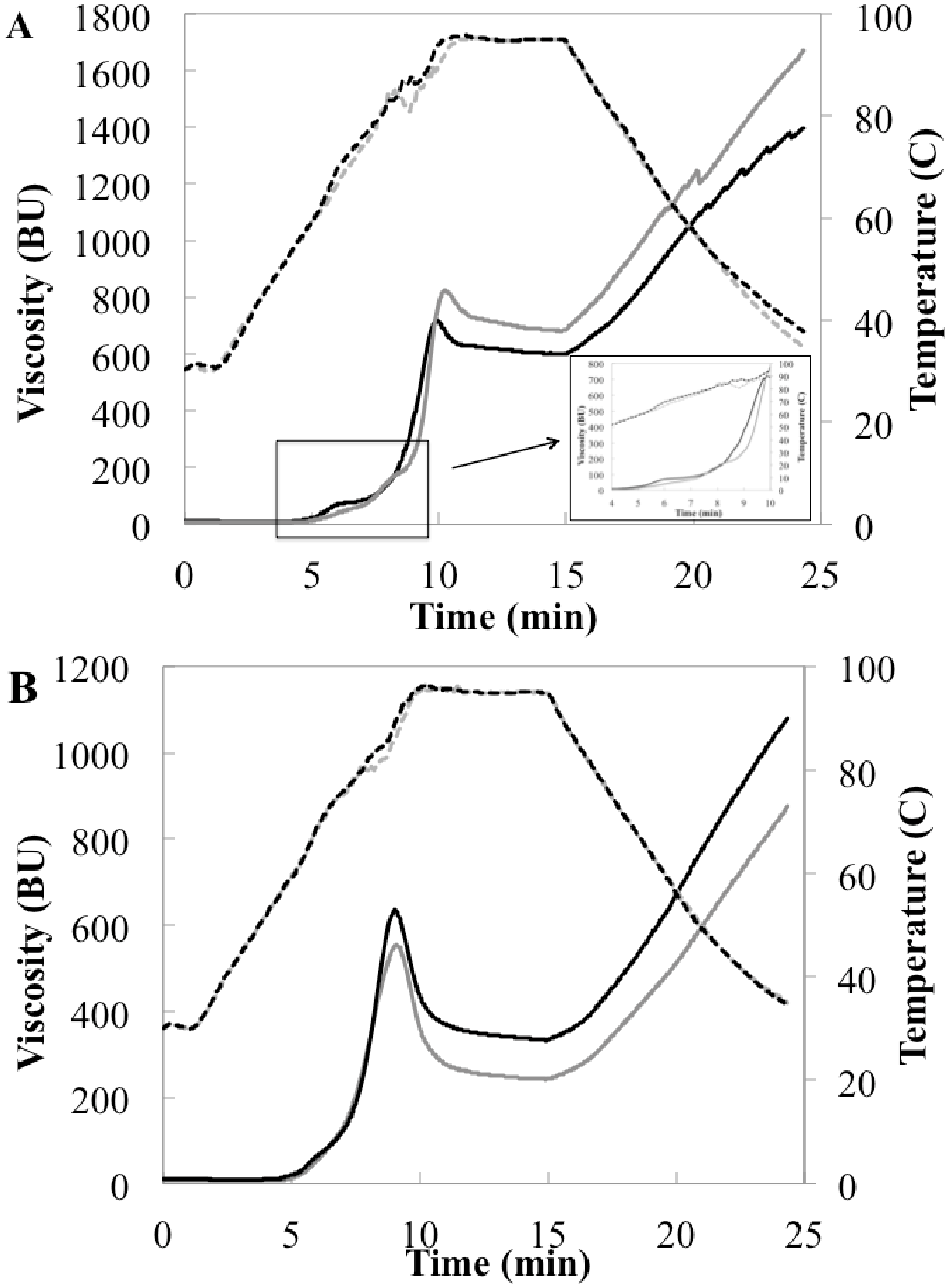


Fig. S1. Pictures showing colors of flours (a) Svevo and (b) Soft Svevo.



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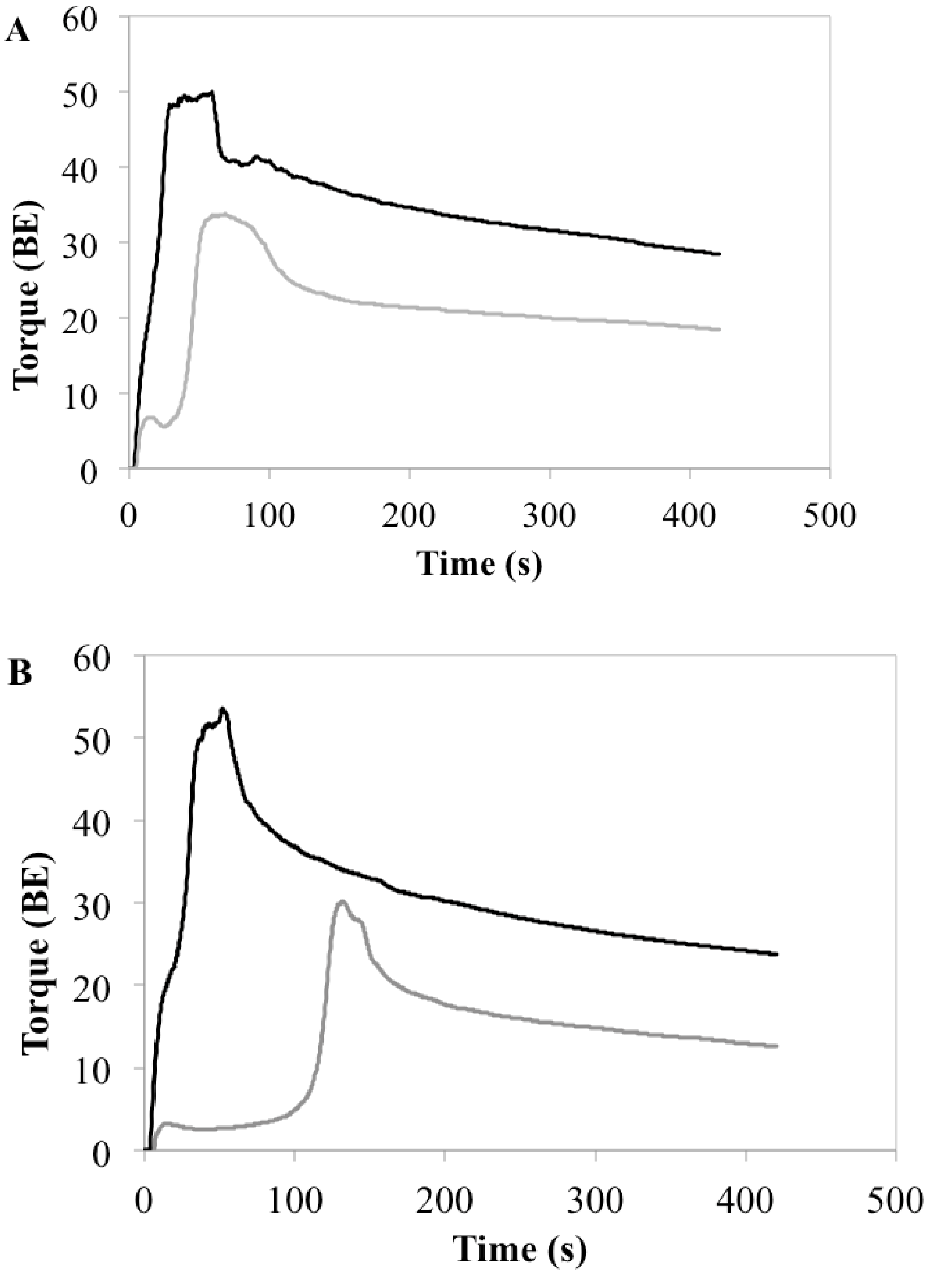

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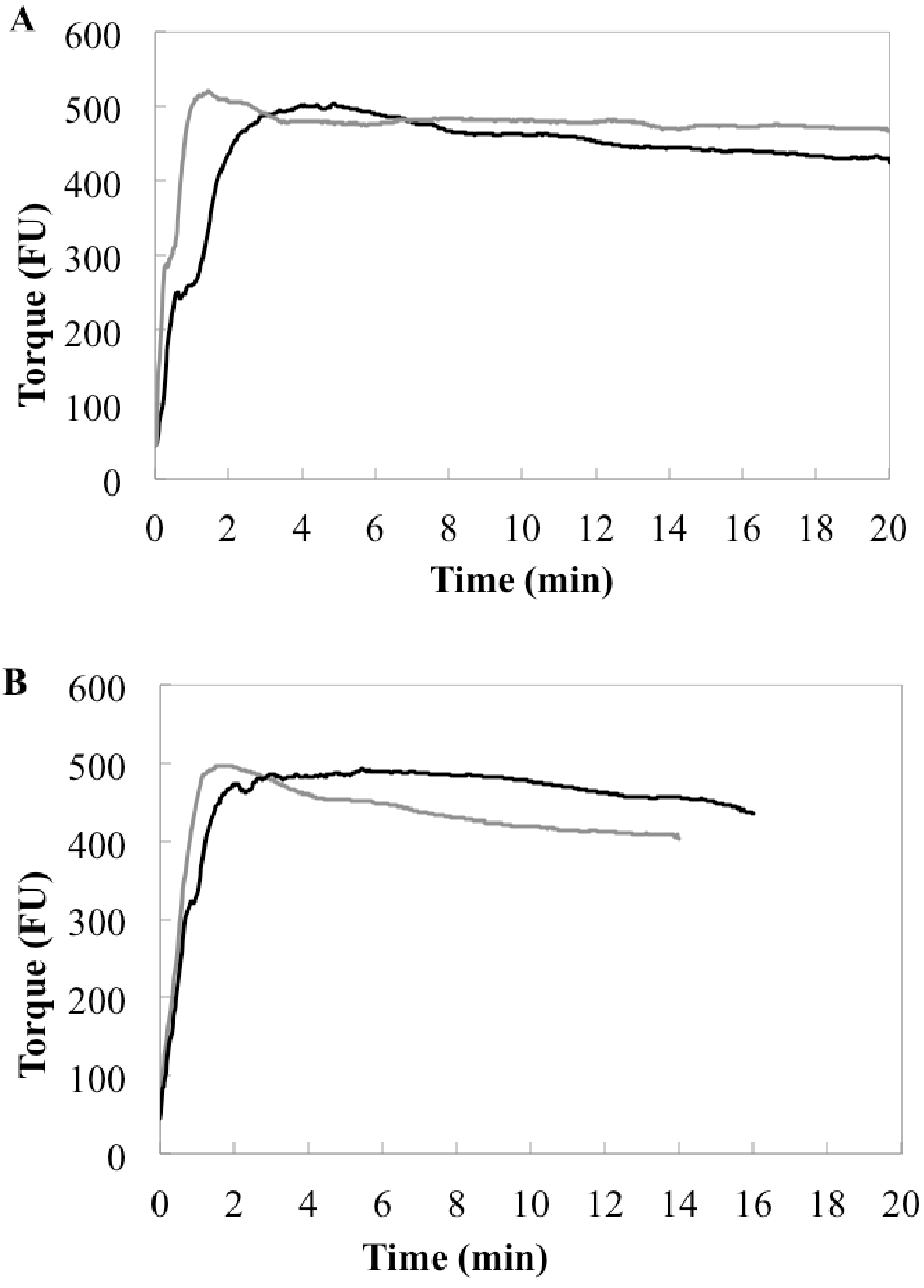

Figure 3. Mixing profile of (a) Svevo (black line) and Soft Svevo (grey line), (b) Alpowa (grey line) and Hard Alpowa (black line).