

Mixing Properties and Gluten Yield of Dough Enriched with Pea Protein Isolates

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Abstract

Over the last few years, many studies were carried out on the use of legume-based ingredients to supplement cereal-based matrices and produce nutritionally enhanced products. However, little is known about the influence of supplementation on the mixing properties of the enriched cereal-based matrices. The objective of this work was to study the impact of supplementing cereal-based matrices with commercial pea protein isolate or pea protein isolate produced by ultrafiltration/diafiltration using a 50 kDa membrane on the dough mixing properties. Studies were performed using a Perten® Glutomatic to estimate gluten yield, namely in terms of gluten index, wet gluten, dry gluten and water binding capacity, and using a Brabender® Farinograph to estimate water absorption, dough development time, stability, mixing tolerance index and minimum and maximum water content for dough formation. Four levels of pea protein isolate enrichment were considered: 0, 5, 10 and 15%. Results indicated that level of enrichment has little effect on measured mixing properties compared to the pea protein isolates considered. Isolate processed by membrane technologies takes part to the dough formation which does not seem to be the case with commercial isolate. Higher amount of water is required for dough formation with matrices enriched with commercial pea isolate compared to membrane processed isolate, while stronger dough properties are observed for matrices enriched with membrane processed isolate. This is attributable to the properties of the isolate, namely solubility and state of the proteins (native or denatured), which could impact how they interact with wheat proteins.

Keywords: Farinograph, Glutomatic, Mixing properties, Gluten yield, Pea protein, Durum wheat semolina

1. Introduction

Over the last few years, many studies were carried out on the use of legume based products to supplement durum wheat semolina and produce nutritionally enhanced pasta (Nielsen, Sumner & Whalley, 1980; Bahnassey, Khan & Harrold, 1986; Yanez-Farias, Bernal-Aguilar, Ramirez-Rodriguez & Barron-Hoyos, 1999; Zhao, Manthey, Chang, Hou & Yuan, 2005; Sabanis, Makri & Doxastakis, 2006; Shogren, Hareland & Wu, 2006; Torres, Frias, Granito, Guerra & Vidal-Valverde, 2007; Wood, 2009; Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, Ochoa-Martinez, Corzo, Bello-Perez, Medina-Torres & Peralta-Alvarez, 2010; Petitot, Boyer, Minier & Micard, 2010; Mercier, Villeneuve, Mondor & Des Marchais, 2011). Supplementation with legume protein ingredients allows to improve pasta protein content and compensates wheat semolina deficiency in lysine and threonine, two essential amino acids (Kies & Fox, 1970; Abdel-Aal & Hucl, 2002; Sánchez-Lozano & Martínez-Llorens, 2009).

It was observed that enrichment affects pasta quality, namely in terms of texture (Nielsen *et al.*, 1980), color (Gallegos-Infante *et al.*, 2010), cooking quality (Zhao *et al.*, 2005), sensorial aspect (Alireza Sadeghi & Bhagya, 2008) and engineering properties (Mercier *et al.* 2011). The impact of fortification is dependant on the level of supplementation and on the legume from which origins the product (Zhao *et al.*, 2005; Petitot *et al.*, 2010).

However, little is known about the influence of the processing of the protein ingredients. Indeed, these ingredients can be made using various processes which can influence proteins structure and as a result the proteins functional properties including their solubility. For example, it is well known that soy protein isolate produced by isoelectric precipitation are more denatured and as a result have lower solubility than isolates produced by membrane technologies (Petruccioli & Anon, 1994; Wagner, Sorgentini & Anon, 2000; Rao, Shallo, Ericson, & Thomas, 2002). As observed by Ribotta, Edel Leon, Pérez & Anon (2005) in the case of soy proteins, the state of the proteins (native or denatured) can impact their solubility and therefore their interaction with wheat proteins.

The objective of this work was to study the impact of supplementing cereal-based matrices with commercial pea protein isolate or pea protein isolate produced by ultrafiltration/diafiltration using a 50 kDa membrane. Effect of enrichment was studied on dough mixing properties and gluten yield, which are properties that are highly correlated to the final product quality, namely in terms of texture and cooking characteristics (Dexter & Matsuo, 1980; Ohm & Chung, 1999; Mason, Navabi, Frick, O'Donovan, Niziol, & Spaner, 2006; Oak, Sissons, Egan, Tamhankar, Rao & Bhosale, 2006).

2. Materials and Methods

2.1 Raw materials

Commercial pea protein isolate (CPPI) was purchased from Nutri-pea Limited (Portage La Prairie, MB, Canada). Isolate produced by membrane technologies (MTPPI) was made from certified #1 Eclipse Yellow peas purchased from Wagon Wheel Seed Corporation (Churchbridge, SK, Canada). Roller-milled durum wheat semolina was purchased from Horizon Milling (Montreal, QC, Canada).

2.2 Production of pea protein isolate by membrane technologies

Pea protein isolate was produced by membrane technologies as previously described by Taherian *et al.* (2011). The peas were first soaked in water (ratio 1:8 w/w) at room temperature for 90 minutes to swell them and detach the grain hull or cuticle so they would be easier to dehull afterwards. After soaking for 90 minutes in water, sodium hypochlorite (NaOCl) was added to the mixture until a concentration of 0.7 g/L was reached and the soaking was carried out for an additional 30 minutes. The peas were then recuperated and rinsed with water (ratio 1:8 w/w) to remove any residual sodium hypochlorite. They were dried overnight in a forced air 4-plate dryer (The National Drying Machinery Co., Philadelphia, Pennsylvania, USA, model 52979) operated at room temperature. Upon removal from the oven, the peas were ground using a Quadro Comil model 196 (Quadro Engineering Inc., Ontario, Canada) equipped with screen 002 for a first pass and with screen 009 for a second pass. The broken peas and their hulls were then passed through a multi aspirator (Kice, Wichita, Kansas, USA, model 6F6), where the hulls were removed from the top while the dehulled peas fell into the reservoir through the force of gravity. The dehulled peas were ground again using the Quadro Comil equipped with screen 106 to produce flour. The flour was placed in aluminium pouches which were hermetically sealed and stored at 4 °C until used.

For the production of the pea protein isolate, 7 kg of flour was dispersed in 105 kg of water (ratio 1:15 w/w) at room temperature and the dispersion was agitated for 30 minutes to allow the hydration of the flour. The pH of the dispersion was then adjusted to 7.5 using 2 M NaOH and the extraction was carried out for 45 minutes under agitation. If required, the pH was readjusted during the extraction. Once the extraction was completed, the insoluble matter was removed using a Basket Centrifuge (Western States, Hamilton, Ohio, USA, model STM-1000). In a first pass, a filter bag with a molecular weight cut-off of 60 µm was used while in a second pass, a filter bag with a molecular weight cut-off of 1 µm was used. The solution obtained after removal of the insoluble matter was then purified by ultrafiltration/diafiltration (UF/DF) using an home-made module equipped with two 50 kDa hollow fibre membranes with a surface of 2.3 m² for each membrane (Romicon (KOCH) model CTG 3", HF 25-60-PM50). The UF/DF sequence used to purify the pea extract was a UF step with a VCR 5 carried out at the extraction pH (i.e. 7.5) and a discontinuous DF step with a re-VCR 5 again at the extraction pH. The resulting pea protein isolate was lyophilized and placed in aluminium pouches which were hermetically sealed in stored at 4 °C until used.

2.3 Solubility of pea protein isolates

Protein solubility of both isolates was determined at pH 7 as follows: dry samples were rehydrated in bi-distilled water in order to obtain a 5% w/w solution (1 g of isolates in 19 g of bi-distilled water). The solutions were vigorously agitated for a few seconds using a vortex mixer and allowed to rest at room temperature for approximately two hours, with a second agitation after one hour. The samples were then centrifuged at 4000 g

for 15 minutes at room temperature. An aliquot of approximately 5 ml of the supernatant was taken, weighed with precision, and transferred to a Kjeldahl flask. Supernatant samples were mineralized and then titrated in the Kjeldahl flask, an official method for proteins determination. Protein relative solubility was determined by multiplying the fraction of protein in the supernatant over total protein content (dry basis) by 100. Moisture content was determined using AOAC Methods 925.09 (AOAC International, 1995) by drying samples overnight in a vacuum oven at 92 °C.

2.4 Farinograph analysis

Farinograph analysis was performed using two methods. The first method was the classical constant dough weight AACC Approved Method 54-21.02. This method measures and records the resistance of dough to mixing. It is used to evaluate the absorption of flours and to determine stability and other characteristics of dough during mixing. Results were expressed in terms of 6 parameters: (1) water absorption, which is the amount of water required to bring the dough to 500 FU; (2) dough development time, which is the time from water addition to the first sign of dough weakening in the range of maximum consistency; (3) stability, which is the difference in time between the points where the top of the curve reach and leave a constant torque line tangent to the middle curve at the point of maximum consistency; (4) mixing tolerance index, which corresponds to the decrease in Farinograph units (FU) between the top of the curve at maximum consistency and the top of the curve four minutes after maximum consistency was reached; (5) overmixing tolerance index, defined here as the decrease in FU between the top of the curve four and eight minutes after maximum consistency was reached; (6) bandwidth, which is the difference in torque between the top and bottom line as measured four minutes after peak time.

The second Farinograph method was a modified version of the continuous water addition method developed by Landillon, Cassan, Morel & Cuq (2008). Water was continuously added to the dough at a rate of 4 mL min⁻¹ with a Masterflex peristaltic pump. Results were expressed as resistance to mixing (in FU) *versus* dough water content. Three parameters were determined: (1) minimum water content for dough formation, which was arbitrarily defined as dough water content when a 100 FU torque level was reached; (2) water content at maximum torque, which corresponds to dough water content at maximum torque; (3) maximum torque, which was the highest value of FU reached.

Both methods were conducted using a Brabender-E Farinograph equipped with a 50-g bowl (Model FA-R/2; Brabender Co., South Hackensack, NJ, USA). All Farinograph tests were conducted in duplicate with durum wheat semolina substituted at a 5, 10 and 15% level with CPPI and MTPPI.

2.5 Gluten yield

Gluten yield analysis was performed according to AACC Approved Method 38-12.02 using a Glutomatic (Perten Instruments, Huddinge, Sweden). Results were expressed in terms of gluten index, wet gluten, dry gluten and water binding capacity. Tests were conducted in triplicate with durum wheat semolina substituted at a 0, 5, 10 and 15% level with CPPI and MTPPI.

2.6 Statistical analysis

Analysis of variance was performed *a priori* on each parameter using SAS software (version 8.2, SAS Institute Inc., Cary, NC, USA). Multiple comparison procedures (Least Significant Difference, $p = 0.05$) were performed *a posteriori* to compare parameters whose variance was significantly different.

3. Results

3.1 Pea isolates protein content and solubility

The protein content of the CPPI is $84.8 \pm 0.4\%$ dry basis, which is lower than the protein content of the MTPPI ($96.1 \pm 0.2\%$ dry basis). However, the solubility of the CPPI is $6.1 \pm 0.2\%$, which is approximately 13.8 times lower than the one of the MTPPI ($85.1 \pm 0.8\%$). This indicates that MTPPI proteins are mostly in their native states, while CPPI proteins are denatured.

3.2 Mixing properties

Typical data obtained using the Farinograph constant dough weight method, with 10% enrichment level, are presented in Figure 1. Dough with substituted CPPI showed a characteristic sharp peak and noise around dough development time (indicated by the vertical line below the curve), while data of dough containing MTPPI were smoother, without sharp peak. This suggests that the protein solubility has a more important impact on dough formation than the protein content of the isolates since the behaviour (shape of the curve in Figure 1) was strongly affected. It is well known that difference in protein content alone would not have affected the shape of the curve but only the intensity of the various parameters (Irvine, Bradley & Martin, 1961).

Measured Farinograph parameters are presented in Figure 2-7. Increasing durum wheat substitution level with both CPPI and MTPPI resulted in higher water absorption (Figure 2) compared to control ($P < 0.01$). This is the trend typically observed when flour or semolina is enriched with proteins (Wood, 2009; Sabanis *et al.*, 2006; Mashayekh, Mahmoodi & Entezari, 2008). Although MTPPI had higher protein content, it overall resulted in dough with lower water absorption than CPPI at an enrichment level of 15% ($P < 0.05$), but not at 5 or 10% level.

Dough development time (Figure 3) increased when CPPI or MTPPI was added to semolina ($P < 0.01$), but only at 5% substitution level, which is similar to the results reported by Ribotta *et al.* (2005) with soybean flour. However, the gluten network may not have been completely formed at the measured dough development time for semolina enriched with high level of CPPI. Indeed, curves were very unstable and wide around this point and another small increase in consistency was observed 3-5 min after dough development time for 15% CPPI enrichment level.

Enriching semolina with CPPI at a 5% level induced a sharp increase in stability (Figure 4) and a diminution of mixing tolerance index (Figure 5) compared to control ($P < 0.05$). Both of those properties are associated to strong flour or semolina. However, a reverse trend was observed at 10 and 15% enrichment level. Indeed, the Farinograph curves for 10 and 15% CPPI level showed just as good stability in the long run after the peak compared to 5%. This is supported by the decrease in overmixing tolerance index (Figure 6) for 10 or 15% CPPI level compared to control ($P < 0.05$). The opposite was observed with the MTPPI: overmixing tolerance index was higher than control when a substitution level of 15% was used ($P < 0.05$).

Dexter and Matsuo (1980) reported that bandwidth correlates well with gluten strength and spaghetti cooking and overcooking quality. Their results indicated that wide bandwidth is linked to strong gluten with good cooking and overcooking qualities. In this study, enrichment with both isolates resulted in lower bandwidth ($P < 0.05$) than control (Figure 7), which might be due to gluten dilution. Decline in bandwidth was more pronounced with MTPPI compared to CPPI ($P < 0.05$).

Dough mixing properties under continuous water addition are presented in Table 1. While the classical Farinograph method allows analysing dough resistance to mixing over time, this procedure allows to study the behaviour of dough at different moisture content. Minimum water content for dough formation was affected by the isolate ($P < 0.01$). MTPPI supplementation caused a decrease of this parameter compared to CPPI or to isolate-free semolina. This effect was enhanced at 10 or 15% enrichment level ($P < 0.01$). This indicates that semolina enriched with MTPPI requires less water for the development of the gluten network. Petitot *et al.* (2010) also reported a decrease in minimum water content for dough formation with durum wheat flour enriched with split pea or faba bean flour.

Similar results were obtained for dough water content at maximum torque. Enrichment with MTPPI resulted in a decrease in water content at maximum torque compared to control ($P < 0.05$), while enrichment with CPPI did not have a significant effect. The decrease in water content at maximum torque with MTPPI was independent of the level of substitution.

In terms of the maximum torque obtained, effects were observed for both the type of isolate and substitution level ($P < 0.01$). The addition of isolate resulted in dough with higher resistance to mixing at the water content of maximum torque, especially in the case of MTPPI.

3.3 Gluten yield

Parameters from gluten yield experiments are presented in Table 2. No results are available for CPPI at a 15% enrichment level because no gluten network developed during the mixing step in the Glutomatic. Similar observations were made by Ribotta *et al.* (2005) with durum wheat flour enriched with soy protein flour. However, a gluten network developed for each substitution level when using MTPPI. This indicates that the CPPI is more harmful to gluten formation than MTPPI especially at higher level of supplementation.

Gluten index represents the ratio of strong gluten to total gluten. Matsuo, Dexter, Kosmolak & Leisle (1982) and D'Egidio, Mariani, Nardi, Novaro & Cubadda (1990) indicated that gluten strength is an important factor influencing pasta quality. In the present study, gluten index was significantly influenced by the protein isolate ($P < 0.01$). Overall, gluten was stronger when semolina was enriched with MTPPI compared to control or dough enriched with CPPI. This effect was independent of the level of substitution. This result is due to the fact that proteins of the MTPPI have more interactions with the gluten proteins compared to CPPI, causing more important changes in the network properties.

Wet and dried gluten, when measured in terms of percentage of dry wheat semolina, were influenced by both the

type of isolate and substitution level ($P < 0.01$). Less gluten was retrieved when semolina was enriched with the MTPPI compared to CPPI or isolate-free semolina. Moreover, the difference was enhanced with high MTPPI substitution level. Thus, MTPPI retrieves some gluten from the network, causing it to pass through the Glutomatic screen with water and starch, which is not the case or at lesser extent for CPPI.

Water binding capacity, when expressed in terms of percentage of wet gluten, was not affected by the protein isolate or enrichment level. Thus, enriching semolina with pea proteins did not affect gluten capacity to hold water.

4. Discussion of the Impact of Supplementing Durum Wheat Semolina with Pea Protein Isolate

Enriching durum wheat semolina with protein isolate altered most dough properties as measured by Farinograph and Glutomatic. However, the impact was dependant on which isolate was used. Enrichment with CPPI did not impact gluten development, the minimum water content for dough formation and the water content at maximum torque while it increased the maximum torque. Enrichment with MTPPI resulted in an increase in gluten strength, and decreases in wet and dry gluten. It also required less water for the development of the network while it increased the maximum torque. In practice, these observations suggest that the MTPPI will take part to the network formation of pasta matrices while the CPPI would remain entrapped into the network limiting the amount of CPPI that can be supplemented into the pasta. In this context, previous work on the bread-making potential of the MTPPI clearly showed that bread matrix can support 10% substitution with MTPPI without harmful effect on the network (Des Marchais, Foisy, Mercier, Villeneuve, & Mondor, 2011). This resulted in bread with $20.9 \pm 0.1\%$ of protein which can be considered as high protein bread.

The impact of the two protein isolates is different due to the conformation state in which their proteins are. MTPPI making process leads to soluble proteins, while CPPI proteins were very less soluble. This indicates that the proteins presents in the MTPPI are less denatured than the ones in the CPPI. This impacts how pea and gluten proteins interact, similarly to what is observed in the case of wheat-soy protein interactions (Ribotta *et al.*, 2005; Rocchia, Ribotta, Pérez & Leon, 2009). Pea proteins in their native state interact more directly with wheat proteins, thus explaining the increase in maximum torque and gluten index observed with MTPPI. Moreover, decreases in wet gluten and dry gluten are due to wheat-pea proteins interactions causing an increase in gluten solubility. On the contrary, proteins from CPPI alter dough properties more indirectly because of water competition and gluten dilution. To strengthen these observations, it would be of interest to perform some chemical analysis on the dough during its formation in order to assess the nature of the interactions between proteins. For example, the quantitative measurements of sulfhydryl (SH) and disulfide (SS) bonds would be of interest.

5. Conclusion

Level of dough enrichment with pea protein isolates (0, 5, 10 and 15%) has little effect on measured mixing properties compared to the pea protein isolate considered for enrichment. Pea protein isolate processed by membrane technologies takes part to the dough formation, which does not seem to be the case with commercial isolate. Higher amount of water is required for dough formation with matrices enriched with commercial pea isolate compared to membrane processed isolate or isolate-free dough, while stronger dough properties are observed for matrices enriched with membrane processed isolate.

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Table 1. Mixing properties of control and enriched dough under continuous water addition Farinograph method

Parameters		CPPI					MTPPI			
		Control	5%	10%	15%	Mean	5%	10%	15%	Mean
MWCDF	g-water	0.54	0.51	0.51	0.51	0.51	0.42	0.38	0.37	0.39
	g-semolina ⁻¹	±0.00	±0.01	±0.03	±0.01	±0.02	±0.01	±0.02	±0.00	±0.03
WCMT	g-water	0.76	0.76	0.79	0.76	0.77	0.65	0.62	0.62	0.63
	g-semolina ⁻¹	±0.02	±0.02	±0.03	±0.01	±0.03	±0.02	±0.01	±0.01	±0.02
MT	FU	521	539	608	687	611	787	>950	>950	>896
		± 17	±49	±48	±100	±86	±63			

Where MWCDF is the minimum water content for dough formation, WCMT is the water content at maximum torque and MT is the maximum torque.

Table 2. Gluten yield of control and enriched dough

Parameters		CPPI					MTPPI			
		Control	5%	10%	15%	Mean*	5%	10%	15%	Mean
GI	%	72.7 ±0.8	71.8 ±0.4	73.9 ±2.2	-	72.9 ±1.3	83.2 ±2.5	85.1 ±1.9	85.6 ±2.3	84.7 ±0.3
WG	%	48.3 ±1.0	49.1 ±0.6	47.7 ±2.2	-	48.4 ±1.1	44.4 ±0.3	44.4 ±0.5	40.0 ±1.1	43.0 ±0.3
DG	%	16.8 ±0.2	16.9 ±0.3	16.4 ±0.9	-	16.6 ±0.4	15.6 ±0.1	15.4 ±0.1	13.6 ±0.2	14.8 ±0.1
WBC	%	65.1 ±1.1	68.7 ±3.6	65.7 ±0.3	-	66.7 ±2.3	64.7 ±0.7	65.4 ±0.4	65.9 ±0.6	65.3 ±0.2

Where GI is the gluten index, WG is ratio of wet gluten to dry durum wheat semolina, DG is ratio of dry gluten to dry durum wheat semolina and WBC is the water binding capacity as % of wet gluten.

* Mean value was calculated from 5 and 10% data since no data were available at 15%.

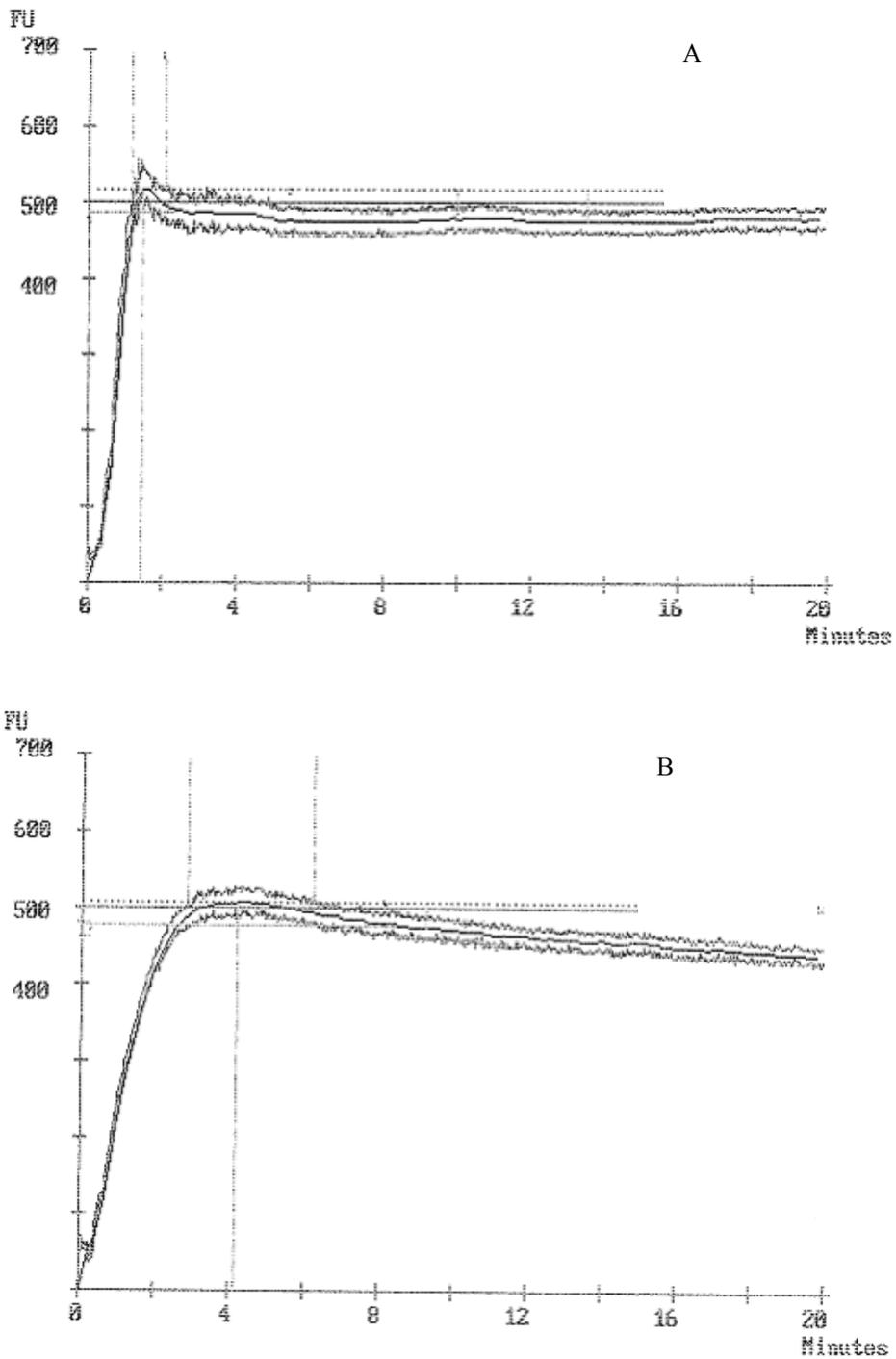


Figure 1. Typical experimental plot of resistance to mixing *versus* time for constant dough weigh Farinograph test with durum wheat semolina enriched with 10% CPPI (a) and MTPPI (b)

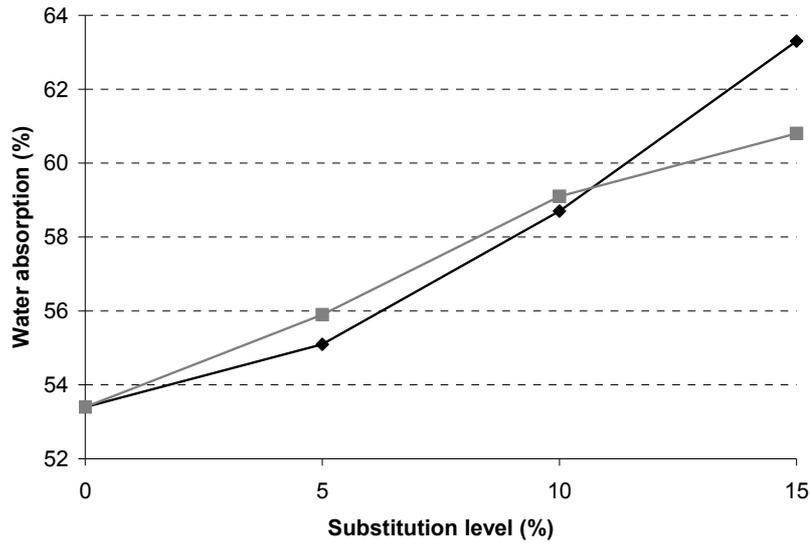


Figure 2. Experimental results of water absorption for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

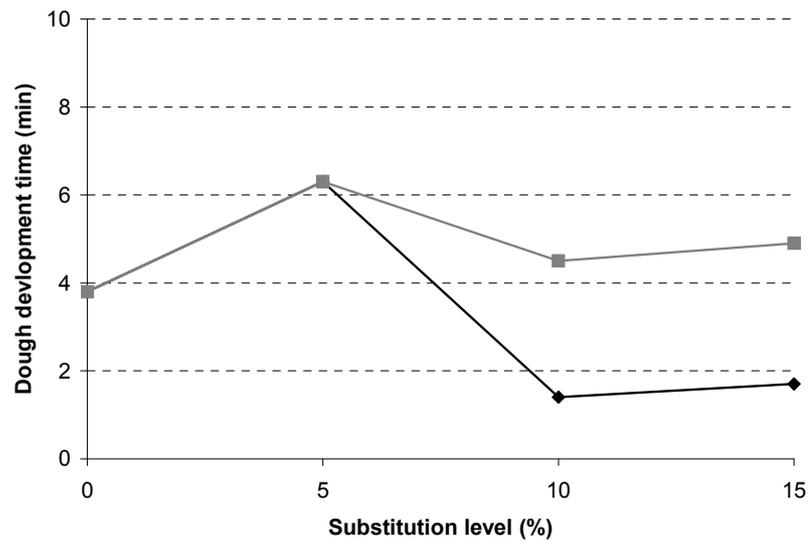


Figure 3. Experimental results of dough development time for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

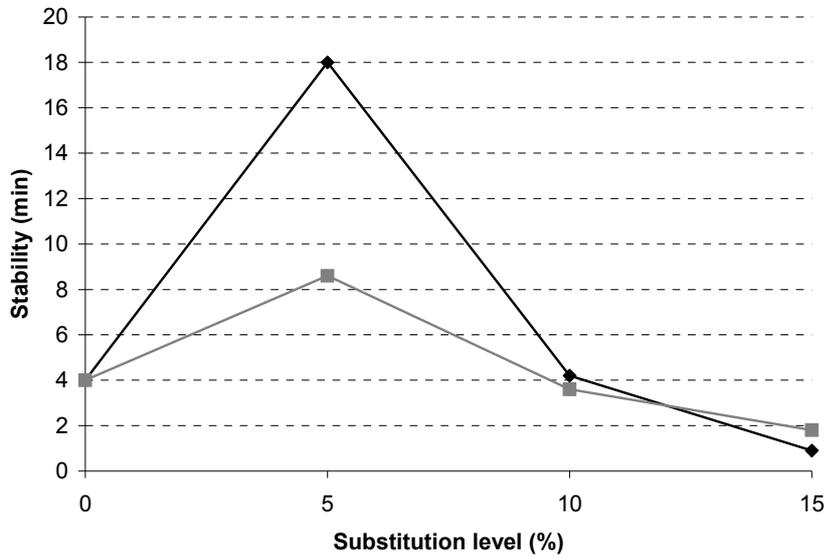


Figure 4. Experimental results stability for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

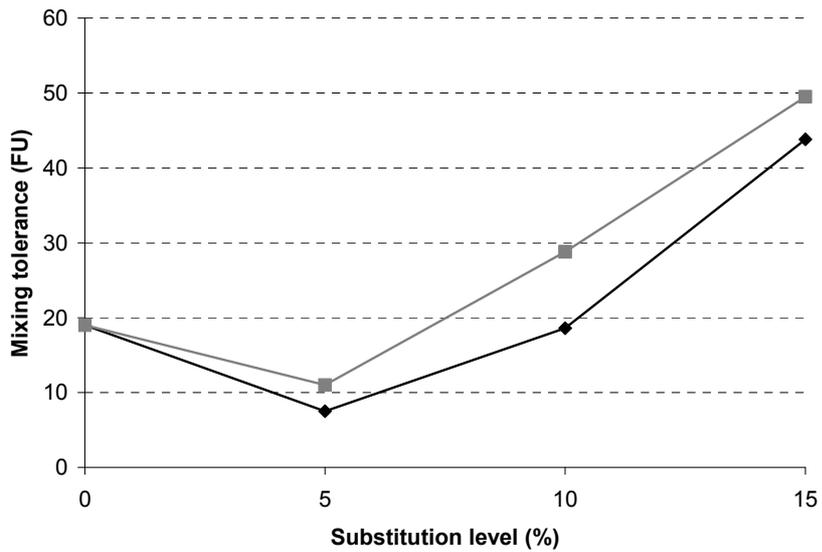


Figure 5. Experimental results of mixing tolerance index for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

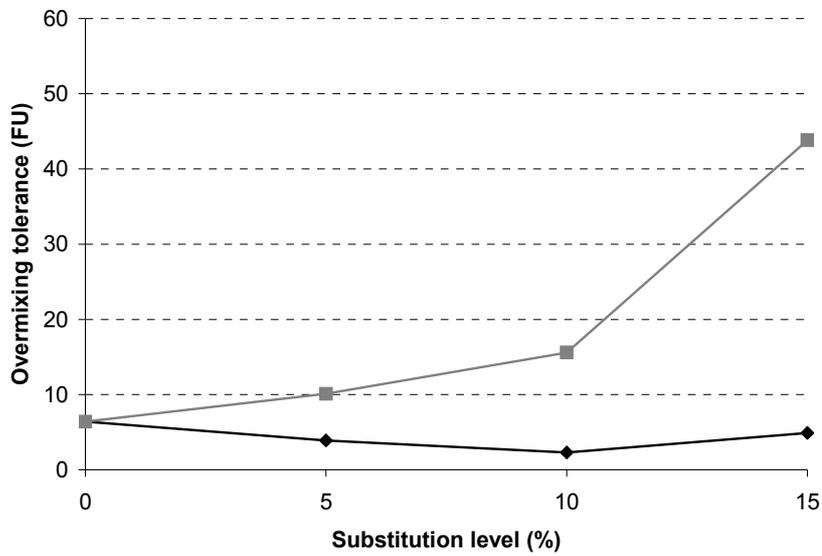


Figure 6. Experimental results of overmixing tolerance index for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

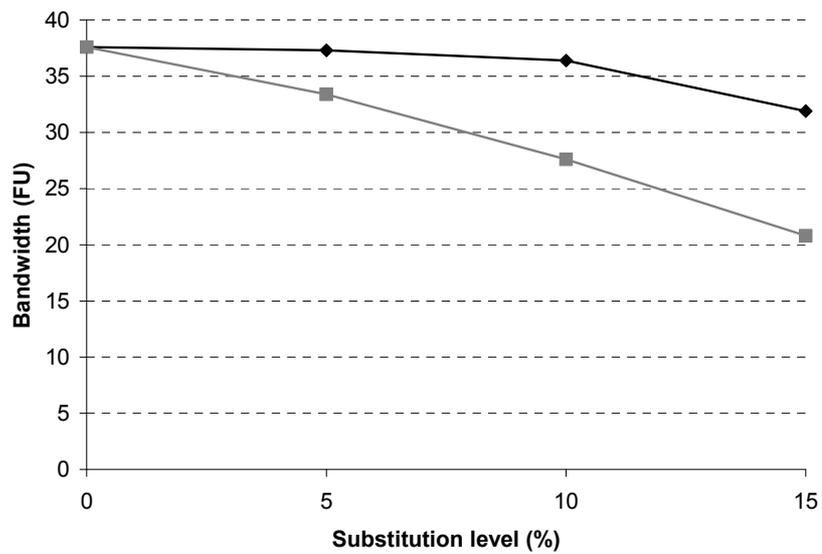


Figure 7. Experimental results of bandwidth for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level