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Kernel chemical composition and flour quality of wheat in response to nitrogen sources and doses

Composição química de grãos e qualidade de farinha de trigo em resposta a fontes e doses de nitrogênio

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Abstract: The chemical composition of wheat kernels (*Triticum aestivum* L.) directly affects the quality of flour by modifying its functional and technological properties, determining its use by the industry. Field trials were conducted with wheat genotypes BRS 208 and BRS Pardela to evaluate the impact of different forms of urea (conventional, with urease inhibitor, and polymer-protected) and doses of nitrogen applied in topdressing (0, 40, 80 and 120 kg ha^{-1}) in kernel chemical composition and flour technological quality. The experimental design was randomized block with 3x4 factorial structure, with four replications. The data were submitted to analysis of variance (ANOVA) with regression for nitrogen doses, and comparison of means for genotypes and forms of urea by Tukey's test ($p \leq 0.05$). Although the genotypes evaluated presented significant differences regarding kernel chemical composition and quality of flour, similar responses were observed for the variables evaluated, forms of urea, and doses of nitrogen. Application of urea with urease inhibitor increased starch content in the kernels, and polymer-protected urea reduced flour yield. A positive linear regression was observed between nitrogen doses and protein ($R^2=0.706$) and ash ($R^2=0.990$; $p<0.01$), and negative with lipid content ($R^2=0.836$; $p<0.01$). However, the application of increasing doses of nitrogen reduced kernel volume density ($R^2=0.864$; $p<0.01$) and brightness of flour (L*; $R^2=0.977$; $p<0.01$). In addition to breeding, the application of adequate doses using various sources of mineral nitrogen could be used as an additional procedure to obtain raw materials with the desired chemical composition profile.

Keywords. Grain quality, polymer-protected urea, *Triticum aestivum* L., urease inhibitor (NBPT)

Resumo: A composição química dos grãos de trigo (*Triticum aestivum* L.) afeta diretamente a qualidade da farinha obtida, modificando suas propriedades funcionais e tecnológicas, determinando seu uso pela indústria. Diante disso, experimentos a campo foram conduzidos com dois genótipos de trigo (BRS 208 e BRS Pardela) para avaliar o efeito de formas de ureia (convencional, com inibidor de urease e protegida por polímero) e doses de nitrogênio em cobertura (0, 40, 80 e 120 kg ha^{-1}) na composição química do grão e qualidade tecnológica da farinha de trigo. O delineamento experimental foi em blocos casualizados, esquema fatorial 3x4, com quatro repetições. Os dados foram submetidos a [illegible]ipos e formas de ureia) pelo teste de Tukey ($p \leq 0,05$). Apesar dos genótipos avaliados apresentarem diferença significativas em relação a composição química do grão e qualidade da farinha, ambos apresentaram respostas similares as variáveis avaliadas, formas de ureia e doses de nitrogênio. Aplicação de ureia com inibidor de urease aumentou o teor de amido no grão, e a ureia protegida reduziu o rendimento de farinha. Observou-se regressão linear positiva entre doses de nitrogênio e teor de proteína ($R^2=0,706$) e cinzas ($R^2=0,990$; $p<0,01$), e negativa com o teor de lipídios nos grãos ($R^2=0,836$; $p<0,01$). Outrossim, a aplicação de doses crescentes de nitrogênio reduziu o peso hectolitro do grão ($R^2=0,864$; $p<0,01$) e luminosidade da farinha (L*; $R^2=0,977$; $p<0,01$). Em adição ao melhoramento genético, a aplicação de doses adequadas utilizando diferentes fontes de nitrogênio mineral pode ser recomendado como procedimento para obtenção de matéria-prima de qualidade.

Palavras-chave. Inibidor de urease (NBPT), qualidade do grão, *Triticum aestivum* L., ureia protegida por polímero

Introduction

Faced with market demand, segments in the production chain should be focused on production of food with quality, which is a determining factor in a global competitiveness. The term quality encompasses a set of physical, chemical and rheological characteristics (dough viscoelastic properties) of wheat, which define its use by the industry (Scheuer et al., 2011). Furthermore, quality dependents on genotype, environment (temperature, precipitation, soil fertility, etc.), and interaction between genotype and environment (Franceschi et al., 2009). Changes in growing conditions not only affect grain yield, but also both physical and chemical parameters of the kernel (Souza et al., 2016; Prando et al., 2012).

Wheat genotypes differ in tiller emission capacity, cycle, architecture and productive potential. These differences may interfere with absorption, assimilation and capacity of conversion of nitrogen into grain yield and technological quality (Ferreira et al., 2016; Xue et al., 2016; Souza et al., 2014; Prando et al., 2012).

Studies have shown that both yield and protein content can be increased simultaneously with increasing doses of nitrogen (Souza et al., 2016; Xue et al., 2016). Soil fertilization and adequate plant nutrition are essential and enable the exploration of the wheat crop. Nitrogen is the most absorbed and exported nutrient by wheat plants. The use of fertilizers with increased efficiency is one of the strategies to reduce losses of nitrogen and maximize grain production (Prando et al., 2012).

Urea with urease inhibitor slows hydrolysis, and polymer-coated urea promotes gradual release of the nutrient. Urease inhibitors are products added to fertilizers, which are referred to as stabilized fertilizers. The inhibitors are not actually fertilizers, but slow bacteria and enzymatic activity in the soil to maintain fertilizers with reduced probability to be displaced from the root zone by leaching or gaseous losses. In polymer-coated urea, when prills are in contact with moisture, the pores in the resin coating allow water to diffuse into the core, dissolving the water-soluble compounds inside. This increases the osmotic pressure and causes the coating to stretch and the pore size to increase, which allows the nutrients to diffuse through the pore (Ozores, 2014). Both products are used to reduce volatilization losses by gradual and late availability, which might have positive influences in wheat quality. Late application of nitrogen is known for improving protein quality and quantity, and predominantly benefits protein build-up over starch in the grain and extends the duration of grain-filling (Xue et al., 2016; Sowers et al., 1994).

Studies evaluate the influence of crop management practices on productivity and, less frequently, their influence in quality. Kernel chemical composition is directly linked to flour quality, and determines its potential in the production of a certain product (Franceschi et al., 2009). For bread production, the quantity and quality of protein is important to produce a high-quality product, since glutenin and gliadin proteins have a role in gluten formation and bread structure development. On the other hand, in the production of cakes, starch is the most important component since there is no significant gluten formation (Cauvain, 2017).

So far is known from this field trial, as reported by Prando et al. (2012), analyzing the same experiment but focusing in agronomic characteristics, that increasing doses of nitrogen applied in topdressing, independently of genotype and form urea evaluated, positively influenced dry mass of flag leaf and number of ears per m^2, which led to an increase of +9.4% in productivity. Therefore, due to the lack of information in literature about the effect of agricultural management practices on kernel chemical composition and flour technological quality, and based on the promising results obtained by Prando et al. (2012), further analyses were performed to evaluate its effect in the quality of the kernel produced.

The objective of this research was to evaluate the effect of forms of urea (conventional urea, with urease inhibitor, and polymer-protected) and doses of nitrogen (0, 40, 80, and 120 kg ha^{-1}) in the chemical composition and technological quality of wheat genotypes BRS 208 and BRS Pardela.

Material and Methods

Location and Characterization of Experimental Area

Individual field experiments were carried out in Ponta Grossa, located in the Southern Plateau of

Paraná State in Brazil (coordinates 25º09'S and 50º06'W), which has an average altitude of 800 m.

The region has subtropical climate conditions, classified as Cfb according to Köppen, i.e., the average temperature in the coldest month is below 18ºC, with temperate summers, and the average temperature in the hottest month is below 22 ºC, with no defined dry season. The data of maximum and minimum daily temperatures and precipitation during the growing (Figure 1).

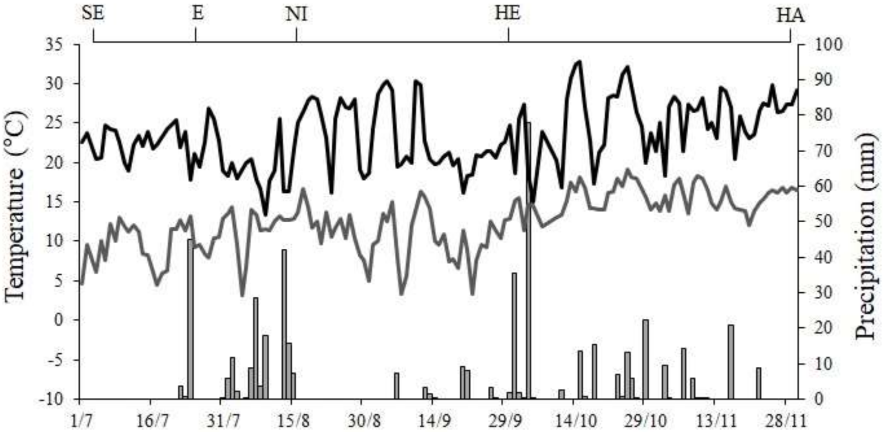

Figure 1. Maximum (black line) and minimum (grey line) temperatures (ºC) and daily precipitation (bars; mm), in Ponta Grossa, Paraná, Brazil. SE: Seeding; E: Emergency; NI: Nitrogen fertilization; HE: Heading stage; HA: Harvest.

The soil in the experimental area is classified as haplic cambisol dystrophic and managed in no-tillage system, following a previous soybean crop. Before the installation of the experiment, soil samples were collected for chemical and physical analyses. The chemical characteristics of the soil to the depth of 0-20 cm are: pH ($CaCl_2$): 5.4; C: 13.2 g dm^{-3}; P (Mehlich 1): 10.1 mg dm^{-3}; H + Al: 4.7 cmol$_c$ dm^{-3}; K: 0.2 cmol$_c$ dm^{-3}; Ca: 6.1 cmol$_c$ dm^{-3}; Mg: 2.1 cmol$_c$ dm^{-3}; Cation exchange capacity of 13.1 cmol$_c$ dm^{-3}and base saturation of 63.8%.

Material and methods

The genotypes used are known as medium-cycle (60 to 70 days from emergence to the heading stage), with contrasting characteristics of plant height, tillering capacity, productive potential and technological quality. The BRS 208 genotype has an average height of 89 cm, is moderately susceptible to lodging, medium tillering capacity, limited productive potential, good soil tolerance to aluminum toxicity, with wide adaptation and good response in low fertility soils, and belongs to the Bread class according to the Brazilian commercial classification of wheat, presenting dough strength average of 292 10^{-4}J. The BRS Pardela genotype has an average height of 79 cm, is moderately susceptible to lodging, presents good tillering capacity and high productive potential, being suitable for soils with medium and high fertility, and belongs to the Improver class according to the Brazilian commercial classification, with dough strength average of 355 10^{-4}J (Bassoi et al., 2014; MAPA, 2010).

Experimental Design

For each genotype (BRS 208 or BRS Pardela), individual field experiments were designed in randomized blocks, in a 3 x 4 factorial structure with four replications. Three forms of urea were evaluated as nitrogen source (conventional, with urease inhibitor and polymer-protected), applied in four doses of nitrogen (0, 40, 80, and 120 kg ha^{-1}). The experimental plot consisted of ten rows, each six meters long and spaced 20 cm. The harvested area in each plot was six central rows, eliminating 0.75 m at the ends of each row (border), and totaling 5.4 m^2.

Operational Procedure

The cultural practices were conducted according to the Technical Indications of the Brazilian Wheat and Triticale Research Commission for the State of Paraná. Seeding was carried out aiming a density of approximately 300 plants per m^2, and application of 20 kg ha^{-1} of N, 50 kg ha^{-1} of P_2O_5, and 50 kg ha^{-1} of K_2O at sowing. Most of the seedlings emerged 20 days after sowing, and 19 days later, when the crop was at the beginning tillering stage, the application of nitrogen in topdressing was performed.

Harvest occurred when the crop was at harvest maturity, with kernels with less than 20% moisture. After cleaning and drying the samples to approximately 13% moisture, part was ground to 30 mesh using a hammer mill (MOD MA-090, Marconi®, Piracicaba, São Paulo, Brazil) and stored at -18 °C (MOD FE26, Electrolux®, Manaus, Brazil) until chemical composition analyses. The remaining of the samples was stored in a cold chamber (5 ± 3 °C) until technological quality analyses.

Chemical Composition Analyses

The chemical composition of samples obtained from milling the kernels to 30 mesh was analyzed and the results expressed on a dry basis. To evaluate the moisture content, the sample was dried for 72 hours at 103 ºC (MOD NV 1.5, Nevoni®, São Paulo, Brazil), with subsequent cooling in desiccant and weighting, as described by AACC International Approved Method 44-15.02. The protein content was evaluated according to AACCI Method 46-12.01, the sample was digested (MOD TE-40/25, Tecnal®, Piracicaba, Brazil) with subsequent distillation (MOD TE-036/1, Tecnal®), and conversion of total nitrogen content into protein as described by AACCI Approved Method 46-19.01. The lipid content (crude fat) was evaluated in prehydrolyzed samples (5 g sample/50 mL of 4M hydrochloric acid), from refluxing 150-200 mL of ether petroleum using Soxhlet extractor (MOD TE-188, Tecnal®; MOD MA-487, Marconi®), as described by AACCI Approved Method 30-25.01. The ash content was evaluated by weighing the muffled incineration residues (MOD 318D24®, Quimis®, Diadema, Brazil; AACCI Method 08-01.01). The starch content was evaluated following the PTF protocol proposed by Walter et al. (2005). The total carbohydrate content was calculated using the formula: total carbohydrate %= 100% - (moisture% + protein % + lipids % + ash %). Analyses were run in duplicate or triplicate if high variation was observed.

Quality Traits Analyses

The hectoliter weight or test weight of the kernels (kg hL-1) was evaluated by weighing a known volume of sample with an electronic scale (225 mL) (MOD Type 40, DalleMolle®, Caxias do Sul, Brazil). For the experimental extraction of flour (percentage of flour yield), the kernels were first conditioned to 14% moisture for approximately 16 hours, to facilitate the extraction and to increase the yield. After conditioning, the milling was carried out in a roller mill (MOD Quadrumat Junior, Brabender®, Duisburg, Germany) according to AACCI Approved Method 26-10.02. The alveography was analyzed in alveograph (MOD MA82 with alveolink accessory, Chopin®, Villeneuve-la-Garenne, France), according to AACCI Approved Method 5430.02. The parameters considered were dough strength (W, 10-4J), tenacity or resistance to dough extension (P, mm), extensibility (L, mm) and elasticity index (I.e., %). The falling number (sec) was evaluated according to AACCI Approved Method 56-81.03 (MOD 1500 Fungal, Perten Instrument®, Huddinge, Stockholm, Sweden). The flour brightness, which values range from white (L*=100) to black (L*=0), was evaluated by CIELAB system in a colorimeter (MOD CR 310, Minolta®, Osaka, Japan), with D65 illuminant and 10° of viewing angle.

Statistical Analysis

The data were submitted to analysis of variance (ANOVA), regression (nitrogen doses) and mean comparison by Tukey test for genotypes and forms of urea ($p<0.05$), using SISVAR version 4.0 (System for Analysis of Variance) and Statistica 13.2 (Dell™ Statistica™ 13.2, Tulsa, Oklahoma, United States). The data were grouped in a single analysis since the ratio between the largest and the smallest mean squared error (MSE) was lower than 7.

Results and Discussion

Table 1 shows the analysis of variance results for kernel chemical composition and technological quality of the flour from wheat genotypes in response to urea sources, application of increasing doses of nitrogen in topdressing, and factor interaction.

No interactions were observed between the factors evaluated in this study (genotype, forms of urea, or doses of nitrogen), only individual or main effect for each factor. The environmental conditions during the experiment were non-optimal for the initial development of the wheat crop. The precipitation during the growing period was 486 mm with uneven distribution (Figure 1). After sowing, a period of drought occurred reducing uniformity of germination and plant emergence, and limiting initial crop development. Still, Prando et al. (2012) observed average grain yields of 4,347 kg ha^{-1}, which is higher than the national (2,482 kg ha^{-1}) and State of Parana averages (2,778 kg ha^{-1}).

.The genotypes differed statistically for most of the characteristics evaluated (kernel chemical composition and flour quality), with exception of moisture content in the kernels, yield, and brigthness (L*) of the flour, presenting distinct kernel chemical composition, which might affect the technological quality of the flour (Table 2). The protein content observed in the kernel was 6.13% higher for BRS Pardela than BRS 208 genotype (Table 2; p<0.01). This shows the genetic difference for quality characteristics evaluated and confirms that the genotypes used, besides the differences in agronomic characteristics observed by Prando et al. (2012), are contrasting in relation to their kernel chemical composition and flour technological quality.

Table 1. Summary of analysis of variance (ANOVA) for the characteristics evaluated in wheat genotypes (BRS 208 and BRS Pardela), in response to forms of urea (conventional, with urease inhibitor and polymer protected) and doses of nitrogen applied in topdressing (0, 40, 80, and 120 kg ha^{-1}).

Source of Variation	MOI	PRO	LIP	ASH	STA	TCA	TWE
BL(Genotype)	0.923^{ns}	0.195^{ns}	0.034*	0.146^{ns}	0.451^{ns}	0.369^{ns}	0.063^{ns}
Genotype	0.242^{ns}	0.000**	0.034*	0.000**	0.000**	0.000**	0.000**
Doses	0.233^{ns}	0.0889^{ns}	0.004*	0.017*	0.255^{ns}	0.983^{ns}	0.007*
Forms	0.696^{ns}	0.3853^{ns}	0.612^{ns}	0.803^{ns}	0.048*	0.596^{ns}	0.810^{ns}
Doses*Forms	0.985^{ns}	0.5665^{ns}	0.105^{ns}	0.968^{ns}	0.387^{ns}	0.967^{ns}	0.777^{ns}
Genotype *Doses	0.566^{ns}	0.058^{ns}	0.091^{ns}	0.677^{ns}	0.701^{ns}	0.343^{ns}	0.258^{ns}
Genotype *Forms	0.812^{ns}	0.359^{ns}	0.341^{ns}	0.774^{ns}	0.818^{ns}	0.390^{ns}	0.805^{ns}
Genotype *Doses*Forms	0.761^{ns}	0.573^{ns}	0.062^{ns}	0.781^{ns}	0.812^{ns}	0.735^{ns}	0.417^{ns}
C.V. (%)	3.55	2.47	12.26	4.44	8.30	1.28	0.51
Source of Variation	FYI	DOU	TEN	EXT	ELA	FAL	BRI
BL(Genotype)	0.001**	0.190^{ns}	0.026*	0.839^{ns}	0.680^{ns}	0.757^{ns}	0.063^{ns}
Genotype	0.304^{ns}	0.000**	0.001**	0.000**	0.000**	0.000**	0.180^{ns}
Doses	0.175^{ns}	0.439^{ns}	0.354^{ns}	0.956^{ns}	0.596^{ns}	0.409^{ns}	0.071^{ns}
Forms	0.018*	0.081^{ns}	0.491^{ns}	0.559^{ns}	0.656^{ns}	0.114^{ns}	0.095^{ns}
Doses*Forms	0.336^{ns}	0.497^{ns}	0.133^{ns}	0.397^{ns}	0.334^{ns}	0.088^{ns}	0.732^{ns}
Genotype *Doses	0.960^{ns}	0.249^{ns}	0.178^{ns}	0.918^{ns}	0.800^{ns}	0.612^{ns}	0.540^{ns}
Genotype *Forms	0.922^{ns}	0.055^{ns}	0.621^{ns}	0.873^{ns}	0.056^{ns}	0.264^{ns}	0.142^{ns}
Genotype *Doses*Forms	0.055^{ns}	0.072^{ns}	0.076^{ns}	0.562^{ns}	0.254^{ns}	0.888^{ns}	0.255^{ns}
C.V. (%)	3.57	14.1	13.93	18.24	3.88	13.05	0.55

ns, ** e *: Not significant and significant to 1% and 5% of probability, respectively, for F test.

BL: Block; C.V.: Coefficient of variation; MOI: Moisture; PRO: Protein; LIP: Lipids; STA: Starch; TCA: Total carbohydrates; TWE: Test weight; FYI: Flour yield; DOU: Dough strength; TEN: Tenacity; EXT: Extensibility: ELA: Elasticity index: FAL: Falling number; BRI: Brightness.

This result partially explains the superior technological quality often observed in BRS Pardela genotype, classified as wheat improver according to the Brazilian commercial classification (Bassoi et al., 2014). Dias et al. (2017), evaluating the protein profile of Brazilian wheat genotypes, observed that genotype BRS Pardela, classified as extra-strong wheat, presents the expression of high molecular weight glutenin subunits coded as 5+10, which is frequently associated with the production of dough presenting high resistance and amount of gluten.

Genotype BRS 208 kernels had a higher concentration of starch (Table 2; $p<0.01$), presenting 9.12% more starch than BRS Pardela. Regarding the quality of the kernels produced, BRS Pardela genotype showed a higher test weight (Table 2; $p<0.01$) compared to the other genotype. However, both genotypes presented values greater than 78 kg hL^{-1}, which classifies them as type 1 (MAPA, 2010). This difference is probably due to the higher grain mass presented by genotype BRS Pardela (Bassoi et al., 2014). Scheuer et al. (2011), while evaluating the technological quality of Brazilian wheat genotypes and their applicability, also observed that BRS Pardela genotype had a higher test weight than other genotypes evaluated in the study (80.80 kg hL^{-1}). BRS 208 genotype showed greater falling number, which means a low activity of alpha-amylase enzyme. However, both genotypes presented values above the minimum required for commercialization. As previously reported, high α-amylase activity (<250 s) promotes excessive degradation of starch resulting in a dense, low volume product with a sticky crumb and dark crust. On the other hand, low α-amylase activity (>320 s) provides insufficient degradation of starch, resulting in low volume and compact product (Barrera et al., 2016; Scheuer et al., 2011).

Table 2. Kernel chemical composition and quality traits of wheat genotypes (BRS 208 and BRS Pardela).

Kernel Chemical Composition*	Genotype		Average	p-value	C.V. (%)
	BRS 208	BRS Pardela			
Moisture (%)	15.32±0.6a	15.19±0.3a	15.26	0.242	3.55
Protein (%)	17.13±0.6b	18.18±0.4a	17.66	0.000	2.47
Lipid (%)	1.54±0.3b	1.65±0.3a	1.60	0.034	12.26
Ash (%)	1.90±0.1a	1.79±0.1b	1.85	0.000	4.44
Starch (%)	59.08±4.2a	54.14±5.1b	56.61	0.000	8.30
Total carbohydrates (%)	64.11±0.9a	63.20±0.7b	63.66	0.000	1.28
Quality Traits					
Test weight (kg hL^{-1})	80.31±0.5b	81.56±0.4a	80.94	0.000	0.50
Flour yield (%)	61.14±3.2a	61.61±1.8a	61.38	0.304	3.57
Dough strength (W; 10^{-4} J)	299.10±40.0b	395.56±65.9a	347.33	0.000	14.10
Tenacity (P; mm)	79.98±11.3b	88.71±14.5a	84.35	0.001	13.93
Extensibility (L; mm)	138.96±27.7a	115.65±13.2b	127.31	0.000	18.24
Elasticity index (I.e., %)	51.23±2.3b	67.76±2.5a	59.50	0.000	3.88
Falling number (sec)	402.94±49.3a	287.73±41.0b	345.34	0.000	13.05
Brightness (L*)	92.58±0.6a	92.72±0.5a	92.65	0.180	0.55

*Expressed in dry basis; Means (n= 48 ± standard error) followed by the same letter within a row (genotype – BRS 208 and BRS Pardela) are not significantly different by Tukey test ($p>0.05$); C.V. = Coefficient of variation.

Dough strength result for BRS Pardela genotype was 32.23% higher than BRS 208 (Table 2; p<0.01), which classifies the first as improver (dough strengh > 300 10^{-4} J) and the former as bread wheat class (300 10^{4} J > dough strengh > 220 10^{-4} J), according to the Brazilian legislation, corroborating with the description of genotypes (Bassoi et al., 2014; MAPA, 2010). BRS Pardela presented a dough with high tenacity but less extensible, with a higher P/L ratio, however, both genotypes had balanced gluten (value ranging from 0.6 to 1.2). This is important since the specific volume of breads is directly related to the total protein content in the flour and its quality, due to the viscoelastic properties attributed to the dough (Scheuer et al., 2011).

Table 3. Kernel chemical composition and quality traits of wheat in response to forms of urea (conventional, with urease inhibitor or coated with polymer).

Kernel Chemical Composition*	Forms of urea			Average	p-value	C.V. (%)
	CU	UI	UP			
Moisture (%)	15.21±0.5a	15.32±0.4a	15.23±0.6a	15.25	0.6957	3.55
Protein (%)	17.54±0.8a	17.75±0.7a	17.67±0.7a	17.65	0.3853	2.47
Lipid (%)	1.61±0.3a	1.56±0.3a	1.54±0.3a	1.57	0.6123	12.26
Ash (%)	1.84±0.1a	1.85±0.1a	1.85±0.1a	1.84	0.8032	4.44
Starch (%)	55.37±4.8b	58.25±0.9a	56.20±5.3ab	56.61	0.0481	8.30
Total carbohydrates (%)	63.79±0.9a	63.46±5.5a	63.72±1.0a	63.66	0.5955	1.28
Quality Traits						
Test weight (kg hL^{-1})	80.95±0.8a	80.90±0.8a	80.96±0.7a	80.94	0.8097	0.50
Flour yield (%)	61.84±1.9a	61.84±2.3a	60.45±3.1b	61.38	0.0179	3.57
Dough strength (10^{-4} J)	350.53±80.9a	332.03±49.1a	359.44±82.7a	347.33	0.0812	14.10
Tenacity (P; mm)	85.03±14.9a	82.34±11.7a	85.66±14.3a	84.34	0.4914	13.93
Extensibility (L; mm)	129.34±26.8a	123.69±22.2a	128.91±24.9a	127.31	0.5585	18.24
Elasticity index (I.e.; %)	59.51±9.7a	59.23±8.0a	59.76±8.3a	59.50	0.6558	3.88
Falling number (sec)	356.81±81.2a	333.00±65.1a	346.19±73.4a	345.33	0.1141	13.05
Brightness (L*)	92.49±0.5a	92.71±0.6a	92.76±0.5a	92.65	0.0952	0.55

*Expressed on a dry basis; Means (n = 32 ± standard error) followed by the same letter within a row (forms of urea – conventional, with inhibitor and coated with polymer) are not significantly different by Tukey test (p>0.05); CU = Conventional urea; UI = Urea with urease inhibitor; UP: Urea coated with polymer; C.V. = Coefficient of variation.

The forms of urea applied in topdressing (conventional urea, with urease inhibitor, and polymer-protected) did not influence the kernel chemical composition and the flour quality, except for starch content and flour yield (Table 3).

This result is probably due to the interference of climatic conditions at the time of application of the nitrogen fertilizers, since it was carried out two days after a period of three days of rain with a total accumulation of 65 mm, followed by a dry season of 18 days (Figure 1). The excess water present in the soil and in residues from previous crops (no-till system) resulted in a rapid incorporation of the fertilizers into the soil, impairing the expression of its effect on reducing the loss of nutrients to the environment.

Martins et al. (2014) have identified similar behavior when applying conventional urea and slow release source under optimal conditions in maize culture. As reported, under high soil moisture conditions, both conventional urea and slow release source provide their nitrogen stock rapidly, in about 4.5 minutes. Thus, the application of slow release source in soil with adequate condition, presents a performance similar to urea, because the fertilizer is solubilized in the presence of moisture. However, when in conditions of low soil moisture, sources of slow release have better results, because of their layers of inhibition retaining the nitrogen.

Despite the results observed, is know that a gradual or later release of nitrogen provided by alternative forms of urea can influence the kernel composition and flour quality (Xue et al., 2016; Sowers et al., 1994). Although the cause is not clear, higher starch content in wheat kernels was observed when urea with urease inhibitor was applied, and lower flour yield (-2.25%) when polymer-protected urea was used compared to conventional urea application (Table 3).

According to Xue et al. (2016), the application of increasing doses of nitrogen mainly enhance the amount of protein but the gradual application can improve the quality of those proteins found in the kernels, since more nitrogen is available to kernel protein synthesis after anthesis, by enhancing the percentages of gliadins and glutenins as well as certain high molecular weight glutenin subunits (HMW-GS), which led to an improved baking quality (loaf volume).

As shown in Figure 2B, protein content in kernels increased by +1.7% with the increase in nitrogen doses applied in topdressing ($R^2 = 0.71$; $p<0.05$), comparing the control (0 kg ha^{-1}) with the maximum rate applied (120 kg ha^{-1}). Also, a negative linear regression with lipid ($R^2 = 0.84$; $p<0.01$; Figure 2C) and positive with ash content ($R^2 = 0.99$; $p<0.01$; Figure 2D) were measured with increasing nitrogen doses.

Souza et al. (2014), after evaluating the effect of nitrogen doses in wheat (0 to 120 kg ha^{-1}), also observed significant correlation between increasing doses of nitrogen and protein content in the kernels ($R^2>0.90$). This is caused by nitrogen being a limiting factor for development and productivity of crops due to its importance in the formation of amino acids, proteins, chlorophyll and essential enzymes that stimulate growth and development of the plant (Taiz and Zeiger, 2013; Prando et al., 2012). Furthermore, as observed for other crops, the nutrient is also known for affect other chemical compounds such as lipid, ash and starch contents and safety (Souza et al., 2016).

As shown in Figure 2C, lipids participate in the composition of wheat kernels in a very small percentage and are located mainly in the germ, which is removed at the beginning of milling process (Scheuer et al., 2011). In this experiment the application of high rate of nitrogen (120 kg ha^{-1}) reduced in -12.4% the lipid content in the kernels, compared to control (0 kg ha^{-1}). Opposite result was observed by Souza et al. (2016), were the lipid content in maize kernels was improved with application of high doses of nitrogen ($R^2 = 0.974$; $p<0.01$). As previously reported by Min et al. (2017), clear differences in content and composition of lipids are observed between cultivars, which can be affected by nitrogen fertilization.

The accumulation of minerals, measured as ash content, was also affected by nitrogen fertilization, showing a significant positive correlation with dosage (Figure 2D; $R^2 = 0.990$; $p<0.01$), similar to the results observed by Souza et al. (2016) for maize ($R^2 =0.942$; $p<0.01$). In general, cereals kernels contain about 1.5 to 2.5% of minerals, being phosphorus the highest concentration (Scheuer et al., 2011).

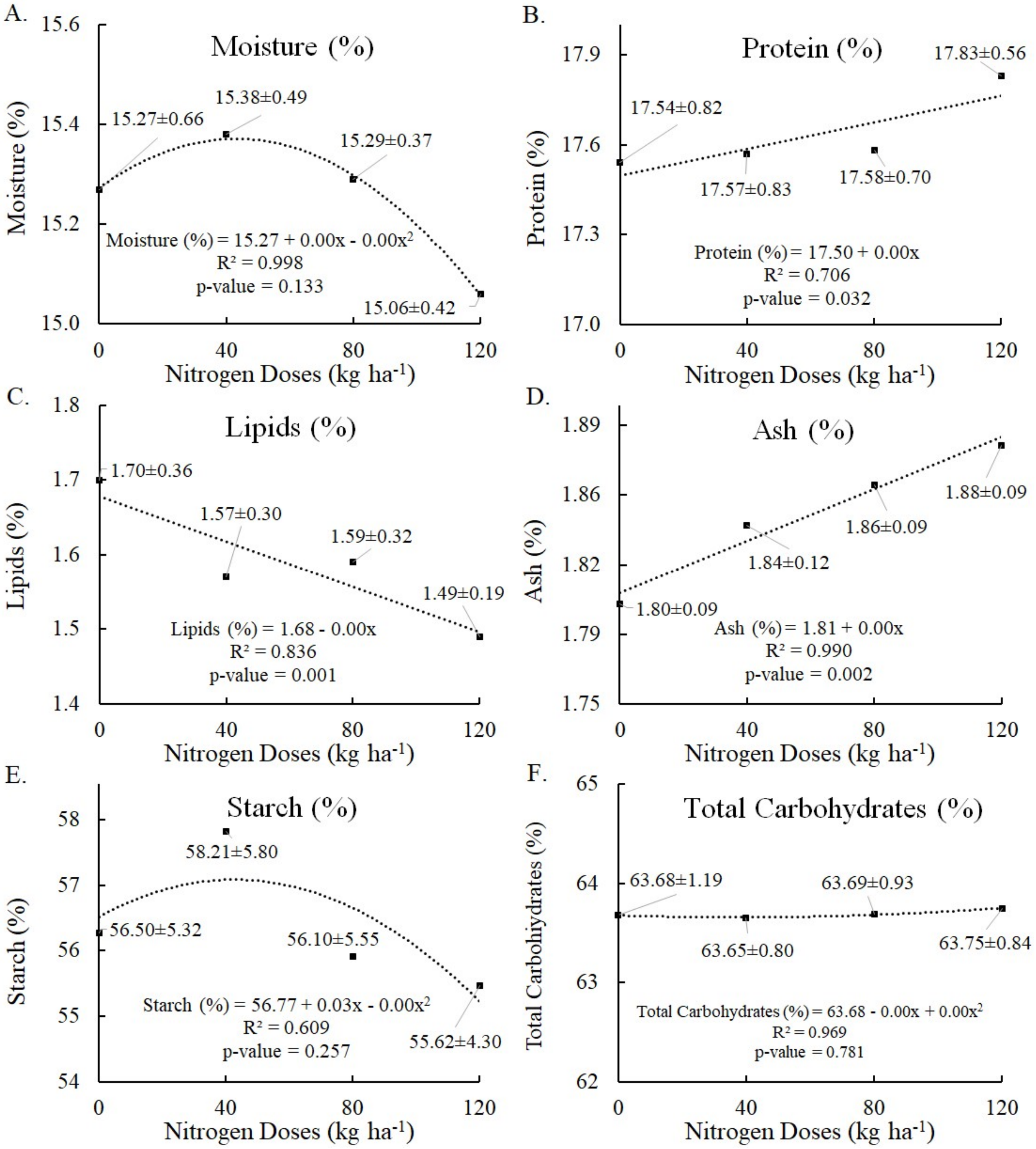

Figure 2. Chemical composition of wheat kernels (*Triticum aestivum* L.) in response to nitrogen application in topdressing (0, 40, 80, and 120 kg ha^{-1}). Expressed on a dry basis; Means (n = 24 ± standard error)

Even though was there was no significant effect of nitrogen doses in starch and total carbohydrates content in the kernels (Figure 2E and F), previous studies have shown that nitrogen deficiency usually results in accumulation of non-structural carbohydrates (Kovačević et al., 2012). According to Wingler et al. (2006), nitrogen deficiency can lead to sugar accumulation by reducing the use of carbon skeletons for amino acid and protein synthesis, resulting in a negative correlation between protein and starch content.

Increasing doses of nitrogen applied at the beginning of tillering stage did not affect the quality traits evaluated, with exception of test weight of the produced kernels (Figure 3). As shown in Figure 3A, a linearly reduction was observed in kernel test weight when increasing doses of nitrogen were applied ($R^2 = 86$; $p<0.01$), without lodging of the wheat plants.

The reduction in test weight can be attributed to a greater competition between kernels for photoassimilates since increasing doses of nitrogen applied in topdressing increase the number of spikes per m^2 and kernels per m^2, known as haying off phenomenon (Hoogmoed et al., 2018). Although no significant difference was detected for weight of a thousand kernels, as reported by Prando et al. (2012), the increase in the number of kernels per m^2 may have produced less dense grains and, consequently, reduced the test weight or kernel volume density. In fact, number of spikes m^2 and productivity showed a significant negative correlation with test weight (r = -0.44 and -0.52, respectively). The reduction in test weight observed was small, around 0.4 kg hL^{-1}, and insufficient to affect the kernel commercial classification (MAPA, 2010).

The test weight is a good predictor of quality potential that positively correlates with flour yield, being higher the higher the value obtained (Deliberali et al., 2010). Although the regression equation between flour yield and nitrogen doses was not significant, as shown in Figure 3B, a trend similar to the observed for test weight was also observed for flour yield, which yielded -2.19% less when applied the maximum nitrogen rate tested (120 kg ha^{-1}), compared to the minimum rate (0 kg ha^{-1}).

Regarding experimental conditions (Figure 1), no significant results were observed for dough strength, tenacity, extensibility, and elasticity index, in response to increasing doses of nitrogen applied in topdressing (Figure 3C to 3F). Even though these baking traits are directly related to protein quantity and quality, the differences observed in protein content in the kernels (+1,7%) was not enough to affect the technological quality of the flour obtained (Dias et al., 2017; Xue et al., 2016).

According to the regression equation in Figure 3G, there was no significant influence of nitrogen doses on the falling number values observed in the flour obtained. Other studies had already reported the absence of results for nitrogen fertilization on falling number values (Boschini et al., 2011). This is due to the fact that falling number, which indirectly indicates the enzymatic activity and germination process in the kernel, is directly influenced by the environmental conditions during the final development stages of the crop, maturation, and harvest (Franceschi et al., 2009).

Regarding flour quality, the increment of nitrogen doses reduced the brightness (L*) of the flour obtained from the kernels (Figure 3H; $p<0.01$). However, the measured values are greater than 92 and close to 93, characterizing the flour as white.

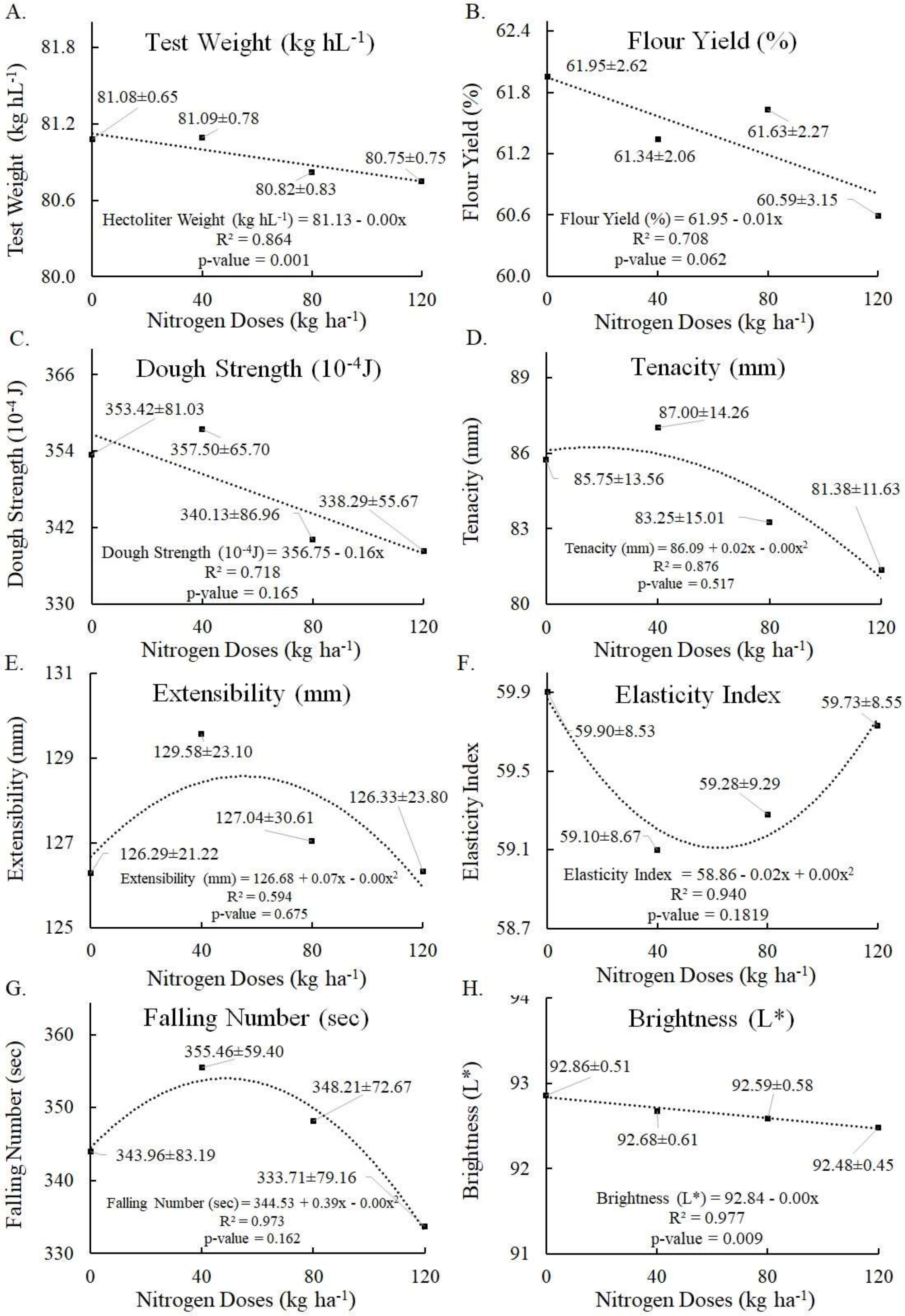

Figure 3. Quality of wheat flour (*Triticum aestivum* L.) in response to nitrogen application in topdressing (0, 40, 80, and 120 kg ha^{-1}). Means (n = 24 ± standard error)

As previously reported, pigments, protein content, mineral content (ash), flour particle size, and kernel hardness are among the factors involved in color changing in flours, which corroborates our results since protein and ash content in the kernels increased after the application of high doses of nitrogen (Figure 2). A significant relationship ($p<0.01$) between flour color and protein content was reported by Nagamine et al. (2003), who observed a significant correlation between the protein content and brightness (L *) of the flour evaluating durum ($r=-0.54$) and soft wheats ($r=-0.54$).

Conclusion

Based on the results obtained from field trials, the quantitative chemical composition of wheat kernels is defined intrinsically by the genetics of the cultivated material. However, strategies such as increasing nitrogen availability to the plants may result in changes in kernel component contents as well as physical characteristics such as density, yield, and quality of the flour obtained.

Increasing doses of nitrogen applied in topdressing at the beginning of tillering stage affected the chemical composition of kernels, increasing protein and ash, and reducing lipid content. Moreover, the application of high doses of nitrogen reduced test weight or volume density of kernels and yield and brightness of flour. However, based on the results and due to the climatic conditions during the crop development, additional studies must be carried out in order to aggregate knowledge about the effect of urea forms as source of nitrogen and its effect on the chemical composition of kernels and quality of wheat flour.

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