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Protein content of grains of different size fractions in malting barley

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The negative relationship between grain size (percentage >2.5 mm) and protein content usually observed in barley grain samples is attributed to the presence of thin grains. The objective of this study was to determine whether, in grain samples from a given environment, thin grains had a different protein content than plump grains. Grain samples from field experiments were analysed for grain yield, size and protein content of the whole sample and of four size fractions within each sample. Grain yield ranged from 1.5 to 6.5 mg ha^{-1} and grain protein (whole sample) ranged from 6.8 to 13.4 %. Most of the variation observed in protein content was explained by the ratio of nitrogen availability to grain yield. Within a grain sample, thin grains had more protein than plump grains (>2.5 mm) only when the protein content of the whole sample was high, that is, when the grain sample came from an environment with a high relative abundance of nitrogen. The fact that grain samples with low grain size tend to have high protein content is not due to the presence of a high proportion of thin grains, because thin grains do not always have more protein than plump grains. Copyright © 2014 The Institute of Brewing & Distilling

Keywords: grain protein content; grain size fractions; nitrogen availability; malting barley; grain yield

Introduction

About 15% of world barley production is converted into malt, which is the primary input for the manufacture of beer *(1)*. The quality of barley grain for malting is directly related to its protein content and grain size *(2,3)*. Grain size is defined by the width of the grain, and is determined by size fractionation with slotted sieves of several sizes (plump grains are >2.5 mm wide). To meet maltsters' quality requirements, barley grain must have a specific protein level and high grain size (i.e. a high proportion of plump grains) *(3)*. These two characteristics related to grain quality are determined during the crop cycle, and nitrogen fertilization may affect both *(4–7)*.

In the malting industry, it is known that grain samples with low grain size usually have a high grain protein content. This negative relationship between grain size and protein content is attributed to the presence of a high proportion of thin grains, because it is known that the thin grains in a batch tend to be richer in protein than the plump grains. The scientific literature on barley use reflects this general concept *(3,8)*. A negative relationship between grain size and protein content has been observed in studies where different cultivars, levels of water and nitrogen availability were compared *(6,9)*. This relationship may have several causes. Drought and high temperature during grain filling simultaneously increase the proportion of thin grains and grain protein content *(10–12)*. Therefore, grain samples from crops that have suffered thermal or water stress during the grain-filling period have lower grain size and higher protein content than grain samples from barley crops that have not suffered stress. Nitrogen fertilization of deficient barley crops may also increase protein content and the proportion of thin grains *(4,5,7,13)*. When a crop is fertilized, the reduction of grain size is due to the increase in the number of grains coming from tillers and apical positions within the spikes. These grains tend to be thinner than the ones from the main stem or the centre of the spikes *(14,15)*. These causes may explain why a negative association between grain size and protein content is observed when grain samples coming from different environments are compared, such as grains from different fields within the same or different agricultural regions (i.e. grain samples from crops grown with different nitrogen availability or environmental conditions during their crop cycle).

In grain samples of a given environment, even of a single plant, there are grains of different sizes. Few authors have measured the protein content of grain of different sizes coming from a given environment. Li *et al. (16)* separated grains from four grain samples into several grain size fractions (>2.8, 2.8–2.4 and 2.4–2.0 mm), and did not observe differences in protein content between these three grain size fractions within each sample. Within a barley spike, grains from the centre are heavier than those from apical positions *(14,17)*. As grain weight and width are closely related *(6)*, it is reasonable to assume that grains from apical positions are thinner than those from the centre. Ellis and Marshall *(14)* observed that grains from apical positions had protein levels slightly higher than grains from the centre. Yin *et al. (17)*, however, observed that the protein content of grains of different positions was affected by the timing of nitrogen fertilization: in barley crops fertilized at tillering, grains from central and apical positions of the spike had similar protein content, while when fertilized at booting, grains from the centre had less protein than those from apical positions (thin grains).

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The difference in protein content between grain size fractions has a practical consequence owing to post-harvest grain management. Malting barleys are usually screened over sieves to retain the plump grains. Maltsters remove thin grains and usually do not process them *(3)*. Therefore, if the thin grains have higher protein levels than the plump grains, sieving will decrease the mean grain protein content of the grain batch. This is the case when the batch is composed of a mixture of grain samples from environments with different nitrogen availability or post-anthesis stress. What happens when grains from a given environment are sieved will thus depend on whether or not thin and plump grains have different protein contents.

The objective of this study was to determine whether, in grain samples from a given environment, thin grains had a different protein content than plump grains. The working hypothesis was that grains of different size fractions from the same environment would have the same protein content. Therefore, differences in grain protein content would be more related to environment characteristics (e.g. nitrogen availability) than to grain size.

Materials and methods

Four field experiments with malting barley (*Hordeum vulgare* L., cultivar Scarlett) were conducted in the Pampean region, in the north of Buenos Aires province, Argentina, between 2005 and 2008 (Table 1). The climate of this zone is humid temperate, with 850 mm mean annual rainfall, and 16 °C mean temperature. Table 1 shows the location and soil characteristics of each experimental site. Experiments were established at farmers' fields, and management practices were those normally used by the farmers, based on no-tillage systems (chemical weed control and seeding directly into the standing residue). Experiments were sown at the beginning of July (row distance of 17.5 cm) and harvested at the end of November. The previous crop was soybean at every site.

Treatments were three levels of nitrogen availability at sowing: N0, no nitrogen added; N1, nitrogen added to reach 100 kg ha^{-1} of available nitrogen (N_{avail}) considering soil nitrogen as nitrate up to 60 cm deep (N_{soil}) plus fertilizer nitrogen ($N_{fertilizer}$); and N2, nitrogen added to reach 160 kg ha^{-1} of N_{avail} ($N_{avail} = N_{soil} + N_{fertilizer}$). Nitrogen fertilizer was applied as broadcast urea at sowing. Soil nitrogen as nitrate up to 60 cm deep was similar in all sites, and ranged from 46 to 53 kg ha^{-1} of nitrogen. Randomized complete block designs with four replications were used for all experiments, and plots were 39 m^2. Phosphorus (15–21 kg P ha^{-1}) and sulfur (10 kg S ha^{-1}) fertilizers were broadcast on all plots at sowing to prevent these particular nutrient deficiencies.

Rainfall was measured during the crop cycle with meteorological stations placed close to each experimental site (Table 1). At commercial maturity, when grain moisture was approximately 12%, 1 m^2 from each plot was harvested and threshed by hand. Grain samples were oven dried at 65 °C to determine grain yield, size and nitrogen content. Grain yield was adjusted to a standard moisture content of 12%. Grain size was determined by size fractionation with a screening machine (Sortimat) with three slotted sieves of different widths (2.8, 2.5 and 2.2 mm). A sample of 100 g of grain was placed onto the top sieve (2.8 mm) and shaken for 5 min. Each grain sample was separated into four grain size fractions: >2.8 mm (fraction 1), 2.8–2.5 mm (fraction 2), 2.5–2.2 mm (fraction 3) and <2.2 mm (fraction 4). Grain size (percentage >2.5 mm) was calculated as the percentage by weight of plump grains (fractions 1 plus 2) within each sample. Nitrogen content of each size fraction was measured in Kjeldahl digests by colorimetry *(19)*, and protein content was calculated by multiplying the N content by a factor of 6.25. The grain protein content of the whole sample of each plot was calculated as the weighted average of the four size fractions considering the proportion (by weight) of each size fraction within each sample.

Data collected was statistically analysed by analysis of variance, and relationships between different variables were

Table 1. Location of experimental sites, soil characteristics and rainfall during crop cycle

	Experimental site			
	1	2	3	4
Growing season	2005	2006	2006	2008
Name of the site	Junin	La Agraria	Baigorrita	Junin
Latitude (S)	34°38′10″	34°39′57″	34°46′02″	34°38′24″
Longitude (W)	60°56′57″	60°54′57″	61°02′31″	60°57′00″
Soil type *(18)*	Entic Hapludolls	Entic Hapludolls	Typic Hapludolls	Entic Hapludolls
Soil analysis at sowing:				
pH (0–20 cm)	5.6	5.5	5.8	5.5
Organic matter (%, 0–20 cm)	1.4	2.4	2.5	2.1
N-NO_3 (kg ha^{-1}, 0–60 cm)	47	49	53	46
S-SO_4 (ppm, 0–20 cm)	5.3	9.4	11.5	13.8
P (Bray1, ppm, 0–20 cm)	5.4	4.4	5.5	12.6
Water content (mm, 0–100 cm)	177	243	263	201
Rainfall during crop cycle (mm)				
Total	310	320	373	243
30 days before flowering to flowering	57	73	198	53
Flowering to maturity	120	190	117	76

assessed by correlation and regression analysis (Statistix, version 9, Analytical Software). A factorial ANOVA was used to analyse the effect of site and N level on grain yield, grain protein (whole sample) and size, and to analyse the effect of N level and grain size fraction on grain protein at each experimental site. When an effect was significant ($p < 0.05$), differences between means were compared using the LSD test (least significant difference, $p < 0.05$). Pearson's correlation (r) and linear regression were used to evaluate the degree of association between two variables. A regression model with dummy variables was used to evaluate the relationship between grain protein (whole sample) and grain yield or grain size considering each N level *(20)*. Slopes of regression lines of grain protein content of each size fraction as a function of the protein content of the whole sample were compared using a *t*-test.

Results

The combination of experimental sites and nitrogen treatments generated a wide variation between environments for crop growth, which affected both grain yield and protein content. Grain yield ranged from 1.5 to 6.5 mg ha^{-1} and grain protein (whole sample) ranged from 6.8 to 13.4%. Grain yield and protein content varied significantly between sites (Table 2). Soil water content at sowing was not related to grain yield. In general, sites that received more rainfall during the crop cycle (and in the period from 30 days before flowering to maturity) had more grain yield and less protein content (Tables 1 and 2). Grain size (percentage >2.5 mm) also varied between sites. The lowest grain size (63%) was observed at site 4, which had the lowest rainfall (total and during the period after flowering). At site 3, with the highest rainfall and grain yield, its low grain size (79%) could be related to the size of the kernels from secondary tillers. Nitrogen availability affected grain yield, with no significant interaction between site and N level (Table 2). Nitrogen addition produced a small increase in grain yield. Averaged over all of the experimental sites, grain yield in N2 treatment was 0.33 mg ha^{-1} higher (8.2%) than the check treatment, while there was no difference between the check and N1 treatment (Table 2). Nitrogen addition increased the grain protein content in three of the four experimental sites (significant site × N interaction). Differences in grain yield between sites were

Table 2. Grain yield, protein and size (mean ± standard error)

	Grain yield (Mg ha^{-1})	Grain protein (%)	Grain size* (% > 2.5 mm)
Site 1 N0	4.07 ± 0.15	8.0 ± 0.21 a	91.4 ± 0.40 a
N1	4.05 ± 0.21	9.7 ± 0.62 b	88.1 ± 0.87 ab
N2	3.79 ± 0.19	11.7 ± 0.61 c	81.7 ± 3.17 b
Site 2 N0	4.22 ± 0.22	8.1 ± 0.21	92.4 ± 0.23 a
N1	4.04 ± 0.17	8.2 ± 0.50	87.9 ± 1.38 b
N2	4.73 ± 0.28	8.9 ± 0.36	87.7 ± 0.96 b
Site 3 N0	5.77 ± 0.14	7.8 ± 0.48 a	87.5 ± 1.24 a
N1	5.81 ± 0.22	8.6 ± 0.36 a	77.6 ± 2.17 ab
N2	6.31 ± 0.11	9.8 ± 0.38 b	73.4 ± 4.45 b
Site 4 N0	1.97 ± 0.13	9.5 ± 0.40 a	63.5 ± 0.23
N1	2.18 ± 0.26	11.4 ± 0.50 b	62.7 ± 0.29
N2	2.53 ± 0.08	12.0 ± 0.54 b	62.9 ± 0.29
Means			
Site 1	3.97	9.8	87.0
Site 2	4.33	8.4	89.3
Site 3	5.96	8.7	79.5
Site 4	2.22	11.0	63.0
N0	4.01 a	8.4	83.7
N1	4.02 a	9.5	79.1
N2	4.34 b	10.6	76.4
ANOVA		*p*-Value	
Site	<0.01	<0.01	
N	0.03	<0.01	
Site × *N*	0.26	<0.01	
N effect			
Site1		<0.01	0.02
Site2		0.14	0.01
Site3		<0.01	0.04
Site4		<0.01	0.20

* ANOVA performed by site owing to heterogeneous variances.
Different letters denote significant difference between *N* levels (least significant difference, $p < 0.05$).

greater than between nitrogen treatments, while both factors had similar effects on grain protein. Nitrogen addition also decreased the grain size (% > 2.5 mm) in three sites (Table 2).

Grain protein content (whole sample) was negatively related to grain size ($r = -0.58$) and grain yield ($r = -0.48$), even without considering the effect of N level on the grain protein (Fig. 1). When the three levels of the N treatment were taken into account separately, grain protein was more closely related to grain yield ($R^2 = 0.61$) than to grain size ($R^2 = 0.53$). N availability and grain yield were combined as the ratio of N availability to grain yield (N_{avail}/grain yield). Grain protein (whole sample) increased along with the availability of N per ton of actual grain yield (Fig. 1c).

Grain protein content of all grain size fractions increased owing to N addition at every site (Fig. 2, Table 3). There was no difference in the protein content between grain size fractions at two sites (sites 2 and 3). At site 4, thin grains had higher protein content than plump grains regardless of the N level. At site 1, the difference in protein content between grain size fractions tended to depend on the N level, being greater at N2 than at N0 (Fig. 2, Table 3).

The protein content of each size fraction within a sample increased linearly as a function of the grain protein content of the whole sample (Fig. 3). The slopes of linear regressions fitted to size fractions 1 and 2 were lower than the ones fitted to size fractions 3 and 4 ($p < 0.05$). These results suggest that the protein content of plump grains (>2.5 mm) tended to increase less than that of thin grains when the protein content of the whole sample rose. Therefore, the difference in protein content between plump and thin grains within a sample (the vertical distance between fitted lines in Fig. 3) was higher in grain samples of high protein content. On the other hand, the difference in protein content between plump and thin grains within a sample was not related to the grain size (i.e. the proportion of plump grains of the sample, $R^2 = 0.06$, $p > 0.05$).

The change in mean protein content of each grain sample when thin grains (<2.5 mm) were excluded was calculated. A segmented function was fitted to the relationship between this change in protein content and the protein content of the whole sample (Fig. 4). When the grain protein content of the whole sample was <8.6%, removing the thin grains had no effect on it, while when it was >8.6%, removing the thin grains slightly decreased the protein content of the sample. The change in mean protein content of each grain sample owing to the thin grain removal increased along with the protein content of the whole sample.

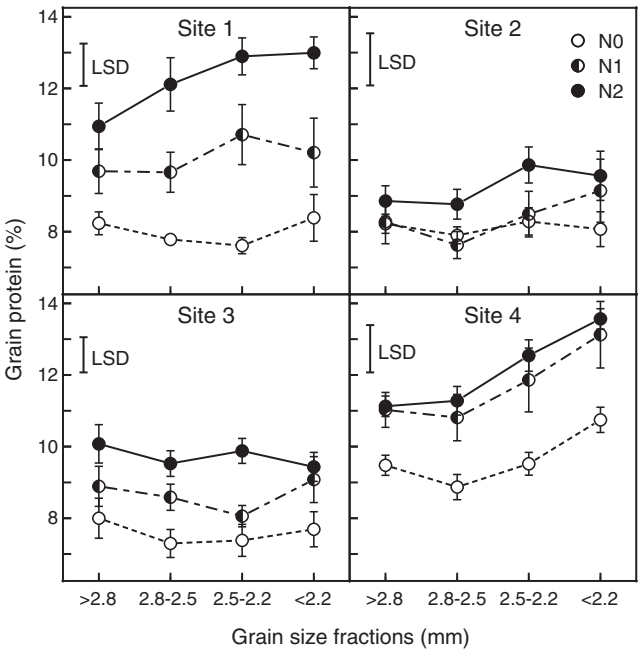

Figure 2. Grain protein content of each size fraction and nitrogen level at each experimental site. Each point is the mean of four replicates. Vertical bars are ± standard errors. LSD, Least significant difference between any two means within an experimental site ($p < 0.05$).

Table 3. *p*-Values from the ANOVA for the effect of nitrogen treatment and grain size fraction on grain protein

	Site 1	Site 2	Site 3	Site 4
N	<0.01	<0.01	<0.01	<0.01
Size fraction	0.02	0.16	0.18	<0.01
N × size fraction	0.07	0.81	0.55	0.83

Discussion

In the Pampean region, as in other agricultural regions of the world, water availability is the major factor affecting grain yield *(21,22)*. It was not unusual that, in our study, experimental sites with more rainfall during the crop cycle had a higher grain yield. The site with the lowest rainfall also had the highest grain

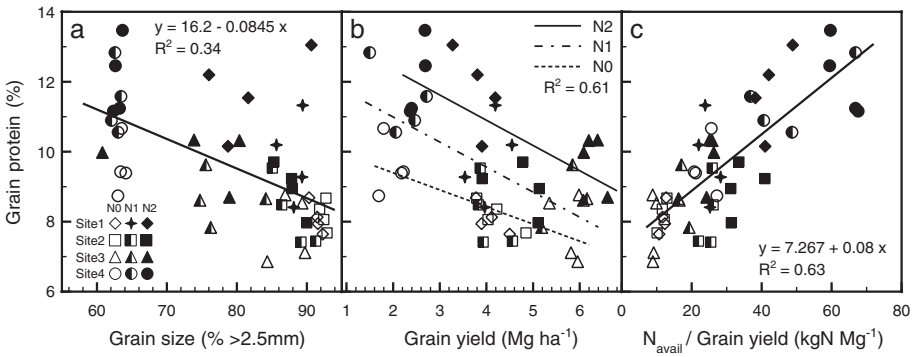

Figure 1. Relationship between grain protein content (whole sample, percentage dry weight) and (a) grain size (% >2.5 mm), (b) grain yield, (c) the ratio of available N (N_{avail}) to grain yield. N_{avail} (kg ha^{-1} of N) is the sum of soil N as nitrate up to 60 cm deep and N added as fertilizer at sowing. Grain yield is based on 88% dry matter. The relationship between grain protein and grain yield in (b) is represented by a model of three lines (one per N treatment). The R^2 in (b) is the coefficient of determination of this model.

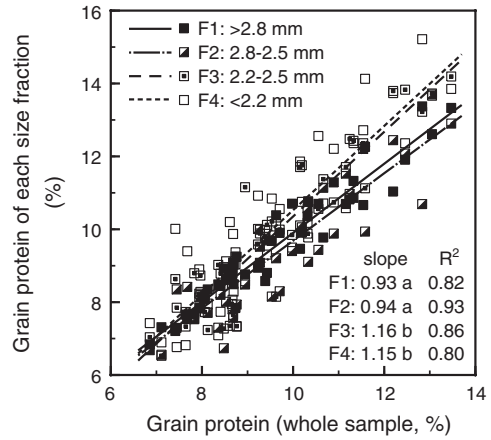

Figure 3. Relationship between grain protein content (percentage dry weight) of each size fraction and the protein content of the whole sample. Each point represents a grain size fraction from a sample. A function (i.e. $y = a + bx$) was fitted for each size fraction. Slope values followed by the same letter are not significantly different (*t*-test, $p < 0.05$).

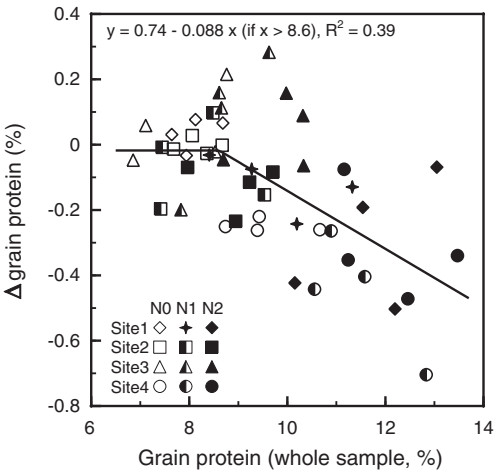

Figure 4. Change in the protein content of each grain sample when thin grains (<2.5 mm) were excluded, as a function of the protein content of the whole sample (prior to the exclusion of thin grains).

protein content and the lowest grain size (percentage >2.5 mm), in agreement with other authors who have reported an increase in protein content and a decrease in grain size in barley crops that have suffered water deficits *(10)*. A possible interpretation is that, for a given amount of available nitrogen, higher grain yields owing to higher precipitation during the growing period may cause a dilution effect on the nitrogen content of the grain *(23)*. As a consequence, the grain protein content of the whole sample is closely related to the amount of available nitrogen per unit of actual yield. This ratio (N_{avail}/grain yield) may be considered an index of the relative abundance of nitrogen for grain protein synthesis. The relative abundance of N may increase owing to nitrogen fertilization or a stress that reduces actual yield. A relationship between grain protein and the ratio of available N to grain yield was also observed by McKenzie *et al*. *(24)*, but they used a different way to estimate N availability.

Within a grain sample coming from a given environment, thin grains had more protein than plump grains only when the protein content of the whole sample was >8.6%, that is, when the grain sample came from an environment with a high relative abundance of nitrogen. Even when these results are restricted to the cultivar used in our study, the results are still in agreement with other author's observations from other cultivars. Yin *et al. (17)* observed that, in grain samples with a mean protein content (whole sample) of 9.8%, the protein content of grains from apical and central positions within the spike was not different, while in grain samples with a higher protein content (11.7%), grains from central positions had a lower protein content than those from the apical positions within the spike. Grain samples with lower protein content came from treatments where N was added at tillering, while those with higher protein content were from treatments where N was added at tillering and booting, increasing the N availability for protein synthesis during grain filling. In two experiments with barley plants grown on a nutrient culture system, Ellis and Marshall *(14)* also observed that grains from central positions had a lower protein content than those from apical positions within the spike. From apical to central positions, grain protein decreased from 12.1 to 11.6% in one experiment and from 11.6 to 10.6% in another. In contrast, Li *et al*. *(16)* did not observe a different protein content between three grain size fractions within each of four samples analysed, even when the whole sample protein content ranged from 11.8 to 13.0%. It is worth noting that grains thinner than 2 mm were not included in their analysis.

Our results indicate that the grain protein content was mostly determined by the relative abundance of N in each environment, and not by the grain size (Fig. 1c, Fig. 3). Differences in protein content between thin and plump grains within a grain sample were much smaller than those between the grain samples from different environments, even considering grains of the same size fraction (Fig. 3). The difference in the grain protein content between thin and plump grains in samples with grain protein (whole sample) >11% was 1.15%, which was small in scale compared with the effect of the environment, as the whole sample protein ranged from 6.8 to 13.4% (Fig. 1, Fig. 3). Therefore, if grain samples are screened and thin grains removed after harvest, in some cases there may be a slight decrease in protein content, but it would not reverse the effect of the environment on the grain protein level of the malting barley.

The results suggest that grain samples with a low grain size (percentage >2.5 mm) tend to have a high protein content and that this is not due to the presence of a high proportion of thin grains, as thin grains do not always contain more protein than plump grains, and where there is a difference, it is a small one. Probably, the samples with a high protein and a low grain size came from environments with a high relative abundance of N owing to fertilization or a low yield caused by a post-anthesis stress. When several samples from different environments are mixed in a batch, samples with high protein and low grain size make a greater contribution to the thin-grain fraction of the batch than those with lower protein and higher grain size. This would explain why most maltsters have experienced thin grains in a batch tending to be richer in protein than plump grains.

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