

Free Asparagine Concentrations in Canadian Hard Red Spring Wheat Cultivars

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Abstract

The amino acid asparagine is considered the rate limiting precursor in the formation of acrylamide in cereal based baked foods. However, there are no data on the quantity of this precursor in Canadian wheat. Wholemeal and white flour samples obtained from 30 Canadian Hard Red Spring (Canada HRS) wheat grown in the Prairie Provinces were analysed for asparagine content by liquid chromatography. The asparagine content varied from 302 to 965 and 116 to 336 $\mu\text{g/g}$ for wholemeal and white flour, respectively. Therefore, wheat grown in Canada has similar asparagine levels to wheat grown in other parts of the world. Analysis of variance suggested that asparagine content is significantly affected by genotype ($p < 0.001$), suggesting that breeding strategies could be investigated to produce cultivars with lower levels of this amino acid. Few significant correlations were observed between wheat and flour quality parameters and asparagine content, although there was a tendency towards weaker dough strength indices with increasing asparagine content.

Key words: wheat, asparagine, quality traits, Canada Hard Red Spring (Canada HRS)

1. Introduction

Acrylamide is found in various heat-treated foods, including cereal extrudates (Masatcioglu et al. 2014), but especially in foods produced by deep frying or baking processes (Mottram et al. 2002). Acrylamide was reported as a food-borne toxicant for the first time by the Swedish National Food Administration in 2002 (Swedish NFA 2002). Due to the health risks associated with the consumption of acrylamide, the European Food Safety Authority (EFSA) has collected data on the acrylamide content of foods sampled from 20 member states of the European Community and Norway; according to EFSA (2011), the mean acrylamide intake for adults (over 18 years),

adolescents (11-17 years), children (3-10 years) and toddlers (1-3 years) in Europe was estimated as 0.31-1.1 µg/kg, 0.43-1.4 µg/kg, 0.7-2.05 µg/kg and 1.2-2.4 µg/kg body weight per day, respectively.

Several researchers have demonstrated that acrylamide is mainly formed from asparagine and carbonyl sources, e.g., reducing sugars, through a complex mechanism in the Maillard browning reaction (Mottram et al. 2002; Yaylayan et al. 2003; Zyzak et al. 2003). The main precursor for acrylamide formation is free asparagine available in raw food materials, such as wheat flour (Capuano and Fogliano 2011). In the wheat kernel, asparagine is mainly present in the aleurone layer and so reducing flour extraction rate will decrease asparagine content in the flour (Capuano et al. 2010); however, at the same time lower flour yield would be undesirable as it would result in economic losses to the mill.

Soil conditions during plant development can affect asparagine content. Asparagine content increases when the plant is exposed to sulphur deficiency conditions (Muttucumaru et al. 2006; Shewry et al. 2009; Halford et al. 2012). In the case of sulphur deficiency in the soil (sulphur level of <0.2% in dry matter), an application of 25-50 kg SO₃/ha as a sulphate-containing fertilizer in early spring before the start of stem extension has been recommended by the Department for Environment, Food and Rural Affairs of the UK (Defra 2010).

In addition, free asparagine content in the wheat kernel is dependent upon both genotype and environment (Curtis et al. 2009b); therefore, selecting genotypes with low amounts of free asparagine would be an approach to acrylamide reduction in the final heat-treated wheat products. The importance of free asparagine content in cereal grains, particularly wheat, is such that the Health and Consumers Directorate-General of the European Commission has highlighted the need

to develop cereal cultivars with low free asparagine content in its 2014 summary report of the Standing Committee on the Food Chain and Animal Health (European Commission 2014). Curtis and Halford (2016) recently asserted that unless wheat breeders addressed asparagine reduction in breeding programs, there was the danger of losing market shares to countries that do.

A recent study presented information on the asparagine content of hard red spring wheat cultivars grown in North Dakota (Ohm et al. 2017). They reported that growing location, genotype and their interaction had a significant effect on the asparagine content in hard red spring wheat. To our knowledge, there has been no research conducted in Canada on the relationship between genotype and environment on the free asparagine content of Canadian wheats or flours milled from these wheats. Therefore, the objectives of this study were to measure asparagine content in wholemeal and flour samples generated from single cultivars of hard red spring wheats grown at one location in western Canada, and to evaluate relationships between asparagine content and standard dough and breadmaking quality indices.

2. Materials and Methods

2.1 Wheat and Flour Samples

Wheat samples were supplied by the Canadian International Grain Institute (Cigi) and consisted of 30 cultivars grown in Canadian Prairie regions (Manitoba, Alberta, and Saskatchewan). A composite sample of the 3 locations was made for each cultivar. Wheat samples were cleaned, tempered (16.5% for 24 hours) and milled at Cigi into straight grade flour using a Bühler laboratory flour mill (MLU-202) with a bran duster (MLU-302). A Perten Laboratory Mill 3100 was used to grind the wheat into a wholemeal for testing of protein, falling number, wet gluten and gluten index. Standard laboratory methods were used to determine amylograph peak viscosity (AACCI

Method 22-10.01) with minor modifications using 65 g of flour instead of 100 g of flour with 450 mL of double distilled water); ash content (AACCI Method 08-01.01); extensograph (AACCI Method 54-10.01); falling number (AACCI Method 56-81.03); farinograph (AACCI Method 54-21.02); wet gluten and gluten index (AACCI Method 38-12.02; single-stage method for flour and two-stage method for wholemeal); moisture content (AACCI Method 44-15.02); protein content (Williams et al. 1998); starch damage (AACCI Method 76-33.01); baking test using a lean no time test baking method (Dupuis and Fu 2017); and bread volume (AACCI Method 10-14.01). Canadian Grain Commission (CGC) guidelines were used to determine weight per 1000 kernels (CGC, 2013) and test weight (CGC 2017).

Water dough colour was determined by mixing flour (300g) with 32% water (w/w) in the GRL 1000 Mixer (6 min at 105 rpm) to produce a homogeneous dough crumb. The dough crumbs were sheeted with an Ohtake laboratory noodle machine (Ohtake, Tokyo, Japan) with an initial gap setting of 3.5 mm, followed by a lamination step at the same gap setting. The dough sheet was then rested for 10 minutes in a plastic bag and then subjected to four reduction passes (2.0; 1.5; 1.2; and 1.0 mm gap settings). The resulting noodle sheet was sealed in a plastic bag to prevent moisture loss until colour measurements were performed. Colour was measured on the folded dough sheet (6 layers) at 3 h and 24 h using a Minolta colorimeter (L^* , a^* , b^*) with a D65 illuminant.

A set of wholemeal samples were also obtained from single cultivars of wheat grown at Glenlea, Manitoba. Since there was insufficient sample to produce flour or to conduct quality analyses, the results for these samples are included to allow a comparison between wholemeal asparagine contents across environments for some cultivars. Wholemeal samples used for asparagine analysis

(both wheat grown in Glenlea, Manitoba and composite wheat samples from Canadian Prairie regions) were prepared by grinding wheat on a Retsch Ultra Centrifugal Mill ZM 200 (Retsch GmbH, Germany) using a 0.5mm sieve.

2.2 Chemicals and reagents

Asparagine standard was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Amino acids, AccQ-Tag Ultra derivatization kit, and AccQ-Tag Eluents A and B were purchased from Waters Limited (Mississauga, ON, Canada). All acids and organic solvents were acquired from Fisher Scientific (Whitby, ON, Canada) and were of analytical or HPLC grade.

2.3 Free asparagine content determination

Two hundred (200) mg of sample was extracted with 5 mL of 0.01 M hydrochloric acid solution as described by Muttucumaru et al. (2006). Asparagine was determined using a Waters' pre-column derivatization amino acid method (AccQ-Tag Ultra). The method involves formation of stable urea following the reaction of 6-aminoquinolyl-N-hydroxysuccinidyl carbamate (Acc-Tag reagent) with free amino acid. An aliquot (10 μ L) of sample was mixed and incubated in a vial at 55 °C for 10 minutes with 70 μ L of AccQ-Tag Ultra borate buffer and 20 μ L AccQ-Tag Ultra reagent.

The derivatized amino acids were separated using a 1.7 μ M Waters AccQ-Tag Ultra C18 column (2.1x100mm) attached to an H class ultrahigh pressure liquid chromatograph equipped with a photodiode array detector (Waters, Mississauga, ON, Canada). The operating conditions were set as described in Waters AccQ-Tag UPLC amino acid H-Class application guide. Briefly, mobile phases consisted of amino acid AccQ-Tag (AAA) eluent A (0.1% formic acid in acetonitrile);

10:90 water: acetonitrile (B); water (C); and Acetonitrile (D). A linear gradient was set as follows with a flow rate of 0.7 mL/min, 0 – 0.29 min (2:0:98:0); 0.29 – 5.49 min (9:80:11:0); 5.79 - 7.10 min (8:15.6:57.9:18.5); 7.10 – 7.3 min (8:15.6:57.9:18.5); 7.3 – 7.69 min (7.8:0:70.9:21.3); 7.69 – 7.99 min (4:0:36.3:59.7); 7.99 - 8.59 min (4:0:36.3:59.7); 8.59 - 8.68 min (2:0:98:0); 8.68 - 10.20 min (2:0:98:0) for A:B:C:D, respectively. Sample and column temperatures were set at 20 and 43 °C, respectively. Asparagine was dissolved in 0.01M HCl and 9 dilutions were prepared (0, 31.3, 46.9, 62.5, 93.8, 125.0, 187.5, 250.0 and 375.0 nM) to generate a standard curve.

2.4 Statistical analysis

JMP 13 statistical software (SAS Institute Inc., Cary, NC) was used to perform a one-way analysis of variance (ANOVA) for the 30 cultivars. Sample means ($n = 3$) were compared using Tukey HSD method and significant differences determined at $p \leq 0.05$. The correlations between asparagine content and quality parameters were done with the Pearson correlation test with significance determined at $p \leq 0.05$.

3. Results and Discussion

3.1 Quality parameters of Canadian Hard Red Spring wheat

The mean and range for the various quality parameters for flours made from the 30 Canadian Hard Red Spring (Canada HRS) wheat cultivars grown in the Canadian Prairie regions are presented in Table 1. All the tested varieties in this study are marketed for their superior milling quality and protein strength and the good scores for most cultivars reflect the protein strength focus for these wheat varieties (Preston et al. 2001). Larger coefficients of variation indicated that genotype differences were more pronounced for falling number, gluten index, amylograph peak viscosity, most dough rheology parameters and loaf top ratio from test baking assessments.

3.2 Asparagine content in Canadian Hard Red Spring wheat wholemeal and flour

The mean asparagine content in the wholemeal samples from the 30 cultivars was 522.8 ± 91.1 $\mu\text{g/g}$ and ranged from $302.2 - 700.3$ $\mu\text{g/g}$ for wholemeal and from $116.1 - 335.5$ $\mu\text{g/g}$ for flours (Table 2). Canada HRS accounts for 65 - 70 % of all wheat grown in Western Canada. Despite being among the world's leading exporters of wheat, there is no published research regarding the asparagine content in Canadian wheat. Our results (Table 2) indicate that the asparagine contents of wheat grown in the Canadian Prairie regions are similar to those of wholemeal made from hard red spring wheat grown in North Dakota, USA ($357 - 1,037$ $\mu\text{g/g}$) (Ohm et al. 2017). The range in asparagine content is somewhat lower than reported for wheat grown in Europe ($320 - 1,560$ $\mu\text{g/g}$) (Corol et al. 2016), but relatively higher than wheats grown in Australia ($137 - 437$ $\mu\text{g/g}$) (Emebiri 2014).

Environment (E), genotype (G) and their interaction (G x E) have a huge influence on the asparagine content of wheat (Corol et al. 2016). Environment explained 32.5 % of the total variance observed in hard red spring wheats in North Dakota with G and G x E accounting for 26 and 4 %, respectively (Ohm et al. 2017). However in Europe, G x E accounted for the majority (43%) of the variance followed by E (36 %) and G (13 %) (Corol et al. 2016). Environmental factors affecting asparagine content in plants include sprout damage (Simsek et al. 2014), pathogen attack, mineral deficiencies and the presence of toxic metals (Lea et al. 2007). Thus, the difference between asparagine content for Glenn (302 $\mu\text{g/g}$) reported in this research and that reported for Glenn grown in North Dakota (357 $\mu\text{g/g}$) (Ohm et al. 2017) can be attributed to environmental differences between the two growing locations (Table 2). Further indication that Glenn is genotypically less prone to asparagine accumulation comes from the wholemeal asparagine content for Glenn grown at the other site in Manitoba (379 $\mu\text{g/g}$). Likewise, wholemeal values

for AAC Brandon and Carberry tended towards the upper end of the range, regardless of location (Table 2).

For the cultivars for which wholemeal and white flour samples were available, the analysis of variance (Table 3) suggested a strong effect of cultivar on asparagine content ($p < 0.001$). Among the tested cultivars, 2 pairs shared the same parents (Table 2): AAC Brandon and AAC Elie (Superb/CDC Osler/ND744) and Carberry and Muchmore (Alsen/Superb). Differences in the mean asparagine content were not substantially different between cultivars in either pair. These observations may suggest that asparagine content is a heritable trait. The asparagine content of Superb was significantly lower than any of the progeny cultivars (Muchmore, Carberry, AAC Brandon and AAC Elie) (Table 2). However, we were not able to find seed of the other parental lines (Alsen, CDC Osler or ND744) to determine if they were responsible for the higher asparagine levels observed in the offspring. Previous experiments on double haploid lines grown under the same conditions as the parent cultivars (Curtis et al. 2009b) gave inconsistent results for the role of parentage on asparagine content (1 higher, 1 lower and 2 no change). A study of 92 Australian wheats indicated heritability of asparagine content as low as 32% (Emebiri 2014) and in Europe it was even lower at 13% (Corol et al. 2016). Thus, more studies are needed to unlock the heritability of asparagine content in Canadian wheat with the objective that this will allow breeders to select for cultivars with low asparagine content.

The majority of wheat is consumed in its refined form and as such we determined the asparagine content in white flour (Table 2). The asparagine content decreased by 40 – 60 %, indicating that much of the wheat's asparagine is accumulated outside the starchy endosperm. This is consistent with reports from Europe (Corol et al. 2016) and Australia (Emebiri 2014). The relationship between asparagine content in the flour and in the whole grain for the 30 cultivars is shown in

Figure 1. Asparagine content varied significantly in different milling streams obtained from hard red spring wheat (Liu et al. 2011). Shewry et al. (2009) reported that the aleurone layer had the highest asparagine content among hand dissected grain fractions. Therefore, flours obtained from wheat cultivars with high extraction rates would have higher asparagine as a result of incorporation of more aleurone layer in the white flour (Claus et al. 2006). We did not observe correlations between asparagine content and flour extraction rate (Table 4), likely due to the limited variation in extraction rate in these samples.

As with wholemeal, the analysis of variance (Table 3) suggested that the asparagine content of white flour was influenced by genotype ($p < 0.001$). The asparagine content in flour ranged from ~ 116 (Glenn) to ~ 336 $\mu\text{g/g}$ (Muchmore) (Table 2). Also, the mean asparagine content in white flour was highly correlated to that of the wholemeals ($r = 0.802$, $p < 0.001$) (Table 4). Similarly in another study, wheat with high asparagine content resulted in white flour with high asparagine content (Corol et al. 2016). Thus, targeting cultivars and agronomic practices resulting in low asparagine content would benefit the consumers of both wholemeal and white flours. Considering the wide diversity in soil nutrition in the Canadian prairies (Angers et al. 1997; Yan et al. 2011), fertilization practices balancing the sulphur (Granvogl et al. 2007; Muttucumaru et al. 2006; Shewry et al. 2009) and nitrogen (Martinek et al. 2009) needs of the plant could prove significant in supplying wheat with low asparagine content. Nevertheless, because of its capacity to substantially reduce asparagine content, the milling process is a primary means of acrylamide mitigation in the food supply.

Acrylamide is a product of the Maillard reaction between asparagine and reducing sugars at high temperatures. In baked products, asparagine appears to be the limiting precursor, but not reducing sugars (Curtis et al. 2009a). Thus, our results imply that wholegrain products will have higher

acrylamide content than those made from the corresponding white flour. Indeed, whole wheat breads had the highest acrylamide content compared to white bread in a study conducted in Turkey (Boyacı Gündüz and Cengiz 2015). These observations pose a dilemma as wholegrain products are considered highly nutritive and healthy by all modern nutrition standards. The literature is inconsistent in reporting the amount of acrylamide formed during the baking of bread. For example, no acrylamide was detected in slices of baked bread except when slices were toasted at $>160^{\circ}\text{C}$ (Surdyk et al. 2004). Granby et al. (2008) reported acrylamide contents of <5 and $7\text{--}23\text{ }\mu\text{g/kg}$ for untoasted wheat and rye bread, respectively, whereas, acrylamide ranged from $13\text{--}74\text{ }\mu\text{g/kg}$ in wheat bread and as high as $175\text{ }\mu\text{g/kg}$ in rye bread in another study (Claus et al. 2006). The calculated conversion ratio of asparagine to acrylamide in heated cereal flours or baked bread is $0.1\text{--}0.6\%$ (Claus et al. 2006; Fink et al. 2006). However, the conversion rate could be reduced in the presence of glycine (Fink et al. 2006) or sodium chloride (Claus et al. 2008), by prolonged dough fermentation (Fredriksson et al. 2004), or by adjustment of yeast type (Katsaiti and Granby 2016), but it is enhanced when starch damage is increased (Wang et al. 2017). Regardless, acrylamide is mostly found in the bread crust but not in the crumb (Ahrné et al. 2007).

3.3 Correlation with quality parameters

The Pearson correlation analysis of asparagine content and quality parameters is presented in Table 4. No correlation was observed between asparagine content and test weight or thousand kernel weight. This is similar to the observation made in 150 wheat genotypes grown in Hungary (Corol et al. 2016) but in contrast to hard red spring wheat grown in North Dakota (Ohm et al. 2017). Even though asparagine is concentrated outside the starchy endosperm, we did not observe a significant association of asparagine to bran and ash. Weak correlations have been reported elsewhere between asparagine content and wheat protein, gluten index and wet gluten (Corol et al.

2016), but we observed no significant correlation between the asparagine content of wheat (wholemeal or white flour) and wheat protein or wet gluten. Gluten index and wholemeal asparagine ($p < 0.05$), and a number of extensograph dough strength parameters were weakly ($p < 0.1$) correlated to asparagine content (wholemeal and flour). Other rheological properties, such as extensograph R_{max} and A , and notably power consumption during mixing (during bread processing), showed significant correlations with asparagine content. Since all correlations of asparagine content to traditional dough strength indices are negative, there is potentially the relief that a class of wheat that has a reputation for good dough strength might not be prone to higher asparagine content. A similar trend was observed in hard red spring wheat grown North Dakota by Ohms et al. (2018). Hence more detailed investigations are warranted.

4. Conclusion

Because nothing is known about asparagine content in Canadian cereal grains, we provide a first report showing that wheat grown in Canada has similar asparagine levels to wheat grown in other parts of the world. Since efforts by global regulatory authorities to reduce asparagine/acrylamide levels in foods continue to mount (European Commission 2017), breeding strategies, agronomic practices and cereals processing measures to mitigate asparagine content in Canadian grain need to be explored to assure continued market access.

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Figure 1: Relationship between flour and wholemeal asparagine contents for Canadian Hard Red Spring Wheat Cultivars grown in locations across Canadian prairie provinces

Table 1. Summary of the quality parameters for Canada HRS wheat cultivars grown in locations across Canadian Prairie Provinces

	DF	Mean	CV, %	Minimum	Maximum
WHEAT					
Test weight, g	29	81.2	1.7	78.7	85.4
Weight per 1000 kernels, g	29	34.2	4.0	32.0	37.0
Falling number, seconds	29	433.5	15.4	280.4	556.1
Flour yield, %	29	77.1	1.4	74.4	78.9
Bran, %	29	18.0	4.8	15.9	19.4
Ash, %	29	1.5	4.1	1.4	1.7
Wet gluten, %	29	40.5	6.3	36.6	47.8
Gluten index, %	29	54.1	21.1	36.0	81.0
Protein, %	29	16.8	4.1	15.7	19.0
FLOUR					
Protein, %	29	14.0	4.2	13.1	15.9
Wet gluten, %	29	41.1	6.5	37.2	48.6
Ash, %	29	0.5	7.9	0.4	0.6
Starch damage, Chopin Dubois Units (UCD)	29	19.7	5.4	17.7	22.0
Amylograph peak viscosity, Brabender Units (BU)	29	525.8	32.6	211.0	854.0
FARINOGRAPH					
Absorption, %	29	63.6	2.0	61.8	67.6
Dough development time, min	29	5.6	16.3	3.9	7.1
Stability, min	29	9.8	21.0	6.6	13.7
Mixing tolerance index, BU	29	38.4	24.7	23.0	60.0
EXTENSOGGRAPH (45 min)					
Rmax, BU	29	311.1	22.4	183.0	507.0
R5, BU	29	173.6	13.7	130.0	222.0
E, mm	29	257.7	6.9	229.0	294.0
A, cm ²	29	108.5	22.0	64.0	183.0
Rmax/E	29	1.2	21.7	0.8	1.8
EXTENSOGGRAPH (135 min)					
Rmax, BU	29	346.6	26.0	200.0	581.0
R5, BU	29	193.6	18.3	137.0	285.0
E, mm	29	252.9	6.9	224.0	295.0
A, cm ²	29	120.3	24.6	70.0	182.0
Rmax/E	29	1.4	26.8	0.8	2.4
TEST BAKING (Lean No Time, LNT)					
Baking absorption, %	29	68.6	1.8	66.8	72.6
Mixing time, min	29	2.5	12.6	2.0	3.4
Power, Watt	29	113.1	6.8	102.4	133.0
Watt*hours/kg (dough)	29	8.3	16.2	6.6	11.8
Loaf volume, cm ³	29	997.7	12.2	767.0	1253.0
Specific volume, cm ³ /g bread	29	4.8	11.4	3.8	5.9
Loaf top ratio	29	0.4	27.1	0.1	0.6
WATER DOUGH COLOR					
3 h - L*	29	73.8	1.5	71.4	75.5
a*	29	2.3	13.4	1.9	3.3
b*	29	25.0	4.6	22.9	27.6
24 h - L*	29	69.4	1.7	66.2	71.3

a*	29	3.8	12.9	3.0	5.3
b*	29	24.8	4.4	23.2	26.8

Table 2. The mean asparagine content $\mu\text{g/g db}$ in measured on a composite flour sample of Canadian Hard Red Spring Wheat Cultivars Grown in locations across Canadian prairie provinces and their Parentage

Cultivar	Wholemeal	Flour	Parentage
5605HR CL	538.7 $\pm$ 5.4	233.7 $\pm$ 2.9	
AAC Brandon	597.2 $\pm$ 2.8	283.5 $\pm$ 3.9	Superb/CDC Osler//ND744
AAC Cameron	485.3 $\pm$ 48.1	268.5 $\pm$ 6.4	D1125/Alsen//BW346/3/BW370/99B60-EJ26
AAC Concord	560.5 $\pm$ 2.2	242.0 $\pm$ 22.2	Lillian / Journey // 9505-LP03A
AAC Elie	700.3 $\pm$ 29.6	327.3 $\pm$ 6.6	Superb/CDC Osler//ND744
AAC Connery	639.0 $\pm$ 6.7	315.5 $\pm$ 1.8	Somerset / BW865
AAC Jatharia	522.2 $\pm$ 20.3	230.2 $\pm$ 4.4	99B60-EJ26/Kane
AAC Redwater	531.3 $\pm$ 22.9	220.7 $\pm$ 11.7	AC Intrepid/Harvest//McKenzie
AAC Viewfield	402.8 $\pm$ 13.2	186.2 $\pm$ 6.2	Stettler / Glenn
AAC W1876	517.6 $\pm$ 17.7	230.1 $\pm$ 11.5	Prodigy/5602HR//Alsen
AC Barrie	460.7 $\pm$ 13.7	215.7 $\pm$ 17.7	Neepawa/Columbus//Pacific
AC Domain	569.3 $\pm$ 30.3	246.2 $\pm$ 10.6	BW83/ND585
Carberry	676.6 $\pm$ 24.5	305.2 $\pm$ 3.2	Alsen/Superb
Cardale	604.1 $\pm$ 37.4	317.7 $\pm$ 11.5	McKenzie/Alsen
CDC Bradwell	583.8 $\pm$ 14.5	236.7 $\pm$ 6.7	5602HR / W02330
CDC Kernen	481.8 $\pm$ 40.8	260.1 $\pm$ 2.6	CDC Bounty/FHB4
CDC Plentiful	466.4 $\pm$ 32.2	210.7 $\pm$ 14.6	BW282/CDC Go
CDC Stanley	475.9 $\pm$ 22.8	230.2 $\pm$ 0.8	W95132/AC Barrie
CDC Titanium	455.8 $\pm$ 25.3	279.8 $\pm$ 21.5	BW282 / P00.06-66
CDC Utmost	346.6 $\pm$ 11.0	175.8 $\pm$ 10.2	AC Elsa//CDC Teal/Seneca DH#10
CDC VR Morris	590.6 $\pm$ 43.6	285.7 $\pm$ 1.7	CDC Bounty/FHB9
Glenn	302.2 $\pm$ 3.6	116.1 $\pm$ 3.6	ND 2831/Steele-ND
Go Early	465.7 $\pm$ 18.1	203.0 $\pm$ 3.6	AC Intrepid' / CDC Go'
Lillian	400.6 $\pm$ 13.0	225.0 $\pm$ 16.7	BW621*3/90B07-AU2B
Muchmore	535.2 $\pm$ 1.3	335.5 $\pm$ 6.7	Alsen/Superb
Parata	499.4 $\pm$ 15.6	204.4 $\pm$ 0.3	AC Splendor/AC Domain//CDC Go
Shaw	559.4 $\pm$ 14.6	270.1 $\pm$ 7.7	Harvest/BW313
Stettler	626.5 $\pm$ 22.6	256.3 $\pm$ 12.6	Prodigy/Superb
Unity	593.5 $\pm$ 20.1	281.5 $\pm$ 6.0	McKenzie*3//BW174*2/Clark
Vesper	496.1 $\pm$ 34.1	215.1 $\pm$ 9.7	Augusta/Hard White Alpha//3*AC Barrie/6/BW150*2//Tp/Tm/3/2*Superb/4/94B35-R5C/5/Superb
Samples grown in Manitoba			
Glenn	378.8 $\pm$ 19.5		ND 2831/Steele-ND
Carberry	679.4 $\pm$ 24.3		Alsen/Superb
Harvest	965.0 $\pm$ 46.4		AC Domain*2/ND640

AAC Brandon	691.8 ± 11.6	Superb/CDC Osler//ND744
Superb	416.9 ± 25.5	Grandin*2/AC Domain
AAC Penhold	883.4 ± 67.6	5700PR / HY644-BE // HY469
Prosper	725.3 ± 59.4	ND2857/Dapps

Table 3. Summary statistics for analysis of variance of asparagine content in Canadian Hard Red Spring Wheat Cultivars Grown in locations across Canadian prairie provinces

Source	DF	Sum of Squares	Mean Square	Prob > F
Wholemeal				
Cultivar	29	669479.02	23085.5	<.0001*
Error	52	46542.23	895.0	
C. Total	81	716021.25		
White flour				
Cultivar	29	182959.22	6308.94	<.0001*
Error	51	8433.37	165.36	
C. Total	80	191392.59		

Table 4. Correlation between asparagine content and quality parameters in Canadian Hard Red Spring Wheat cultivars grown in locations across Canadian prairie provinces

	Wholemeal Asparagine		Flour Asparagine	
	r	p-value	r	p-value
Bran	0.020	0.9074	-0.1978	0.2948
Flour asparagine content	0.802	<.0001	1.000	<.0001
Wholemeal asparagine content	1.000	<.0001	0.802	<.0001
Ash	-0.168	0.3743	-0.107	0.5736
Wet Gluten	0.062	0.7458	-0.027	0.8895
Gluten index	-0.360	0.0498	-0.239	0.2036
Protein	-0.081	0.6588	-0.080	0.6735
Flour protein	-0.140	0.4624	-0.066	0.7300
Flour Wet gluten	-0.034	0.8578	-0.065	0.7334
Flour Ash	-0.111	0.5604	0.187	0.3234
Flour Starch Damage	-0.183	0.3343	0.029	0.8776
Amylograph	-0.171	0.3678	-0.198	0.2936
Flour yield	0.021	0.9139	0.213	0.2585
Test weight	-0.071	0.7113	-0.201	0.2863
Weight per 1000 kernels	-0.269	0.1509	-0.094	0.6197
Falling number	-0.173	0.3611	-0.151	0.4256
Farinograph				
Absorption,	-0.115	0.5436	-0.051	0.7896
Dough development time	-0.207	0.2718	-0.225	0.2310
Stability	-0.190	0.3142	-0.289	0.1216
Mixing tolerance index	0.161	0.3950	0.304	0.1030
Extensograph (45 min)				
Rmax	-0.305	0.1012	-0.370	0.0444
R5	-0.187	0.3236	-0.270	0.1496
E	-0.332	0.0731	-0.142	0.4548
A	-0.389	0.0339	-0.378	0.0394
Extensograph (135 min)				
Rmax,	-0.279	0.1361	-0.361	0.0501
R5,	-0.262	0.1605	-0.376	0.0404
E	-0.285	0.1276	-0.077	0.6868
A	-0.320	0.0845	-0.310	0.0955
Rmax/E	-0.202	0.2853	-0.329	0.0761
Test Baking (Lean no time method)				
Baking absorption	-0.115	0.5436	-0.051	0.7896
Mixing time	-0.112	0.5553	-0.147	0.4391
Power	-0.475	0.0080	-0.459	0.0107
Watt*hours/kg (dough)	-0.152	0.4235	-0.150	0.4284
Loaf volume	-0.131	0.4888	-0.270	0.1497
Specific volume,	-0.134	0.4800	-0.267	0.1532
Loaf top ratio	-0.106	0.5774	-0.212	0.2611
Water Dough Colour				
3 h - L*	-0.002	0.9930	-0.026	0.8923
a*	-0.155	0.4124	0.084	0.6591
b*	0.292	0.1170	0.289	0.1216
24 h - L*	0.338	0.0678	0.196	0.2992

a*	-0.262	0.1620	-0.065	0.7351
b*	0.359	0.0516	0.373	0.0426

Note: Values represent correlation coefficient (r) and probability (p-value) and bold values indicate significant correlation at $p < 0.05$

