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Impact of Breeding on Free Amino Acids of Wholegrain Flour in Wheat and Role of Phenology Genes

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Abstract

Wheat (*Triticum aestivum* L.) is pivotal to global food security, with its energy-rich grains that are also the major vegetable protein source in human diets. Decades of primary emphasis on grain yield improvement have delivered increased gains worldwide, but the grain protein content has declined. Since amino acids are biosynthetic precursors of proteins, this research hypothesized that their contents in the wholegrain flour have also been impacted by past wheat breeding. To test this, the free amino acid content of wholegrain flour in 92 wheat cultivars released in a 20-year period were analyzed by regression against the year of release. The slope of the regression showed positive increases per year in 16 of the 19 individual amino acid considered. Among these, the increases in lysine, aspartic acid and arginine were statistically significant ($P < 0.05$). The level of lysine in wholegrain flour increased by 0.30 mg kg^{-1} yr^{-1} ($R^2 = 0.24$) over the 20-year period of breeding. Similarly, the content of methionine increased by 0.2 mg kg^{-1} yr^{-1} ($P = 0.07$; $R^2 = 0.16$), but free asparagine also increased at the rate of 6.51 mg kg^{-1} yr^{-1} ($P = 0.11$; $R^2 = 0.13$). The study sought further to explore the impact of selection for key developmental genes (*Vrn*, *Ppd*, and *Rht*) that have been targeted for artificial selection since 1840. Wheat cultivars carrying the semi-dwarfing gene, *Rht-B1b*, showed 15% lower content of lysine (the most limiting essential amino acid) and 25% lower

content of free asparagine (precursor of the neurotoxin, acrylamide) in the wholegrain compared to the tall cultivars. At the *Vrn-A1* gene, the winter-type cultivars carrying *Vrn-A1v* allele were 28% lower in free asparagine, and 6% lower in lysine content than their spring-type (*Vrn-A1a*) counterparts. In conclusion, the results indicated that, contrary to the declining trend in grain protein content, free amino acids in wheat wholegrain flour have been increased by past breeding. Selections for semi-dwarfism and vernalization response have had significant impacts on free amino acid content, implying that genetic manipulation of *Rht-B1b* and *Vrn-A1* genes could present a pathway to reducing the acrylamide-forming precursor in wheat wholegrain flour.

Keywords
Wheat; wholegrain flour; free amino acid; phenology genes; genetic breeding

1. Introduction

Wheat (*Triticum aestivum* L.) grain is a major source of energy for a large proportion of the world's population and is also a significant source of other important nutrients including proteins. The grain protein contains variables amounts of free amino acids, some of which are essential for human diet and health. The nutritional quality is determined by the proportion of ten amino acids, which cannot be synthesised, and hence must be provided in the diet. These include lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine, and methionine [1], and amongst these, lysine, isoleucine, and threonine are the most limiting. These are required for growth and maintenance, and if any one of them is limiting, the others will be broken down and excreted resulting in poor growth of livestock and humans [2].

Free amino acids are also linked to several economically important characteristics that contribute to the crop's wide versatility. Some of them, such as serine, asparagine, methionine, and lysine are associated with drought tolerance [3]. Others serve as important substrates for dough micro-organisms [4, 5] and react with sugars to contribute sensory properties such as aroma of bread, flavour, colour, and texture [6]. Arginine, histidine, and leucine produce a characteristic bread flavour, while proline leads to a cracker flavour [7]. However, some, such as asparagine, contribute towards formation of the potentially toxic compound, acrylamide [8-11]. Asparagine is a precursor of acrylamide, an extremely hazardous compound formed in foods via the Maillard reaction during thermal processing. It is classified in group 2A as a probable human carcinogen by the International Agency for Research on Cancer [12], but because the toxicology is not well understood, it is difficult for regulatory agencies to set appropriate limits for intake [11] as the body of evidence is still cloudy [13]. Although no legal legislation has yet been defined on the level of acrylamide in foodstuff [14], there is international agreement that more should be done to reduce the public's exposure to dietary acrylamide, and maximum levels at which a food product cannot be marketed are currently under consideration and may come into force in 2023 [13].

Bread wheat has a high acrylamide risk, based on measured levels in wheat grain-based products [15, 16], and how frequently these foods are consumed [17]. The dietary intake from untoasted bread is relatively low, about 2 μg day^{-1}. However, acrylamide exposure from bread increases

several folds for people eating toasted bread, which was found to contain 27-205 μg^{-kg} acrylamide [18]. Tolerable daily intake is estimated to be 40 μg kg_{bw}^{-1} day^{-1} for neurotoxicity, and for cancer, the estimate is between 2.6 and 16 μg kg_{bw}^{-1} day^{-1} [19]. Additionally, tobacco smoke is a significant source of acrylamide [20], for people who smoke, acrylamide intake can be elevated significantly by 1-2 μg per cigarette [21].

Considerable efforts are now being focused on manipulating the amino acid composition and balance in wheat as an important component of using the grains as medicine. While decades of primary emphasis on grain yield improvement have delivered increased gains worldwide [22-24], the trends are mixed for quality traits [22, 25]. Grain protein content has declined [23, 26, 27], probably due to the strong trade-off with grain yield, but positive genetic gains were found for dough strength related parameters (mixograph mixing time, torque, alveograph) and the overall quality of bread has increased [27, 28]. The wholegrain free amino acid content is not normally targeted in breeding programs, but despite this, Anjum et al. [29] reported that newly released wheat cultivars are nutritionally more superior than the old wheat cultivars, especially in the percentages of essential amino acids, particularly lysine. There is a long-standing effort to increase the lysine content in cereals, but the genes associated with the trait have detrimental pleiotropic effects on yield, which has proved difficult to separate by conventional selection in breeding programs [1]. Because the free amino acids tend to increase linearly as a function of grain nitrogen content [30], we hypothesized that past wheat breeding has consistently reduced the level of free amino acids in modern wheat, which in the case of free asparagine, would be a desirable trend.

Genetic gains in grain yield have relied largely on selection for three major gene complexes that regulate vernalization requirement (growth habit) (*Vrn* genes), photoperiod response (*Ppd* genes) and plant height (*Rht* genes). The *Vrn-A1* gene (formerly referred to as the *Vrn1* gene) exerts the greatest influence on growth habit and is known to promote the expression of other genes that play central roles in abiotic stress tolerance [31-33]. The *Ppd-D1* (formerly *Ppd1*) exerts the strongest influence on photoperiod sensitivity and is associated with other agronomic traits including spike architecture and multiple reproductive traits, such as anther length [34]. These genes account for 53% of the genetic variance for days to heading in southern Australia [35] and are among the many that have been targets for artificial selection since 1840 [36]. The objectives in the present research were to (1) assess impact of breeding on free amino acids by analyzing wheat cultivars bred in a period between 1960 and 2008 and (2) explore any functional link with major adaptation genes (*Vrn*, *Ppd*, and *Rht*), which will be crucial in targeting cultivars to future environments.

2. Materials & Methods

2.1 Experimental Conditions

The ninety-two (92) wheat cultivars used in this study are listed in Table S1 and were previously described in Emebiri [37]. Some of the cultivars were selected for their historical significance, such as the Australian hard wheat cultivar, Halberd, which was popular in the 70's but phased out in favour of higher yielding varieties. Others represent cultivars that are still currently grown, and parents used in breeding programmes by various companies in Australia. The cultivars were grown in a sand culture environment in the glasshouse at the Wagga Wagga Agricultural Institute, Wagga Wagga, Australia (latitude 35.05° S, longitude 147.35° E) to allow for stringent control over plant nutrition, irrigation, weed and disease controls. The experimental units (pots) were arranged in a

randomised complete block design with five replications. Before sowing the seeds, two treatments were applied to generate environmental variability: half of the pots for each variety were watered with a solution containing adequate amounts of phosphorus and calcium ($Ca(H_2PO_4)_2{\cdot}H_2O$), magnesium ($MgSO_4{\cdot}7H_2O$), potassium (KCl), copper ($CuSO_4{\cdot}2H_2O$), Zinc ($ZnSO_4{\cdot}7H_2O$), sodium and molybdenum ($Na_2MoO_4{\cdot}2H_2O$) and boron (H_3BO_3). Thereafter, these pots received a standard application of "Miracle-Gro", a commercial all-purpose plant food every fortnight, until physiological maturity. The other half of the pots were watered with the same medium described above but with dolomite ($CaMg(CO_3)_2$) replacing the sources of calcium and magnesium. Thereafter, these pots were fed with a solution containing ammonium nitrate (NH_4NO_3) every fortnight, until physiological maturity. Throughout the growing period, all plants were kept under natural solar radiation, air temperature (27 ± 3°C) and relative humidity (70-80%).

2.2 Trait Measurement

Grains were milled using a coffee grinder, and approximately 500 mg of flour was accurately weighed, and free amino acids extracted with 5 ml 10 mM HCl for 30 min with gentle mixing. Two replicate samples were centrifuged and 20 µl aliquots analyzed at the Australian Proteome Analysis Facility Ltd (Macquarie University, Sydney, Australia). All amino acids were derivatised using the Waters AccQTag Ultra chemistry and analysed by Waters Acquity UPLC. The amino acid content in wholegrain flour of each genotype was quantified in milligram per unit dry matter (mg kg^{-1}).

2.3 Statistical Analysis

The cultivars released in the years between 1960 and 1987 were excluded to achieve balance in consecutiveness and to estimate changes on yearly basis [28]. Trends in genetic changes were quantified as the slope (b value) [38] of the best fitting curve between each amino acid as the dependent variable and the year of release of the cultivar as the independent variable. The equation $y = \alpha + \beta(\text{Year}) + \varepsilon$ was used to estimate absolute genetic gain per year, where y is the average value of cultivars in the year of release, intercept was estimated by α, β is the regression coefficient, and ε is the residual error.

2.4 Functional Alleles at Major Phenology Genes

The wheat cultivars were classified for specific alleles at phenology genes (*Ppd-B1*, *Ppd-D1*, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Rht-B1* and *Rht-D1*) according to Eagles et al. [31, 39] and Martin et al. [40]. The allele designation (Table 1) was cross-checked against the more recent reports of Harris et al. [41] and Bloomfield et al. [42] and analyzed for allelic differences using analysis of variance tests. The functional alleles were uneven in the cultivars (Table 1), and alleles with less than 10% frequency were replaced with missing values.

Table 1 Description of major phenology genes and alleles categorised in the panel of wheat cultivars used for the study.

Gene	Allele type*	No. of cultivars	Allele frequency (%)	Allele description
Rht-B1	Rht-B1a	36	48.00	Tall allele.
	Rht-B1b	39	52.00	Semi-dwarf allele.
Rht-D1	Rht-D1a	40	53.33	Tall allele.
	Rht-D1b	35	46.67	Semi-dwarf allele.
Ppd-B1	Ppd-B1a	18	40.91	Insensitive to photoperiod.
	Ppd-B1b	17	38.64	Responsive to photoperiod.
	Ppd-B1c	2	4.55	Insensitive to photoperiod.
	Ppd-B1d	7	15.91	Insensitive to photoperiod.
Ppd-D1	Ppd-D1a	42	65.62	Insensitive to photoperiod, promotes early flowering.
	Ppd-D1b	13	20.31	Responsive to photoperiod.
	Ppd-D1c	2	3.12	Insensitive to photoperiod, associated with late flowering.
	Ppd-D1d	6	9.38	Insensitive to photoperiod, associated with late flowering.
Vrn-A1	Vrn-A1a	30	48.88	Spring allele, unresponsive to vernalisation.
	Vrn-A1b	5	7.81	Spring allele, unresponsive to vernalisation.
	Vrn-A1v	29	45.31	Winter allele, responsive to vernalisation.
Vrn-B1	Vrn-B1a	37	58.73	Spring allele, unresponsive to vernalisation.
	Vrn-B1v	25	39.68	Winter allele, responds to vernalisation.
Vrn-D1	Vrn-D1a	20	32.26	Spring allele, unresponsive to vernalisation
	Vrn-D1v	42	67.74	Winter allele, responds to vernalisation.

*Adapted from Eagles et al. [31], Harris et al. [41].

Normality checking was performed on residuals, and as all traits failed the normality tests, analysis of variance (ANOVA) was performed using the R package, *lmPerm* [43], which calculates a *P* value based on a permutation procedure that is robust to non-homogeneity of variance. Differences between means were tested for significance using Tukey's Honestly Significant Difference (HSD) at $P < 0.05$.

3. Results

Genotypic values of the free amino acids are summarised in Figure 1, which showed that the grains were high in tryptophan but deficient in the other essential amino acids. The mean values (Table S2) showed that the most deficient were methionine, which accounted for 0.42% of the total, histidine (0.84% of the total), threonine (0.84%), and isoleucine (0.99%), all of which are essential for human health. Free asparagine, the precursor of acrylamide, was the most abundant in the wholegrain flour, accounting for 20.3% of the total.

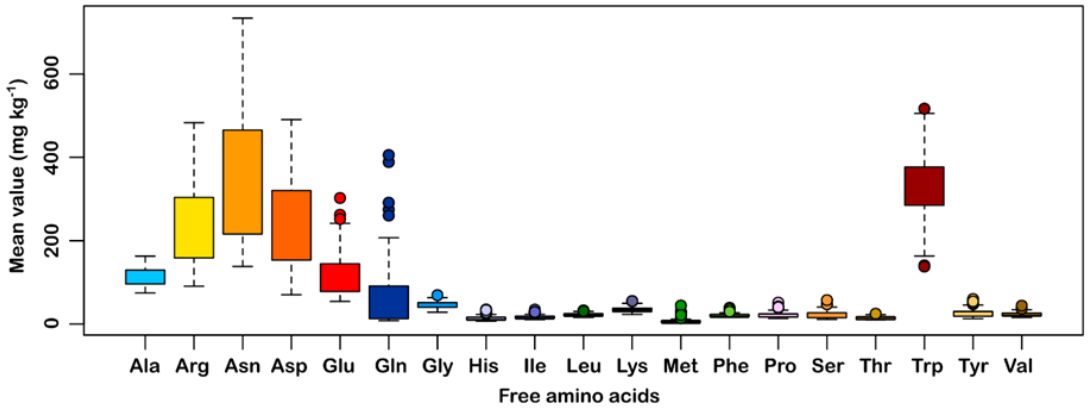

Figure 1 The content of free amino acids in the wholegrain flour of 92 wheat cultivars released between 1960 and 2008.

The slope of the regression showed positive increase per year for 16 of the 19 individual amino acid considered (Table 2). Of the 10 essential amino acids, slightly negative changes were observed in tryptophan and isoleucine, but these are not significant, and the r-squared values were close to zero (Table 2). Remarkably, the level of lysine in wholegrain flour of wheat increased significantly ($P = 0.02$) over the 2 decades of breeding (Figure 2). The regression analysis showed an increase of 0.30 mg kg^{-1} yr^{-1} ($R^2 = 0.24$) for lysine, and 0.2 mg kg^{-1} yr^{-1} for methionine ($P = 0.07$; $R^2 = 0.16$), two of the most limiting essential amino acids, but also an increase of 6.51 mg kg^{-1} yr^{-1} in asparagine ($P = 0.11$; $R^2 = 0.13$), the precursor of acrylamide formation in wheat products, and of 6.53 mg kg^{-1} yr^{-1} ($P = 0.02$; $R^2 = 0.24$) in aspartic acid, which is in the asparagine biosynthetic pathway (Table 2; Figure 2).

Table 2 Estimates of parameters of change in free amino acid content of wholegrain flour in wheat cultivars released during the period of 1988 and 2008 in Australia.

Free amino acid	b-value	SE	t-value	Pr(>|t|)	R-squared
Alanine	0.79	0.58	1.36	0.189	0.09

Arginine	4.52	1.79	2.52	**0.021**	0.25
Asparagine	6.51	3.93	1.66	0.114	0.13
Aspartic acid	6.53	2.64	2.47	**0.023**	0.24
Glutamic acid	2.17	1.09	1.99	0.061	0.17
Glutamine	2.51	2.05	1.23	0.235	0.07
Glycine	0.15	0.21	0.74	0.470	0.03
Histidine	0.00	0.14	-0.02	0.982	0.00
Isoleucine	-0.01	0.12	-0.09	0.931	0.00
Leucine	0.05	0.07	0.70	0.490	0.03
Lysine	0.30	0.12	2.45	**0.024**	0.24
Methionine	0.19	0.10	1.90	0.073	0.16
Phenylalanine	0.10	0.08	1.23	0.233	0.07
Proline	0.31	0.19	1.68	0.110	0.13
Serine	-0.02	0.22	-0.11	0.910	0.00
Threonine	0.08	0.09	0.86	0.402	0.04
Tryptophan	-0.43	1.91	-0.23	0.823	0.00
Tyrosine	0.28	0.18	1.55	0.138	0.11
Valine	0.15	0.13	1.16	0.260	0.07

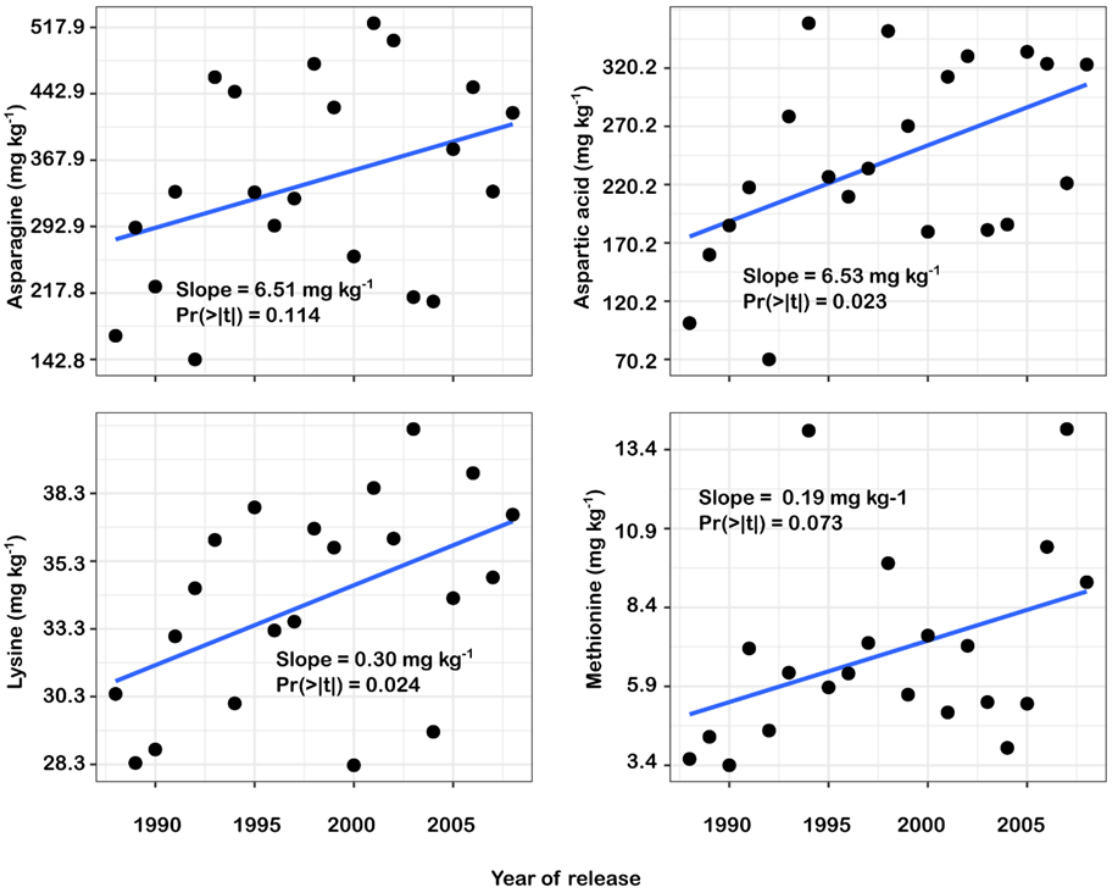

Figure 2 Genetic changes in free amino acids measured in wholegrain flour of Australian wheat cultivars released during a 20-year period of 1988 to 2008. Blue line represents the regression line against year of release.

3.1 Allelic Effect of Phenology Genes

Allelic variation at major phenology genes was assessed for its effect on free amino acid content of wholegrain flour. Four of the free amino acids (histidine, methionine, serine, and tyrosine) were unaffected by allelic variation at any of the phenology genes (Table 3). Of the two major semi-dwarfing gene, alleles at the *Rht-B1* gene locus showed multiple associations with both essential and non-essential amino acids, but the alleles at *Rht-D1* only influenced the non-essential amino acids, particularly asparagine and components of its biosynthetic pathway, aspartic acid, glutamic acid, and glutamine (Table 3). The *Rht-B1* gene alleles showed significant influence on five of the essential and eight non-essential amino acids. In all cases, the tall-height conferring alleles (*Rht-B1a* and *Rht-D1a*) were associated with increased contents, and the alternate dwarfing alleles (*Rht-B1b* and *Rht-D1b*) with the reduced contents of free amino acids (Figure 3).

Table 3 The P-values indicating statistical significance of allelic effect of major phenology genes on free amino acids measured in wholegrain flour of wheat cultivars used in the study.

Free amino acid	Rht-B1	Rht-D1	Ppd-B1	Ppd-D1	Vrn-A1	Vrn-B1	Vrn-D1
Alanine	**<0.001**	0.160	0.241	0.528	1.000	0.134	0.500
Arginine	**<0.001**	0.377	0.137	0.855	0.485	**0.021**	0.824
Asparagine	**0.020**	**0.033**	0.580	0.548	**<0.001**	0.473	0.139
Aspartic acid	**0.032**	**0.037**	0.836	0.138	**<0.001**	0.278	0.784
Glutamic acid	**0.014**	**0.031**	0.285	0.416	0.119	0.497	0.541
Glutamine	**0.023**	**0.033**	0.506	0.669	0.177	0.401	0.394
Glycine	**0.017**	0.667	0.802	0.527	**0.043**	0.355	0.667
Histidine	0.660	0.804	0.573	0.061	0.722	0.882	0.686
Isoleucine	0.271	0.221	0.673	0.381	0.644	0.718	**0.007**
Leucine	**0.038**	0.980	0.100	0.557	1.000	0.268	**0.006**
Lysine	**0.001**	0.941	**0.011**	1.000	0.299	**0.052**	**0.053**
Methionine	0.278	0.490	0.777	0.439	0.267	0.939	0.824
Phenylalanine	**0.004**	0.250	0.681	0.560	1.000	0.665	0.108
Proline	**<0.001**	**0.046**	0.422	0.621	0.238	0.282	0.765
Serine	0.564	0.745	0.694	0.723	0.143	0.576	0.863
Threonine	**0.019**	0.233	0.181	0.309	0.213	0.664	0.332
Tryptophan	0.686	0.643	0.253	0.284	0.140	**0.024**	**0.007**
Tyrosine	0.298	0.804	0.186	0.195	0.299	0.279	0.149
Valine	**0.004**	0.065	0.131	0.995	0.657	1.000	**0.001**

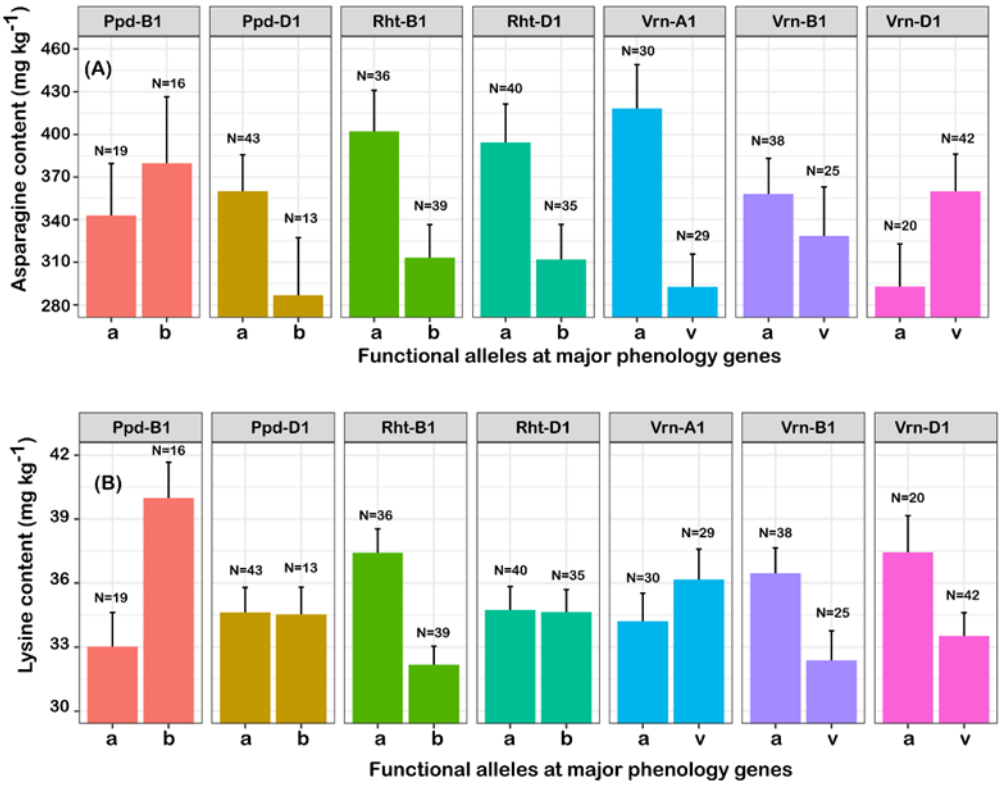

Figure 3 Allelic effect of major phenology genes on (A) free asparagine and (B) lysine content of wholegrain flour in wheat cultivars used in this study. The level of statistical significance is presented in Table 3, along with those for other amino acids measured in wholegrain flour of wheat. N = number of cultivars carrying the respective alleles.

Amongst flowering time genes, the photoperiod response genes (*Ppd-B1* and *Ppd-D1*) did not show any association with the free amino acids, except with lysine content (Table 3). The essential and non-essential amino acids were differentially affected by vernalisation genes, with *Vrn-A1* and *Vrn-B1* mainly affecting the non-essential amino acids, while the *Vrn-D1* affected the essential amino acids. Of special interest was the highly significant association of *Vrn-A1* with asparagine, such that cultivars with spring-type allele at the locus were 50% higher in asparagine content than the counterparts carrying winter-type alleles (Figure 3).

4. Discussion

4.1 Selection for Height Genes has Affected Wholegrain Free Amino Acids

Free amino acids in the wheat wholegrain flour are rarely targeted for selection in traditional breeding programs [44] and it was rather surprising, therefore, to find positive increases in most of the individual free amino acids of wheat cultivars released over the 20-year period, including the content of free asparagine. A similar positive trend was reported by Poudel et al. [26], but Rapp et al. [45] found no change in asparagine content with year of release. In contrast, Corol et al. [46] observed an inverse relationship between asparagine content and year of cultivar registration, which the authors attributed to breeders selecting for reduced heights. Breeders have been selecting for reduced plant heights since semi-dwarfing genes were introduced into commercial

wheat cultivars in the 1960s, and the findings of Corol et al. [46] suggests that this may have also resulted in reduced total grain asparagine content. These changes may be due to unconscious selection, as free amino acids are rarely targeted in breeding programs.

To explore the functional link between adaptation and free amino acids deposited in the grains, molecular analyses of the cultivars was undertaken with gene-based markers linked to phenology genes. The results showed a highly significant impact of alleles at the *reduced height* (*Rht*) and vernalisation (*Vrn*) genes, which have been among the many that have been targets for artificial selection since the green revolution was initiated in the 1960s. Alleles at the *Rht-B1* and *Rht-D1* loci showed significant ($P < 0.05$) statistical associations with multiple free amino acids including lysine (the most limiting essential amino acid) and of free asparagine, non-essential but important as precursor to the processing contaminant acrylamide. The association of *Rht* genes with free amino acids was reported by Oddy et al. [47], who identified a locus near *Rht-B1* that overlapped with quantitative trait loci (QTL) for asparagine, glutamine, glutamic acid, and glycine, and another locus near *Rht-D1* that controls aspartic acid. In the current study, alleles that confer tall heights (*Rht-B1a* and *Rht-D1a*) were associated with increased contents, and the alternate dwarfing alleles (*Rht-B1b* and *Rht-D1b*) with the reduced contents of free amino acids (Figure 3). The implies that tall wheat cultivars would be prone to accumulate higher amounts of asparagine in the grains than the semi-dwarf types. This supports the findings of Corol et al. [46] who reported higher asparagine in taller wheat plants but contrasts with Oddy et al. [47], where the mutated allele at *Rht-B1* (which confers reduced height) was associated with increased asparagine content. The *Rht* genes encode mutant DELLA proteins that are negative regulators of several gibberellic acid responses (see review by [48]). Because gibberellic acids are involved in many developmental processes, *Rht-B1b* and *Rht-D1b* have a range of effects on the plant in addition to reducing plant height [49], and the impact on free asparagine suggests a pathway to reducing the precursor of the food contaminant. However, many side-effects associated with the genes are undesirable, and as new gibberellic-acid sensitive alternative dwarfing genes (eg. *Rht18*) are now available, a detailed analysis is required to comprehensively assess the impact of *reduced height* genes on free asparagine, given that the choice of cultivars is currently the only validated crop management strategy to reduce the acrylamide risk.

4.2 Effect of Vernalisation Genes

The wide adaptability of wheat is governed by the genes that control vernalization response (*Vrn*), photoperiod sensitivity (*Ppd*) and the genes controlling earliness *per se* (*Eps*) [50]. In wheat cultivars released over the 20-year period of wheat breeding, there was no significant effect of the photoperiod response genes, *Ppd-B1* and *Ppd-D1*, on wholegrain free amino acid contents (Table 3). In contrast, there was a highly significant effect of the vernalisation response gene, *Vrn-A1*, on asparagine content (Table 3; Figure 3). The *Vrn-A1* gene, located on chromosome 5AL, encodes a MAD-Box transcription factor [51], and allelic variations at the gene locus have been shown to affect other plant traits including freezing tolerance [32] and grain yield [52]. It is tightly linked to *TaNUE1*, a gene known to influence nitrogen use in wheat [53]. Nitrogen availability is a major factor in free asparagine accumulation [54, 55] and nitrogen treatments for crude protein contents in flours above 13% have been shown to cause an increase of 130% to 270% in free asparagine in winter wheat, depending on year and cultivar [55, 56]. The major vernalisation genes, *Vrn-A1* and *Vrn-D1*,

have been found to co-localise with chromosomal regions in wheat that control nitrogen-use efficiency [57], and thus, it can be argued that improvements in nitrogen-use efficiency would affect the content of free asparagine in wholegrain flour.

5. Summary and Conclusions

The content of free amino acids in wholegrain flour of wheat has increased due to breeding, despite not being a target for selection. The rate of increase was positive for majority of the free amino acids considered (Table 2), suggesting a correlated response to selection during the 20-year period of cultivar release. A functional link to phenology genes was established, particularly involving genes that control plant height and vernalisation response. Wheat cultivars carrying the semi-dwarfing gene, *Rht-B1b*, showed significantly reduced content of free asparagine in the grain than those carrying the tall-height conferring allele. Similarly, cultivars carrying the winter allele at the vernalization response gene, *Vrn-A1*, were significantly lower in free asparagine than their alternate counterparts. This suggests that genetic manipulation of these genes could present a pathway to reduce acrylamide-forming potential in heat-processed wheat products.

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Author Contributions

The author did all the research work of this study.

Competing Interests

The author has declared that no competing interests exist.

Additional Materials

1. Table S1: Names of cultivars used in the experiment, including date of release as a commercial variety and pedigree.
2. Table S2: Mean content of free amino acids in mg/kg of grains in 92 wheat cultivars released for commercial cultivation between 1960 and 2008.

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