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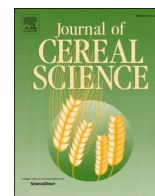
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Relationship between specific weight of spring barley and malt quality

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ABSTRACT

The assessment of malting barley to determine if it meets grain quality requirements is an integral step in ensuring an efficient malting process and a good quality malt output. Specific weight (SW) is an industry standard criterion, however links between SW and malting are not well understood. In this study the effect of a changing SW on malting was investigated. Samples were manipulated according to both grain size and weight, creating grain fractions with a range in SW. Prior to malting, grain quality traits were measured, and after malting, malt quality traits were examined. Increased SW resulted in a reduced number of whole, unmodified corns in malt, implying increased levels of modification. Specific weight correlated with both hot water malt extract ($r = 0.82$, $P < 0.01$) and predicted spirit yield ($r = 0.84$, $P < 0.01$), this highlights an increased malt output. Furthermore peak gelatinisation temperature of extracted starch from the malt correlated with both SW ($r = 0.69$, $P < 0.05$) and grain density ($r = 0.65$, $P < 0.05$). This could benefit malt efficiency by increased conversion of starch to fermentable sugars, but with the same energy input. The changes in SW and consequently malt output in this study are a result of changing grain density rather than packing efficiency.

1. Introduction

Barley (*Hordeum vulgare* L.) is an ancient cereal crop; it was domesticated in the fertile crescent 10,000 years ago and has remained an important crop ever since. In 2018 the global harvest of barley was 141 Mt, placing it fourth in terms of crop production worldwide (FAOSTAT, 2020). The primary use for barley is as a livestock feed which accounts for roughly two thirds of its usage, one third is used for malting and 2% is used directly for human consumption (Baik and Ullrich, 2008). However in Scotland, barley is the main cereal crop grown, accounting for 68% of the total area of cereal grown in 2019, of this, 80% is planted with spring barley (The Scottish Government, 2019).

Barley is the preferred cereal crop for the malting industry. Its physical, physiological and biochemical characteristics are well suited to malting and downstream processes such as brewing and whisky distilling. The barley-malt-whisky supply chain forms an important part of the Scottish economy (Gupta et al., 2010). The key difference between barley destined for malt or feed are the quality requirements which the grain has to meet to be accepted for malting. Quality requirements include germination rate, protein content, moisture content, grain uniformity, specific weight (SW), quantity of screenings and levels of

damaged grains (caused by disease, mechanical damage or weathering) (Brewing and Malting Barley Research Institute (BMBRI), 2010). These requirements are decided upon in contracts between growers and maltsters, if met a premium is paid for the grain. It is understood that these traits are directly related to the processing efficiency and/or malt output (hot water extract and predicted spirit yield). Despite SW being used as a grain quality trait in malting for many years, direct links between this trait and malt quality are not well understood. However, SW remains a breeding target, which is routinely measured and listed alongside screenings and nitrogen content in the Agriculture and Horticulture Development Board's (AHDB) Recommended List (RL), as one of the few grain quality traits for spring barley.

Specific weight is the mass of grain per unit volume and is measured in kilograms per hectolitre (kg hl^{-1}). It is also referred to as test weight, bushel weight and hectolitre mass in the literature. Specific weight is quick and easy to measure during the intake of barley at a maltings, in comparison to other measures of grain quality, such as starch content. Specific weight is measured using either a chondrometer or devices calibrated against this instrument (Manley et al., 2009). Previous work to enhance the understanding of SW has demonstrated that this trait is a product of barley grain density (GD) and its packing efficiency (PE)

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(Hoyle et al., 2018). These two components of SW can change independently, therefore both need to be considered jointly in future studies on SW. Grain density is thought to be determined by grain composition and the internal architecture of the grain. A previous study showed that when a grain sample is stratified by ascending GD, this is associated with an increase in grain nitrogen (Hoyle et al., 2019). However, when different cultivars without this stratification process are compared, the relationship between GD and grain nitrogen is not maintained. This demonstrates that even though within a cultivar this relationship is maintained, it is not maintained across different cultivars (Hoyle et al., 2019). The PE of grains on the other hand is thought to be determined by a combination of the following parameters: grain dimensions, ratios of these dimensions, grain shape, uniformity of these within a sample and also surface textures (Hoyle et al., 2018).

Malting is the controlled germination of cereal grain which takes place in three stages i) steeping, ii) germination and iii) kilning. During germination the starchy endosperm undergoes modification, a key step in achieving good malt quality. Modification of the endosperm is a result of the activity of enzymes primarily produced within the aleurone layer (Palmer, 2017). Modification involves the breakdown of both cell walls by hydrolytic enzymes, and hordein proteins by proteolytic enzymes, into soluble peptides and associated amino acids (Baxter, 1981; Palmer, 1993). Modification is an essential part of malting, which makes starch stored within endosperm cells available for later gelatinisation during mashing, and also releasing nutrients which are metabolised by yeast. It is the result of grain quality traits which determine to what extent this modification occurs and therefore how successfully this malt is processed downstream. Diastase enzymes (α -amylase and β -amylase) are also produced during endosperm modification, these are later utilised in mashing to convert the dissolved starch into maltose and glucose. The power of these enzymes to breakdown starch is referred to as the diastatic power (DP), a malt quality parameter. However, how SW influences the malting process as whole or individual parts of it is unknown.

A range of additional quality assessments are carried out on malted grain. Hot water extract (HWE) is an important malt quality parameter which measures the amount of dissolved solids within the wort, the sugary liquid created by the mashing of ground malt (grist) and hot water (Briggs, 1998). These dissolved solids are primarily fermentable sugars but also consist of nitrogenous compounds and polyphenols. Mashing is typically carried out at 65 °C, which is just higher than the typical gelatinisation temperature of barley starch 62 °C (Macgregor et al., 2002). This is an integral step in both beer and whisky production, which gelatinises complex starch into simpler fermentable sugars, which can then be utilised by yeast. The temperature at which barley starch begins to gelatinise, the peak of its gelatinisation and also the conclusion of gelatinisation, are influenced by both barley genotype and environmental growth conditions (Tester, 1997). Therefore these gelatinisation properties of starch show seasonal variation and are also be considered malt quality parameters. The SW of barley adjuncts (additional unmalted grains) have previously been reported to show a positive correlation with HWE, but this has not been demonstrated for the SW of the malting barley itself (Agu, 2008). Specific weight is thought to be associated with a higher starch content. Therefore this positive relationship is predicted to be maintained with malting barley SW because of an increased amount of starch contributing to more dissolved sugars in the wort, and hence a higher HWE. Predicted spirit yield (PSY) is another malt quality parameter and is calculated using the fermentable extract, but does not include unfermentable dissolved solids, such as complex sugars. Therefore PSY is influenced by the total quantity of dissolved solids but also the levels of gelatinisation of the starch. The interaction between SW and PSY has not been established, but a positive relationship is proposed because of increased mass per unit volume than high SW confers. To understand links between SW and malting output or efficiency, the influence of GD and PE have to be considered, as grain composition and grain packing within the bulk both influence malting.

The primary focus of this work is to study the effects of SW and its components GD and PE on the malting process. This will be addressed through the following aims: (1) alter SW and its components through the manipulation of grain size and grain weight, (2) determine the malting quality of grain samples with different SWs and/or components and (3) examine correlations between grain parameters and malt quality parameters to establish links between SW and malt quality. This work should help provide improved information about how SW can be used in the grading of malting barley and the impact it has on the malting process.

2. Materials and methods

2.1. Plant material and sample preparation

Commercial spring barley (*Hordeum vulgare* L.) samples were obtained from Bairds Malt (Witham, UK); 20 kg of the cultivar Concerto and 5 kg of the cultivar Sienna. The samples were harvested from across Scotland in the 2018 season. Samples were cleaned over a 2.25 mm slotted sieve with 19.05 mm long slots to remove screenings. Sienna was used as received with no further selection for different grain sizes. Concerto was used both as received, and also after sorting based on both size and weight as described in the following sentences, in order to create fractions of grain with different SWs. Firstly, 1.5 kg of Concerto was removed for the “as received” fraction to maintain its natural grain size distribution. The remaining 18.5 kg of Concerto grain was sequentially sieved over 2.25, 2.50, 2.75, 3.00 and 3.25 mm wide slotted sieves with 19.05 mm long slots in order to sort the grain based on size. Grains retained by these sieves were labelled as size fractions A, B, C, D and E respectively. Additional fractions were then created by separating fractions B and D into two; first the mean grain weight of fractions B and D were measured, then grains were sorted individually (weighed grain by grain) based on whether their weight was above or below the mean weight of the corresponding fraction. This extra separation was performed to create fractions of similarly sized grains but different weights. The mean grain weight was calculated from three separate 100-grain subsamples from fractions B and D (Mettler AE 160 electronic balance, Mettler-Toledo, accuracy \pm 0.0001 g), giving mean individual grain weights of 35.50 and 49.99 mg for fractions B and D, respectively. Fraction B1 contained grains weighing less than 35.50 mg, and fraction B2 contained grains weighing more than that weight. Fraction D1 contained grains that weighed less than 49.99 mg, and fraction D2 contained grains that weighed more than that weight. This resulted in the production of the 10 fractions listed in Table 1.

2.2. Grain analyses

Specific weight of each fraction was measured using a scaled down method in a 25 ml measuring cylinder which was previously shown to be representative of the industry standard (Hoyle et al., 2018). Two 100-grain samples were removed from each sample. One of these samples was milled into a fine flour using a ball mill (Mixer Mill MM 200, Retsch, Germany). This flour was used to determine the proportion of carbon (C) and nitrogen (N) in the grain with a FLASH 2000 Organic Elemental Analyser (Thermo Scientific). Using the other 100-grain sample, grains were individually weighed on a Mettler AE 160 electronic balance. Grain volume was also measured on these 100-grain samples according Archimedes' principle using a previously described technique, and from this GD was calculated (Hoyle et al., 2019). Packing efficiency was then calculated using the same method as previously described (Hoyle et al., 2018).

2.3. Micromalting

Laboratory micromalting and malt analyses were performed using equipment at the Scotch Whisky Research Institute (SWRI, Roberston

Table 1
Descriptors of sample fractions for miromalting.

Cultivar	Fraction	Size (mm)	Weight selected by (mg)	Contribution to mix (%) ^a
Concerto	A	2.25 to 2.50		5.5
Concerto	B1	2.50 to 2.75	≤35.50	14.5
Concerto	B2	2.50 to 2.75	>35.50	
Concerto	C	2.75 to 3.00		26.4
Concerto	D1	3.00 to 3.25	≤49.99	35.5
Concerto	D	3.00 to 3.25		
Concerto	D2	3.00 to 3.25	>49.99	
Concerto	E	>3.25		9.1
Concerto	Mix	Mix		100
Sienna	Mix	Mix		100

^a % Contribution is by fraction weight to show the relative contribution of each fraction to the natural mix.

Trust Building, Research Avenue North, Riccarton). Five hundred grams of grain was used for each micromalting run, from each of the 10 fractions after SW and grain analyses undertaken. The micromalting was performed in three runs for each fraction of grains. Micromalting was carried out in a Curio Malting (Milton Keynes, UK) MMSG Steep and Germinator 4 tank system, each tank containing space for four grain samples. In each run the position of the different fractions of grain samples both within the tanks and across tanks was randomly allocated. The same micromalting regime (Agu, 2003) was used for all batches, which consisted of a first steep for 8 h at 17 °C, 16 h of air rest at 17 °C, a second steep for 24 h at 17 °C and finally 96 h of germination at 17 °C. Malt was then kilned in a MMK four unit kiln (Curio Malting) at 55 °C for 16 h, then 75 °C for 10 h. This was followed by deculming over a 2.2 mm sieve for 2 min. This created a total of 30 malt samples for malt analyses. Prior to analysis samples were stored in sealed bags to preserve their integrity.

2.4. Malt analyses

2.4.1. Moisture and nitrogen analysis

Malt samples were first analysed by NIR using an Infratec 1241 Grain Analyser instrument (Foss Analytix, UK). From this, malt moisture, total N and soluble N were determined using a barley malt specific calibration based on data from spectral libraries, pairing NIR and laboratory based techniques.

2.4.2. Friability and homogeneity

A subsample of malt (50 g) was loaded into a Friabilimeter (Pfeuffer, Germany) and the machine ran for 8 min. The material retained by the drum was weighed (accuracy ± 0.01 g) and friability (%) assessed (Baxter and O'Farrell, 1983). The non-friable fraction was then shaken over a 2.2 mm slotted sieve until no more material would pass through. Material retained by the sieve was weighed (accuracy ± 0.01 g) and homogeneity (%) calculated (Baxter and O'Farrell, 1983). Any remaining whole grains were then counted and weighed (accuracy ± 0.01 g) and recorded as the number of whole corns (Wc) and weight of whole corns.

2.4.3. Viscosity

The viscosity of samples was also measured using a Newport Scientific Rapid Visco Analyser (RVA). Malt was milled to 0.2 mm and then 0.1 mm to ensure a fine grind using a Bühler Miag disc mill. Approximately 9.3 g of this was adjusted for moisture in accordance with the manufacturer's instructions and was mixed with approximately 18.7 g of water and processed in the RVA, using a previously described malted barley specific 30 min program (Agu et al., 2007). Three variables from the RVA were analysed: i) peak temperature, which is the temperature at

which peak viscosity was reached for the sample, ii) pasting temperature, which is the temperature at which the viscosity starts to increase and iii) pasting time, the time to peak viscosity.

2.4.4. Hot water extract and predicted spirit yield

To determine HWE and PSY 50 g of malt was milled to 0.7 mm and then mashed for 1 h in 360 ml of water at 65 °C using the Mash Bath – R8 (1-CUBE, Czech Republic). Samples were gradually cooled over a 20 min period to 20 °C and held at this temperature for 10 min. Samples were then made up to 450 g with water and shaken for 4–5 min, followed by filtering using Ederol 12 folded filter paper (Rudebeck). The density of 50 ml of the filtered wort was measured using a Paar DMA 5000 density meter (Anton Paar Ltd, UK). A 200 ml volume of wort was then pitched with 1.00 g of distiller's yeast "M" type, supplied by Kerry Bio-Science (Menstrie, Clackmannanshire, UK), and the 44 h fermentation carried out in a water bath at 33 °C. This wash was then filtered using Whatman 2V folded filter papers and the density of the solution collected was measured with an Anton Paar 5000 density meter.

2.5. Statistical analysis

All data analysis was carried out in R software version 3.6.1 (R Core Team, 2019). Data were analysed by using analysis of variance (ANOVA) ($\alpha = 0.05$) using linear models to determine whether grain fraction had a significant effect on either grain parameters or malt quality parameters. Where a significant effect was indicated by the ANOVA, a post-hoc Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$) test was used to show which fractions differed from each other in the parameters measured. Pearson product-moment correlation coefficients were calculated between all variables measured in this study to produce a matrix using the 'corrplot' package (Wei and Simko, 2016). Principal component analysis (PCA) was used with mean values for Wc, SW, PSY, HWE and homogeneity. Plots of scores were created using the 'factoextra' package (Kassambara and Mundt, 2019) to investigate the relationship between grain fractions and grain characteristics and malt parameters.

3. Results

3.1. Grain parameters

Prior to malting, grain parameters including weight, volume, density, SW, C content, N content and C:N were measured on ten fractions across three micromalting repetitions. The mean values of each fraction, and significant differences among fractions for these parameters, are displayed in Table 2.

As expected, in fractions with increasing grain size, grain weight and grain volume increased from 29.06 mg to 26.66 mm³ in fraction A to 57.94 mg and 50.87 mm³ in fraction E. Significant differences were also observed between the two mixed fractions with Concerto Mix having a mean grain weight of 45.83 mg and volume of 40.73 mm³, compared to Sienna Mix having a mean grain weight of 53.65 mg and volume of 45.21 mm³. Grain density ranged from fraction A with 1.09 g cm⁻³ to fraction D2 and Sienna Mix both with densities of 1.17 g cm⁻³, however this difference was not significant. Through sequential sieving and creating these fractions SW was significantly affected (Fig. 1a). Fractions A and B1 were significantly lower than all other fractions, with SWs of 58.97 and 60.82 kg hl⁻¹, respectively. Fraction D2 had the highest SW with 66.98 kg hl⁻¹ which was significantly higher than Concerto Mix, fraction D1, C, B2, B1 and A. Both mixed fractions, Concerto and Sienna had the highest packing efficiencies of 59.12 and 59.30% respectively. These were significantly higher than fraction A with 54.91%. No significant differences were observed between fractions for C content or C:N. Nitrogen content was lowest in the Sienna Mix fraction with 1.23%, this was significantly lower than all other fractions excluding fraction B1.

Table 2
Mean values^a for grain parameters measured on the ten grain fractions^b used in this study.

Fraction	Weight (mg)	Volume (mm ³)	Density (g cm ⁻³)	Packing Efficiency (%)	Specific Weight (kg hl ⁻¹)	Carbon (%)	Nitrogen (%)	C:N
A	29.06 ± 0.76h	26.66 ± 0.18h	1.09 ± 0.02a	54.91 ± 0.42b	58.97 ± 0.96d	40.05 ± 0.14a	1.41 ± 0.03a	28.37 ± 0.82a
B1	32.54 ± 0.28g	30.63 ± 1.42g	1.10 ± 0.01a	57.52 ± 2.66 ab	60.82 ± 0.18d	39.86 ± 0.28a	1.32 ± 0.02 ab	30.19 ± 0.49a
B2	39.08 ± 0.62f	34.18 ± 0.44f	1.15 ± 0.03a	56.57 ± 1.16 ab	64.97 ± 0.68bc	39.66 ± 0.32a	1.33 ± 0.04a	29.88 ± 1.16a
C	43.04 ± 0.38e	37.72 ± 0.28e	1.11 ± 0.06a	56.91 ± 0.71 ab	64.73 ± 0.51c	39.94 ± 0.03a	1.35 ± 0.03a	29.68 ± 0.34a
D1	46.63 ± 0.22d	41.02 ± 0.92d	1.14 ± 0.02a	56.77 ± 1.20 ab	64.02 ± 0.79c	39.76 ± 0.22a	1.35 ± 0.05a	29.50 ± 0.79a
D	50.27 ± 0.22c	44.01 ± 0.65c	1.14 ± 0.02a	56.96 ± 0.89 ab	65.25 ± 0.75abc	39.51 ± 0.45a	1.34 ± 0.05a	29.41 ± 0.60a
D2	53.95 ± 0.09b	46.22 ± 0.07b	1.17 ± 0.00a	56.87 ± 0.60 ab	66.98 ± 0.32a	39.69 ± 0.39a	1.40 ± 0.10a	28.51 ± 1.27a
E	57.94 ± 0.54a	50.87 ± 0.63a	1.14 ± 0.01a	57.29 ± 1.09 ab	65.80 ± 0.33abc	39.52 ± 0.22a	1.35 ± 0.01a	29.32 ± 0.20a
Concerto Mix	45.83 ± 2.32de	40.73 ± 1.88c	1.15 ± 0.03a	59.12 ± 0.47a	64.02 ± 0.47c	39.85 ± 0.21a	1.34 ± 0.02a	29.77 ± 0.34a
Sienna Mix	53.65 ± 1.81b	45.21 ± 1.31b	1.17 ± 0.02a	59.30 ± 0.98a	66.83 ± 0.98 ab	39.32 ± 0.35a	1.23 ± 0.08b	32.02 ± 1.50a

^b Fractions which do not share a letter for each of the measured parameters are significantly different from one another ($P < 0.05$).

^a Mean values are expressed as mean ± standard deviation, calculated from three independent replicates.

3.2. Malt quality parameters

Malt quality parameters including PSY, HWE, friability, homogeneity and nitrogen were measured on ten fractions across three micro-malting repetitions. The mean values of each fraction, and significant differences among fractions for these parameters, are displayed in Table 3.

All measures of malt N content which included soluble N, total N and the soluble N ratio showed no significant differences between fractions. There were also no significant differences in friability between the fractions with an overall mean of 92.53% indicating all fractions

underwent successful modification. Results were similar with homogeneity, where no significant differences were found with an overall mean of 99.09%. The number of whole corns ranged from 4.7 in fraction E, the largest grain size fraction, to 18.7 in fraction A, the smallest grain size fraction (Fig. 1b). Fraction A was significantly higher than all D fractions, fraction E and the two remaining mixed fractions. Hot water extract was lowest in fraction B1 with 80.57% and highest in fraction E with 83.74% (Fig. 1c). No significant differences were observed between malt moisture contents. Predicted spirit yield showed interesting differences across the fractions created in this study (Fig. 1d), fraction A had the lowest PSY with 411 L of alcohol per tonne (LA tonne⁻¹) which

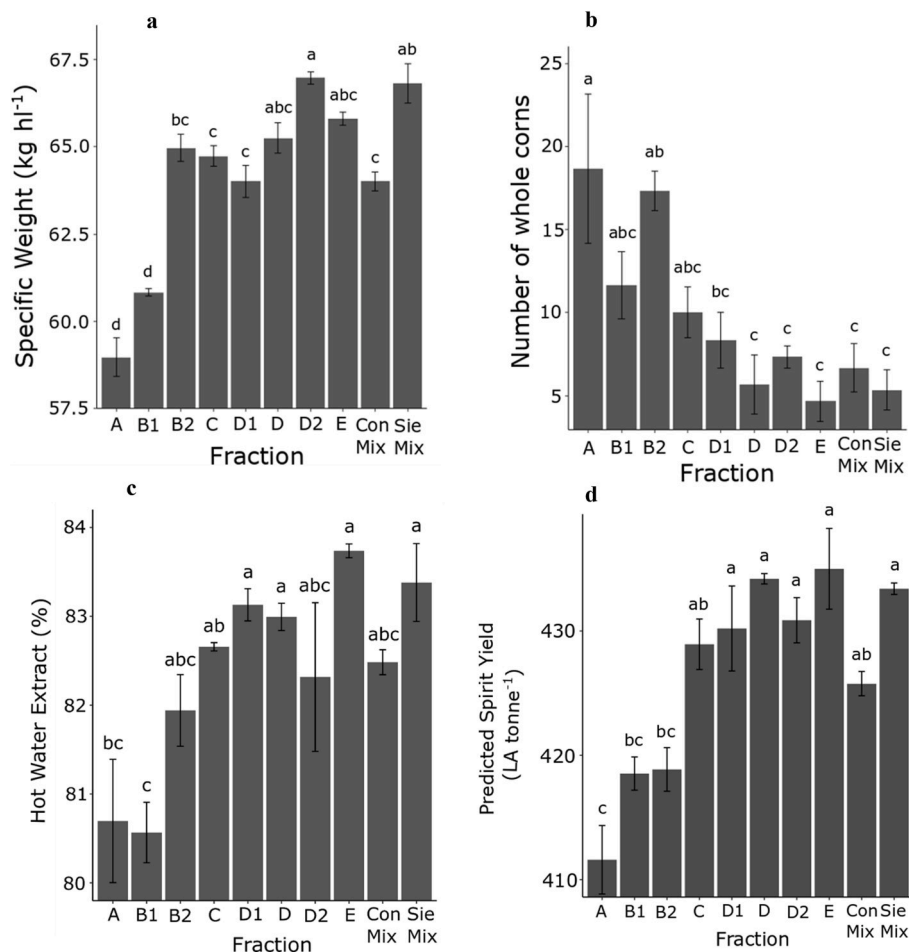


Fig. 1. Mean values of (a) whole corns, (b) specific weight, (c) hot water extract and (d) predicted spirit yield. Error bars represent ± standard error of the mean (n = 3). Grain fractions with different letters are significantly different at $P < 0.05$.

was significantly different from all other fractions apart from B1 and B2. Fraction E had the highest PSY with 435 LA tonne⁻¹. The rheological properties of starch in the ten fractions were investigated through RVA. Fraction A had the highest peak gelatinisation temperature with 61.17 °C and fraction D2 the lowest with 60.27 °C. The temperature for the onset of gelatinisation varied from 54.77 °C with fraction A, to 57.38 °C with fraction C.

3.3. Correlations between grain and malt quality parameters

Table 4 summarises the correlations between both grain and malt quality parameters which are displayed in a matrix of the Pearson correlation coefficients (r).

The friability of the malted samples negatively correlated with malt nitrogen (r = -0.65, P < 0.05) and positively with both predicted extract (r = 0.65, P < 0.05) and soluble nitrogen ratio (r = 0.64, P < 0.05). Friability also correlated with the key malt quality parameters PSY (r = 0.79, P < 0.01) and HWE (r = 0.64, P < 0.05). Malt homogeneity exhibited a strong positive correlation with predicted extract (r = 0.89, P < 0.001) but not HWE. Homogeneity did however show a strong positive correlation with PSY (r = 0.77, P < 0.01). Furthermore, the homogeneity of the fractions also correlated with the packing efficiency of the grain (r = 0.66, P < 0.05). The PSY of fractions strongly correlated with the SW of the sample (r = 0.84, P < 0.01) and also one of the components of SW, GD (r = 0.65, P < 0.05). However PSY did not correlate with the other component of SW, PE (r = 0.5, P > 0.05). Hot water extract showed much the same relationship as PSY with grain parameters positively correlating with SW (r = 0.82, P < 0.01) and GD (r = 0.67, P < 0.05). Starch rheological properties showed correlations with both malt quality parameters and grain parameters. Peak gelatinisation temperature negatively correlates with PSY (r = -0.65, P < 0.05), SW (r = -0.69, P < 0.05) and GD (r = -0.65, P < 0.05). Whereas, the temperature for the onset of gelatinisation shows a positive correlation with HWE (r = 0.76, P < 0.05).

In order to explore the relationships between parameters further, PCA was used to examine trends in multiple parameters together. Principal component (PC) 1 contributed 94.6% of the total variance, fractions with a high score in PC1 have an increased PSY and reduced Wc. PC2 contributed 4% of the total variance, fractions with a high score in PC2 have a high Wc, high SW, high HWE and low homogeneity. A PC biplot of PC1 and PC2 (Fig. 2) displays how grain fractions differ according to the aforementioned parameters. Fig. 2 separates the grain fractions of poorer malting quality from the clustered higher quality fractions. Fraction B2 is separated as a result of its high Wc resulting in a higher score in PC2. Fraction A and B1 are separated due to both a low SW and PSY resulting in negative scores for both PCs. Concerto mix is closest to the group of good malting quality fractions which is representative of its quality status, but is separated along PC2 as a result of a combination of lower SW and PSY.

4. Discussion

Specific weight of barley is an established measure of grain quality, used by maltsters to determine if the grain is of high enough quality for acceptance to malting and consequently have a premium paid for it (www.ukmalt.com). The effect of changing SW, GD or PE of spring barley on either the malting process or outputs has not been investigated prior to this. Specific weight or either of its components could influence this process, or the malt product across numerous stages. Grain composition has been shown to determine GD, therefore different SWs can arise as a result of varying composition. Composition is directly related malt quality (Fox, 2010). Starch complexes determine gelatinisation temperature and also the amount of sugars available for conversion into alcohol (Evers et al., 1999). High protein content has also been associated with a limited modification of the barley endosperm (Agu, 2003). Furthermore, the endosperm structure has the potential to

Table 3
Mean values^a for malt and starch quality parameters measured on the ten grain fractions^b used in this study.

Fraction	Malt		Soluble Nitrogen Ratio (%)	Friability (%)	Homogeneity (%)	Number of Whole Corns	Moisture (%)	Predicted Spirit Yield (LA/tonne)	Hot Water Extract (%)	Starch	
	Soluble Nitrogen (%)	Total Nitrogen (%)								Peak Gelatinisation Temperature (°C)	Onset of Gelatinisation Temperature (°C)
A	0.590 ± 0.02	1.40 ± 0.03	42.15 ± 1.25	89.17 ± 3.10	98.71 ± 0.61	18.7 ± 7.8a	5.93 ± 0.81	411.6 ± 4.77c	80.70 ± 1.20bc	61.17 ± 0.38	54.77 ± 0.29
B1	0.597 ± 0.02	1.32 ± 0.02	45.11 ± 2.25	92.43 ± 3.72	99.07 ± 0.41	11.7 ± 3.5abc	6.27 ± 0.65	418.5 ± 2.32bc	80.57 ± 0.59c	61.03 ± 0.63	54.82 ± 1.09
B2	0.600 ± 0.02	1.39 ± 0.02	43.16 ± 1.01	90.09 ± 2.62	98.43 ± 0.20	17.3 ± 2.1 ab	5.60 ± 1.04	418.9 ± 3.04bc	81.94 ± 0.70abc	61.02 ± 0.46	55.70 ± 2.26
C	0.587 ± 0.02	1.37 ± 0.04	42.82 ± 0.54	93.99 ± 2.95	99.07 ± 0.27	10.0 ± 2.6abc	5.67 ± 0.74	428.9 ± 3.52 ab	82.99 ± 0.27 ab	60.87 ± 0.45	56.70 ± 0.75
D1	0.600 ± 0.02	1.35 ± 0.05	44.34 ± 0.76	93.47 ± 3.41	99.17 ± 0.19	8.3 ± 2.9bc	6.07 ± 0.76	430.2 ± 5.92a	83.13 ± 0.31a	60.95 ± 0.35	57.38 ± 0.84
D	0.610 ± 0.02	1.34 ± 0.04	45.55 ± 2.01	94.89 ± 3.49	99.29 ± 0.45	5.7 ± 3.1c	5.87 ± 0.70	434.2 ± 0.72a	82.99 ± 0.27a	60.38 ± 0.03	56.47 ± 0.73
D2	0.597 ± 0.02	1.40 ± 0.04	42.66 ± 2.15	91.80 ± 2.67	99.16 ± 0.12	7.3 ± 1.2c	5.80 ± 0.75	430.9 ± 3.15a	82.32 ± 1.45abc	60.27 ± 0.08	55.40 ± 0.56
E	0.593 ± 0.01	1.40 ± 0.02	42.28 ± 0.46	92.30 ± 2.85	99.29 ± 0.17	4.7 ± 2.1c	5.70 ± 0.36	435.0 ± 5.63a	83.74 ± 0.13a	60.98 ± 1.09	56.43 ± 0.31
Concerto Mix	0.587 ± 0.02	1.33 ± 0.04	44.12 ± 1.01	92.47 ± 0.91	99.33 ± 0.29	6.7 ± 2.5c	5.67 ± 0.83	425.8 ± 1.69a	82.48 ± 0.24abc	60.97 ± 1.05	56.57 ± 0.42
Sienna Mix	0.587 ± 0.01	1.32 ± 0.07	44.63 ± 2.30	94.65 ± 0.79	99.35 ± 0.35	5.3 ± 2.1c	5.80 ± 0.87	433.4 ± 0.80a	83.38 ± 0.76a	60.58 ± 0.18	55.85 ± 0.22

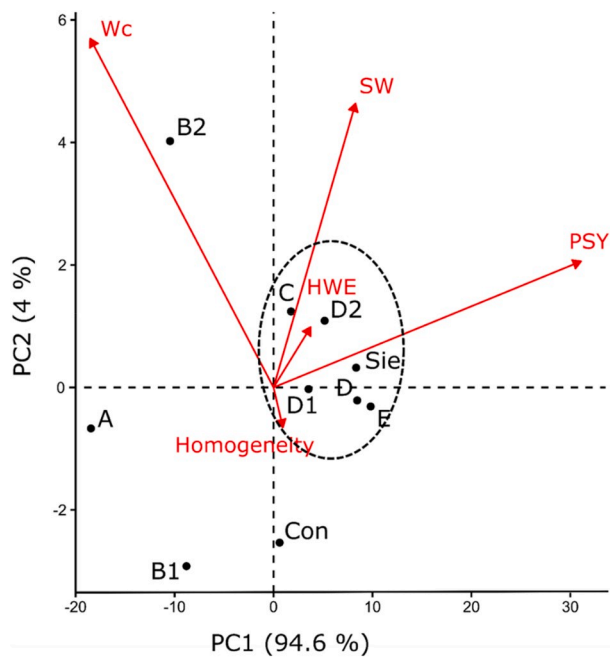
^a Mean values are expressed as mean ± standard deviation, calculated from three independent replicates.

^b Fractions which do not share a letter for each of the measured parameters are significantly different from one another.

Table 4
Correlation matrix of Pearson's correlation coefficients (r) for grain and malt parameters.

		Malt							Starch				Grain					
		Total Nitrogen (%)	Moisture (%)	Predicted Extract (NIR)	Soluble Nitrogen (%)	Soluble Nitrogen Ratio (%)	Friability (%)	Homogeneity (%)	Whole corn number	Whole corn weight (g)	Predicted Spirit Yield (LA/tonne)	Hot Water Extract (%)	Peak Gelatinisation Temperature (°C)	Onset of Gelatinisation Temperature (°C)	Nitrogen (%)	Specific Weight (kg hl ⁻¹)	Density (g cm ⁻³)	Packing Efficiency (%)
Malt	Total Nitrogen (%)	1	-0.37	-0.25	0.04	-0.89***	-0.65*	-0.5	0.38	0.51	-0.18	-0.06	0.12	-0.2	0.71*	-0.01	-0.12	-0.71*
	Moisture (%)		1	0.05	0.28	0.47	0.07	0.11	0.09	-0.06	-0.26	-0.47	0.12	-0.28	0.04	-0.56	-0.47	-0.17
	Predicted Extract (NIR)			1	0.02	0.24	0.65*	0.89***	-0.9***	-0.81***	0.79***	0.57	-0.53	0.43	-0.06	0.53	0.44	0.51
	Soluble Nitrogen (%)				1	0.42	0.11	-0.12	-0.02	0.1	0.14	0.03	-0.35	0.08	0.19	0.1	0.08	-0.33
	Soluble Nitrogen Ratio (%)					1	0.64*	0.41	-0.36	-0.42	0.23	0.07	-0.28	0.2	-0.56	0.05	0.15	0.49
	Friability (%)						1	0.77***	-0.8**	-0.74***	0.79**	0.64*	-0.53	0.59	-0.57	0.53	0.34	0.59
	Homogeneity (%)							1	-0.94***	-0.97***	0.77**	0.59	-0.46	0.42	-0.31	0.42	0.39	0.66*
	Whole corn number								1	0.93***	-0.92***	-0.78**	0.57	-0.52	0.37	-0.68*	-0.59	-0.68*
	Whole corn weight (g)									1	-0.75**	-0.58	0.43	-0.34	0.4	-0.42	-0.38	-0.69*
	Predicted Spirit Yield (LA/tonne)										1	0.91***	-0.65*	0.62	-0.34	0.84**	0.65*	0.5
	Hot Water Extract (%)											1	-0.41	0.76*	-0.37	0.82**	0.67*	0.45
	Starch	Peak Gelatinisation Temperature (°C)											1	-0.01	0.1	-0.69*	-0.65*	-0.24
		Onset of Gelatinisation Temperature (°C)												1	-0.2	0.42	0.29	0.29
Grain		Nitrogen (%)													1	-0.39	-0.37	-0.77**
	Specific Weight (kg hl ⁻¹)														1	0.87**	0.5	
	Density (g cm ⁻³)															1	0.58	
	Packing Efficiency (%)																1	

***, **, * were significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively.



Trait	PC1	PC2
Homogeneity		
Number of whole corns (Wc)	-0.496	-0.738
Predicted spirit yield (PSY)	0.833	-0.267
Hot water extract (HWE)	0.101	-0.128
Specific weight (SW)	0.222	-0.601

Fig. 2. Biplot of the principal component analysis of specific weight and malt quality parameters of the ten grain fractions used in this study. Arrows starting at the centre of the plot represent the loadings of specific weight and malt quality parameters, with the length of the arrows representing the relative importance of each trait. Loadings for PC1 and PC2 are shown in the table beneath the figure.

contribute to GD, which in turn influences water distribution within the endosperm and consequently the spatial distribution of enzyme activity (Chandra et al., 1999). Packing efficiency will influence the flow of water between grains during steeping which may affect the rate at which water is imbibed into the grain and the rate or uniformity of germination.

In this study, a bulk of grain of the cultivar Concerto was manipulated into different fractions through sequential sieving and additional sorting by grain weight. An additional Sienna bulk was also used, without sorting (i.e. with its natural variation in grain size). Therefore ten fractions were investigated in total. This resulted in significant differences in both grain characteristics and malt quality parameters among fractions. Of these grain characteristics, grain volume and weight increased in fractions with increasing grain size as expected. Specific weight generally increased in fractions with increasing grain size, which was expected since larger and plumper grains traditionally are associated with a higher SW. Packing efficiency was highest in the two mixed fractions, indicating that too much homogeneity of grain size may be detrimental for SW despite being a favoured trait by maltsters to ensure uniform modification (Wade and Froment, 2003). Fraction had little effect on grain composition, with N content differing between Sienna and all Concerto fractions apart from Fraction B1, highlighting that

cultivar rather than sieve fraction had a greater influence on composition. This is in accordance with other studies which demonstrated that when creating fractions by sieving, weighing alone or pneumatic classification, cultivar had a greater effect on protein content than the effect of the parameters by which the fractions were sorted (Elfverson et al., 1999).

Malting regimes were not optimised for all ten fractions, therefore all fractions were micromalted using the same malting regime. It is possible that optimising malting regimes for each fraction may have yielded different results, this would need further investigation. However, due to the high friability scores, high homogeneity scores and low number of whole corns across all fractions it is unlikely that optimisation would significantly influence how effectively these fractions malted.

Fractions had no effect on the levels of soluble N, total N or the soluble nitrogen ratio. Friability is effectively a measure of how crumbly a material is, and for malt this is one indication that the grains have malted successfully and undergone sufficient modification. Friability did not vary with fractions suggesting all fractions malted well and achieved similar levels of modification. However, the high Wc in the smallest fractions, particularly fraction A indicated that these fractions did not malt as effectively as the larger fractions. Hot water extract and PSY were significantly affected by fraction. In general, smaller grain size fractions with lower SWs had both reduced PSY and HWE. This was particularly evident in fraction A which had the lowest SW, PSY and HWE. Despite fraction A only contributing 5.5% to the overall mix by weight, its significantly lower malt quality will be detrimental to the total mix fraction. On the other hand fraction B2 achieved a relatively high SW with lower levels of PSY and HWE, in comparison to fractions which had a similar SW, such as fractions D and E. Apart from this exception, the general pattern agrees with the concept of a higher SW being beneficial for malt output. Attempts to link GD and PE to malting output have not been reported before. However a previous analysis of DP from different grain fractions has shown that larger grains have an increased DP, which is beneficial for converting complex starch into fermentable sugars during mashing (Agu et al., 2007). Therefore this enhanced malt output could be contributed to by the increased enzymatic activity in larger grains, which also had an increased GD.

In order to understand how grain attributes that are associated with an increased SW influence malt quality, relationships between and among grain and malt parameters were investigated. Both SW and GD correlated with the two main measures of malt output used in this study, PSY and HWE, however PE did not. This implies that it is the GD aspect of SW which influences malting output to a greater degree than PE. In addition to this the SW correlation is greater than the GD correlation for both PSY and HWE, therefore GD is not explaining all of this observed variation. Packing efficiency explains some of the variation in HWE and PSY which may explain why variation in GD alone is not as good a predictor of malt output as SW. Samples with a higher SW also have a reduced Wc implying a greater level of modification in comparison to low SW samples. Interestingly a high SW and GD results in a reduced peak gelatinisation temperature which could contribute to the explanation of why higher SW fractions have an increased malt output. A lower peak gelatinisation temperature means that during mashing at 65 °C there is an increased chance of full conversion of starch to fermentable sugars. Therefore as well as malt output, SW could be related to an increased malt efficiency with an increased conversion of starch, resulting from the same energy input to reach mashing temperature. Again PE doesn't share this correlation, further highlighting the importance of GD over this component.

This study has shown that through the manipulation of grain size and grain weight in a bulk, SW can be altered. In general, samples formed from the lower size fractions have a lower SW. When SW is altered in this way it is a good indicator of malt quality in the majority of cases. Higher SW fractions on the whole had increased malt output demonstrated by an increased HWE and PSY. Furthermore, these data suggests that efficiency in downstream processes, where the malt undergoes a mash,

could be improved with a higher SW, as it was associated with a reduced peak gelatinisation temperature. However, it is important to note that in this study the changes in SW were due to GD rather than PE. Therefore it is the GD component of SW that is responsible for changes in malt output, rather than PE. If SW had been altered through a change in the PE, we cannot yet tell if it is likely to have the same effect on malt output.

Declaration of competing interest

There are no conflicts of interest in this manuscript.

CRediT authorship contribution statement

Aaron Hoyle: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Maree Brennan:** Supervision, Formal analysis, Writing - review & editing. **Nicholas Pitts:** Methodology, Investigation. **Gail E. Jackson:** Supervision, Writing - review & editing. **Steve Hoad:** Supervision, Writing - review & editing, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcs.2020.103006>.

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