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Understanding the components of specific weight in barley grains: Opportunities for improving grain quality and processing efficiency

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1. Abstract

Quality requirements for malting barley include germination rate, per cent admixture (other grains and contaminant particles), nitrogen levels, cultivar, moisture content, uniformity, skinning level, disease/weathering damage and specific weight (SW). Whilst the majority of these malting barley quality requirements are well understood in terms of end use, SW is not. Specific weight is one of the longest-standing measures of grain quality for cereals and oilseeds; it is a measure of the weight of grain per unit volume and is reported in kilograms per hectolitre (kg hl^{-1}). An increased SW is thought to be beneficial for malt output, however, the relationships between SW and measures of malt output or efficiency are not well understood. The aim of this research is to enhance the understanding of SW as a measure of grain quality and establish which aspects of barley grain determine this measure, then relate this to the malting process and outputs, to understand how SW influences malting. Firstly, SW has been broken down into its two components: grain density and packing efficiency. This is a key part of the research because both components can change independently. Different grain parameters influence each of the components, therefore, both need to be considered together when investigating SW differences or similarities between samples. The packing efficiency and grain density of nine spring barley cultivars were investigated, this demonstrated that grain density contributed 48.5% and packing efficiency 36.5% to the variation in SW. It was hypothesised that the packing efficiency of grains was primarily influenced by grain morphometrics, and grain density influenced by composition. The ways in which composition changed with grain density was investigated by stratifying grains according to their density, resulting in several fractions with different densities. Compositional analyses were carried out on these groups which showed that grain nitrogen level and the proportional volume of starch B-type granules contributed 47% to the observed variation in grain density. Specific weight is also known to be affected by growing conditions, with year-to-year variation observed. Therefore, the effect of a moderate, but prolonged water stress on SW was investigated under glasshouse conditions. Plant development was altered by the stress, but SW was maintained through compensatory mechanisms. To investigate how changes in SW affect malt quality parameters, SW was manipulated through selection for different grain size and weights. Specific weight was shown to be strongly correlated with the predicted spirit yield and hot water extract of the malt. These are two fundamental measures of malt quality. Grain density also correlated with these two malt quality measures, but grain packing efficiency did not. This indicates that it is grain density rather than the packing efficiency of the grain that is beneficial component of SW for malting. Therefore, if breeding is continued to enhance malt quality through increasing SW, this should be targeted through increasing the grain density component rather than packing efficiency.

2. Introduction

2.1. Overview

Barley is the main cereal crop used in the malting industry. The quality of barley grain used for malting is of utmost importance, ensuring both an efficient malting process and a high quality product. Therefore, prior to malting, barley grain is assessed for certain quality attributes which are indicative of future malt quality. One of these quality attributes is specific weight (SW); the weight of grain in a given volume. The underlying grain characteristics associated with this trait are not well understood. Detailed links between SW and the malting process have yet to be shown, however, it is currently used as an industry-wide quality attribute for trading and processing.

This research sought to increase the current understanding of SW in barley and provide links between SW and malting. This introduction is followed by four experimental papers (published or submitted research papers). The main project findings are summarised in a general discussion, placing the results in context of the wider industry.

2.2. Background

Barley (Hordeum vulgare L.) has been an important cereal crop since its domestication 10,000 years ago (Badr et al., 2000). Barley ranks fourth globally in terms of both production quantity and land area; behind wheat, maize and rice (FAOSTAT, 2020). This equates to a global production of 142 million tonnes over 48 million hectares (FAOSTAT, 2020). The success of barley as a cereal crop is a result of both: its ability to be grown across a diverse range of environments, from 70ºN to 65ºS, and also its wide variety of uses (Schildbach, 1986). Its primary uses are for livestock feed and for malting, these account for roughly two thirds and one third of its usage, respectively. In addition, 2% of the global barley crop is grown for direct human consumption, with the majority of this consumption occurring in areas of Asia and North Africa (Baik and Ullrich, 2008). Barley can be subdivided into two types depending on its growing season. Winter barley is planted in autumn and harvested the subsequent summer, requiring a vernalizsation period. Spring barley is planted in spring and harvested the same summer. Winter barley is characteristically high-yielding, with a longer growing season in comparison to spring barley. In the UK, spring barley typically yields 20% less than winter barley (AHDB, 2015). In addition to winter and spring barley cultivar types, cultivars can also be either two-row or six-row. These differences between two-row and six-row cultivars arise as a result of spikelet fertility. In two-row barley, only the central spikelet is fertile, whereas, in six-row barley all three spikelets are fertile (AHDB, 2015).

2.3. Scottish Context

Barley and its downstream uses of grain and straw are of particular importance to Scotland. In 2019, spring barley accounted for 48% (241,000 ha) of the total cereal crop by area in Scotland, and winter barley for 12% (49,000 ha) (The Scottish Government, 2019). Since 2010, the production area, yield and consequently, production quantity of spring barley in Scotland have been relatively stable. The prime arable land in Scotland is mainly situated on the eastern with both reduced rainfall and increased hours of sunshine in comparison to the west. This makes eastern Scotland some of the highest value land for growing barley in the UK, in particular, malting barley. Despite the yield differences between spring and winter barley, spring barley is preferred for malting. This is because, typically, only spring barley cultivars are better suited to producing grain that meets the stringent requirements for malting. This includes grains bulks that process well and lead to a more efficient malting process and higher quality product. Therefore, of the tonnage of barley bought in 2018 by the Scottish members of the Maltsters Association of Great Britain (MAGB), 96.4% of this was spring barley (www.ukmalt.com). However, there is one example of a winter barley which was particularly prominent in the malting industry during the 1970s, Maris Otter. Due to the higher yielding nature of winter barley, there is a potential to increase the malt output per hectare of barley grown. As a result of the need to strive for increased sustainability, there has been a recent resurgence of interest in breeding a winter barley of malting quality. Researchers have started to try and transfer malting quality attributes from spring to winter barley, to reduce or even eliminate this gap in quality between the two types (Thomas and Impromalt Consortium, 2018).

Malt and associated products are used in the: food industry, brewing sector and distilling sector. However, it is the distilling sector that is of particular importance to Scotland. At the time of writing this thesis, there are currently 133 Scotch Whisky distilleries throughout the country, resulting in the highest concentration of whisky producers in the world (O'Connor, 2018; Scotch Whisky Association, 2019). Scotch whisky exports in 2019 were worth £4.9bn to the economy, and account for 70% of the total Scottish food and drinks exports (Scotch Whisky Association, 2019). It is not only these direct measures which are of value to the economy, the whisky industry also benefits tourism and supports jobs in related supply chains.

2.4. The Barley Grain

2.4.1. Barley Grain Anatomy

Barley is harvested for its grain which comprises the caryopsis (or fruit) enclosed within an outer coat or husk caryopsis or grain. In this thesis, it will be solely described as the grain. A typical barley grain is composed of an embryo, endosperm, nucellus, testa, pericarp which comprise the caryopsis, and the lemma and palea, the husk (Evers and Millar, 2002). The embryo consists of two parts, the embryonic axis and the scutellum. The embryonic axis is the next generation containing: the shoot,

mesocotyl and radicle. The scutellum is positioned between the embryonic axis and endosperm and is involved in the secretion and absorption of both water and solutes during germination (Evers et al., 1999). This regulates the supply of nutrition to the embryonic axis (Evers et al., 1999). The largest tissue within barley grains is the endosperm, most of which is the starchy endosperm, the main storage tissue of the grain. The main constituents of the starchy endosperm are starch and protein. The second part of the endosperm is the aleurone, a layer which surrounds the starchy endosperm, consisting of between two and four thickly walled cells, typically three cells thick. This layer is rich in proteins and lipids and is responsible for the production of endosperm cells. It also plays an important role in germination through the secretion of hydrolytic enzymes which solubilise carbohydrate reserves within the starchy endosperm (Evers et al., 1999). Adjacent to the aleurone layer, is the nucellus, a maternal tissue within which the endosperm and embryo developed (Evers and Millar, 2002). The testa is the true seed coat and is composed of a single layer of cells, which is enclosed by an outer cuticle. Grain tissues which are outside of the testa are therefore, no longer part of the seed, but part of the fruit. The pericarp originates from ovary walls which have ripened. Finally, in typical hulled barley two outer bracts, the lemma and palea, protect the floral parts and later further layers and become adhered to the grain caryopsis., the lemma and palea, these act to protect the grain.

2.4.2. Barley Grain Composition

The endosperm is responsible for starch storage and is composed of a cell wall-protein matrix with semi-crystalline starch granules embedded within the cells (Chandra et al., 1999). Starch is the most abundant constituent of barley grains, accounting for roughly 60% of grain weight (Fox, 2010). Starch is composed of two polysaccharides, amylose and amylopectin (James et al., 2003). Amylose is composed of a chain of α-glucose units, which are primarily unbranched, amylopectin is also composed of α-glucose units, but these are highly branched. Barley starch is stored within the endosperm in granules which come in two distinct forms; A-type and B-type starch granules. Although some studies suggested that there are three forms of granules small, medium and large, it is largely agreed that in barley there are these two distinct types (Takeda et al., 1999). Despite this disagreement, it is well established that larger granules (A-type) are initiated earlier in grain development and have a higher proportion of amylose than later developing, smaller (B-type) granules (Takeda et al., 1999). The matrix within which these granules are embedded, can either be densely or loosely packed, leading to grains with either a ‘steely’ or ‘mealy’ texture respectively (Chandra et al., 1999). Differences in endosperm texture as a result of this influences the downstream processing of barley. Barley endosperm cell walls are primarily composed of β-glucans (75%) and arabinoxylans (20%) (Fox, 2010). The protein content of barley is typically between 8 and 13%, with different requirements within this range for different end-uses (Fox, 2010). In brewing, a protein content of between 10% and 10.9% is typically demanded, whereas in distilling, a lower range of 9.4% to 10.3% is demanded (MAGB, 2020). Protein content is important for these end users

because it is the valuable starch granules that are embedded within the matrix, and there is typically an inverse relationship between starch content and protein content. The main proteins in barley grains are hordeins, but albumins, globulins and glutelins also contribute to the overall protein content (Fox, 2010). Additional minor constituents of barley grains include lipids, which are between 2% and 4% of the total grain weight. These are present in the forms of nonpolar lipids, glycolipids and phospholipids (Shewry and Ullrich, 2014).

2.5. Grain and Malt Quality

In the UK, barley is the main crop used for malting, with both its biochemical composition and physical characteristics contributing to a desirable malt. The AHDB's (Agriculture and Horticulture Developmental Board's) RL (Recommended List) for cereals and oilseeds provides crop and cultivar specific information for: market options (feed, brewing and distilling), yield, agronomy, grain quality and disease resistance. The yearly updated RLs guide the decision-making procedure undertaken by growers and maltsters. The RL also assists the selection of cultivars to sow or demand in a given season, which to a large extent is driven by the listing of agronomic and grain quality attributes of the currently recommended cultivars. Appropriate cultivar selection helps to ensure the grain harvest will be acceptable for the intended end market. In the malting industry, barley cultivars are selected on additional 'grain quality' traits which influence the malting process. The grain quality traits that are listed in AHDB's RL are: screenings (a measure of grain size), specific weight (SW) and nitrogen content. The RL does not contain an exhaustive list of grain quality characteristics, there are many more grain quality characteristics that contribute to malt quality and malting efficiency (Table 2-1).

In both the literature and among industry, the term 'malting quality' comprises a composite of grain features that influence the efficiency of the malting process, output and the quality of the end product. Therefore, it is often difficult to assign specific factors or traits lead to either a high malt quality or high malt output. Here, malting quality has been split into i) Grain traits that influence quality and efficiency as shown in Table 2-1 and ii) Malt quality parameters that can be used to define the quality of the malt product in Table 2-2. The target values for the parameters listed in Table 2-2 will vary depending on the product being made, however, these are the target values for malt used in the brewing industry. Knowledge of how grain traits relate to malting efficiency and output is essential, so maltsters can make informed decisions about the cultivars they demand from growers.

Table 2-1 Factors affecting malt quality and their desirable levels, information collected from a range of sources.

Grain Traits	Desirable Level	References
Grain	No frost or heat damage	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
Condition	Bright grains, free from disease	(Gupta et al., 2010; Martin, 2015)
	No admixture of weeds/insects/chemicals	(Martin, 2015)
	No tolerance of mycotoxins e.g. DON	(Brewing and Malting Barley Research Institute (BMBRI), 2010; Martin, 2015; Nielsen et al., 2014)
	Pure batch of one cultivar	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
	<5% of skinned/broken grains	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
Grain Size	90% retention through 2.5mm Screening	MAGB
	Favour high SW	AHDB RL
	Favour uniform grains (homogeneity)	(Wade and Froment, 2003)
Germination	No pre-harvest germination	(Martin, 2015)
	Fully mature grains	(Brewing and Malting Barley Research Institute (BMBRI), 2010; Martin, 2015)
	>98% germinative energy	MAGB
Moisture Content	<13.5%	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
Composition	Protein Content 11-12.5%	(Brewing and Malting Barley Research Institute (BMBRI), 2010)

Table 2-2 Malt quality parameters and target values for good brewing malt (adapted from Verstegen *et al*. 2014; Brennan *et al*. 1997).

Malt quality parameter	Target values
Protein content	<10.8%
Kolbach Index, or soluble protein	38% to 42%
Hot water extract	305-315 L˚/kg
Extract difference	1.2% to 1.8%
Viscosity	<1.55 mPa s
β-Glucan	<300 mg/l
Wort colour	<3.4 EBC (European Brewery Convention)
Boiled wort colour	<5.0 EBC
Soluble Nitrogen (dry matter)	>0.65g/100 g MTrS
Friability	>87%
Viscosity 65 °C	<1.65 mPa s
β-Glucan 76 °C	<400 mg/l
DMS-P (Dimethyl sulphide)	<6 ppm

2.6. Malting

Malting is the controlled germination of cereal grains. It has been suggested that malting is the oldest biotechnology in the world. Since the cultivation of barley, accidental germination in the harvested crop probably lead to noticeable flavour changes in products made from the grain. This would have given rise to the deliberate germination of cereal grains, then ancient methods of producing bread and beer (Briggs, 1998). Malting is a batch process, meaning the product is not produced continuously. Malting broadly occurs in three stages: i) steeping, ii) germination and iii) kilning. Steeping involves the soaking of grains in water to increase their moisture content from <12% to >40% (Gupta et al., 2010). In a maltings, the temperature of the steep water is often controlled because this will subsequently impact on germination time. The steep water becomes dirty and is changed at least once during a steep, therefore, this initial stage in malting can be a very water-intensive part of the malting process. In seeking for a reduction in the environmental impacts of the high water use, of this step, some maltings filter and re-use steep water. The whole steeping process takes between 48 and 72 hours. Water uptake by the grain is affected by endosperm structure. Grains with a less dense matrix or ‘mealy’ texture are likely to have more uniform uptake of water during the steeping process and later movement of enzymes (Gupta et al., 2010).

Once barley grains have imbibed enough water to increase the moisture content to >44%, steeping is complete. Water is drained and grain enters the next stage of the process, germination. This can either be in: a different vessel, the same vessel or traditionally, the grain was spread across a maltings floor. In either of these methods, germination is triggered and a cascade of biochemical changes within the grain. Hydrolytic enzymes such as α-amylase, which are produced in the scutellum and aleurone layer begin to degrade components of the endosperm (Briggs, 1998). This results in the breaking down of cell walls and weakening of the endosperm structure. Consequently, previously bound starch granules are released from the matrix (Gupta et al., 2010). The combination of these changes to the grain are termed 'modification'. Both the accumulation of enzymes and modification of the endosperm are crucial to produce a good quality malt. However, during the natural process of uncontrolled germination, the plant would further degrade the starch and use the resultant sugars to begin growth. Therefore, the degree of modification and accumulation of enzymes needs to be balanced with embryo growth. Otherwise, the sugars that are required to make the downstream products (e.g. beer, whisky, malt extract) from malt will be metabolised by the embryo, equating to malting losses. When fermentable sugars are lost as a result of this, the endosperm has undergone 'over-modification'. Consequently, germination is arrested before full degradation and excessive embryo growth occurs, by the final stage of the process, kilning. The time taken for the required level of germination in a maltings can vary between 84 and 144 hours.

After germination is judged by the maltster, the final stage of kilning can begin. Kilning dries the grains and stabilises the changes in biochemistry, so all the necessary starch degrading enzymes are still present in the resulting malt, the product of the malting process (Gupta et al., 2010). Kilning regimes can vary depending on the type of malt being produced. Kilning can produce large differences in characteristics of malt, from pale lager malts through to darker ale malts. Colour and flavour can be further enhanced by roasting malts, this is typical for caramel or chocolate malts. However, it is vital that for all types of malt, the initial kilning temperature is not too high, as this causes enzyme denaturing while the moisture content of the malt is still relatively high. These enzymes are then utilised in the downstream uses. Upon rehydration and elevated temperatures, the enzymatic degradation of starch through amylase activities continues, producing fermentable carbohydrates (Gupta et al., 2010). This highlights the need for only this partial degradation of starch and the maintenance of starch degrading enzyme integrity during the malting process. Kilning is the most energy intensive part of the malting process. Malting industries have taken steps to reduce energy consumption through heat recovery systems and the introduction of continuous kilning. However, there is a lot more scope for decreasing the environmental impact of the malting industry.

2.7. Specific Weight

2.7.1. Definition and Applications

Specific weight is defined as the mass of grain per unit volume and is measured in kilograms/hectolitre (kg hl^{-1}). Specific weight describes the bulk density of grain and is thought to be primarily determined by: grain weight, grain density (GD) and packing efficiency (PE) of a bulk (Clarke et al., 2004). However, on a finer scale, these are in turn thought to be influenced by the following grain traits: size, morphology, compaction, composition, surface friction and moisture content, which are themselves influenced by both genotype and environment (Pushman and Bingham, 1975). Specific weight is a measurement used on all cereal grains; however, with the different end uses of grain, it has more relevance for certain processes. For example, SW has come under criticism in terms of its use for valuing animal feed (McCracken et al., 2002). This study demonstrated that there was no relationship between the SW of wheat grain and the feed value for poultry. However, SW has applications in the transportation and storage of grain around the world, describing the mass of grain that can be transported in a given container (Grain Trade Australia, 2013).

The terminology surrounding SW is inconsistent in both the literature and industry, where it can be referred to as ‘grain density’, ‘test weight’, ‘bushel weight’, ‘hectolitre mass’ or ‘hectolitre weight’ (Manley et al., 2009). Throughout this thesis and all subsequent work, this grain quality measurement shall be referred to as SW. In addition to this, units for measuring SW and techniques often vary, further complicating this measurement (Wychowaniec et al., 2013). The typical piece of equipment used to measure SW is a chondrometer. “Chondro-” originates from the Greek word ‘khondros’ meaning grain, and “-ometer” is an instrument used in measuring something. Hence, a chondrometer is an instrument used for measuring grain. In the UK, this consists of two stacked cylinders separated by a sliding gate. The upper cylinder is filled with grain, the separating gate is withdrawn and re-inserted once the grain has fallen into the bottom portion. The grain in the lower cylinder, of known volume (500 ml), is weighed and from this SW in kg hl^{-1} is calculated. However the exact apparatuses of this equipment vary from country to country. Some use funnels of differing diameters to pour the grain; others use a collection cylinder of varying shapes and sizes. It has been demonstrated that the use of these different techniques and equipment across different countries leads to different SW values (Manley et al., 2009). Furthermore, when different personnel use the equipment, different results can be obtained (Manley et al., 2009). This highlights the need for an increased awareness of this variation. In this work, only one scaled-down version of a chondrometer will be used, this will allow work on smaller grain samples, and ensure consistency between measurements. The absolute values obtained will not be directly comparable with the industry standard, however, will allow comparisons to be made between samples when the same system is used to estimate SW.

2.7.2. Specific Weight and Malting

At present, high SW is considered a desirable characteristic of barley cultivars approved for malting. Hypothetically, SW could play a role in the amount of extract produced per batch and consequently, efficiency. If grains of a high SW are purchased; an increased weight of grains could be included in the malting vessels, increasing the output per processed batch.

The link between SW and malt quality parameters in Table 2-2 has yet to be made. Any quantitative relationship between SW and hot water extract (HWE) or predicted spirit yield (PSY), the main predictors of malt output used in industry, remains to be shown. Specific weight has been included as a grain quality characteristic in AHDB's RL without the necessary evidence to support that this is indicative of malt output. Therefore, SW has been included in Table 2-1 without the knowledge of what SW is beneficial for in terms of either malting efficiency or quality. Work in this project will address this link between SW and malt quality parameters. Its inclusion may be a result of it being one of the longest standing measures of grain quality and the simplicity and speed in measuring it.

The literature is vague when describing links between SW and potential malting benefits. Often, grains with a high SW are thought to be plumper and, therefore, have a larger proportion of endosperm, particularly the starchy endosperm (Dimmock and Gooding, 2002), resulting in more starch available for hydrolysis to maltose. However, Yu et al. (2017) has recently shown that a high SW does not result in increased starch content in barley grains. Therefore, it is important to know if an increased SW is due to a change in the PE of grains, grain composition or a combination of both.

If SW is altered by changing the proportion of the protein matrix in the starchy endosperm, is SW a good measure of malt quality? If a higher proportion/density of endosperm protein increases SW, a lower SW may result in more efficient malting and higher quality malt. The ranges of acceptable levels of protein have been previously mentioned. High protein contents can lead to a slow rate of endosperm modification during malting and also a reduced extract yield from the malt produced (Agu, 2003) and deteriorate final beer quality. Not only does this reduce the output of this batch of malt, but because of the slower rate of germination, the next batch of malt will be delayed. Therefore, the throughput of batches of malt would be reduced in the maltings. However, if a lower protein content increases SW, the opposite may occur with enhanced levels of modification, increased extract yield and higher throughput. This lack of knowledge of which grain traits contribute to SW needs to be addressed, to be confident that SW is a relevant quality indicator for malting and which aspect of malting.

As mentioned previously, grain with a high SW is thought to be associated with well-filled plump grain (Gooding et al., 2003). However, the precise ways in which grain morphology and composition contribute to SW are not fully understood. Furthermore, variation in SW is observed among barley

cultivars and between growing locations, demonstrating SW is influenced both the genotype and the environment (AHDB, RL harvest data 2016). This highlights another avenue of interest, how the environment influences grain traits and consequently, SW. The environmental conditions during the growth of barley are known to influence both composition and morphology. Starch biosynthesis under different environmental stressors has been widely studied. The effect of an environmental stress on starch biosynthesis and accumulation is dependent upon: the severity of the stress; timing and duration of the stress; and also the sensitivity of the genotype to the stress (Thitisaksakul et al., 2012). Globally, water stress is the most common stress, responsible for most of the observed reductions in yield, with starch content correlating well with yield (Worch et al., 2011). Alongside yield impacts, water stress is also known to affect the malt quality of barley grain changing composition (β-glucan) and enzyme activity (β-amylase) (Wu et al., 2017).

In addition, the use of SW as an indicator of potential malt quality needs to be tested to determine the effect of different SWs on the malting process. One of the difficulties in testing this is that SW is thought to be influenced by numerous grain characteristics simultaneously. A mixture of experimental and statistical work will aim to quantify the key contributors to SW and examine which are likely to impact on malt quality. This could enhance the understanding of whether or not SW is an important grain trait to measure when evaluating the malting potential of spring barley cultivars. This aims to provide the malting industry with a clear description of the contributing factors to SW and the consequences which these may have for both malt quality and efficiency.

2.8. Report Outline

The overarching goals were as follows:

1. Describe how grain packing efficiency and grain density contribute to SW (Paper 1 – Section 3)
2. Investigate associations between grain composition and the grain density component of SW (Paper 2 – Section 4)
3. Explore the effects of a changing SW as a result of manipulated grain size and weight on malting quality (Paper 3 – Section 5)

In Paper 1, grain dimensions, weight, volume and two-dimensional area of 100 individual grains of nine cultivars were measured to develop a detailed grain-level understanding of cultivars with a range of SWs. This described the contribution of grain packing efficiency and grain density to SW. Through detailed grain and bulk analysis, it was shown that SW is the product of grain density and packing efficiency. The findings of this paper provide the basis for all following papers. It highlights that, in all future work on SW (in all cereal species), both components grain density and packing efficiency should be taken into consideration.

Paper 2 builds on Paper 1 by further investigating the components of SW. In this paper, the grain density component of SW which was not dissected in the previous paper is examined. It was hypothesised that unlike packing efficiency, which is influenced by grain morphology, grain density will be related to the composition of the barley grain. This was investigated by addressing the three following aims:

1. examine the correlations between quantitative changes in grain composition and single grain density
2. build an equation to predict single grain density from grain composition to understand the contributions of compositional aspects to single grain density
3. test the accuracy and efficacy of the equation using an independent grain sample

Analysis of the compositional changes across a range in grain densities related single grain density within a cultivar to the composition of these grains. Grain density and composition in barley have not been linked before.

Paper 3 utilises information gained across the previous three experimental papers, particularly on the components of SW and what influences these. This paper investigates links between SW and the malting process, in terms of either efficiency or output. To study how SW and its components; grain density and packing efficiency effect malting three aims were addressed:

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
3. examine correlations between grain parameters and malt quality parameters to establish links between SW and malt quality

3. Specific weight of barley grains is determined by traits affecting packing efficiency and by grain density (Paper 1)

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Authors: Aaron Hoyle, Maree Brennan, Gail Jackson and Steve Hoad.

3.1. Background

Specific weight influences the market value of barley grain, and in malting barley a high specific weight is thought to result in an increased malt output. However, links between specific weight and malt output have not yet been established. We hypothesised that packing efficiency and grain density will each contribute to specific weight. These traits would have implications for the malting process, highlighting the need for understanding what grain traits contribute to specific weight before we can predict its effect on malting performance and efficiency.

It is clear there is a knowledge gap in identifying what attributes of spring barley grains influence SW. This needs to be addressed prior to investigating the effect of grain attributes on the malting process and product. In this study, we measured grain dimensions, weight, volume and two-dimensional area of 100 individual grains of nine cultivars to develop a detailed grain-level understanding of cultivars with a range of SWs. Grain density and PE were calculated and grain size manipulated to determine how these contribute to the SW of barley grains. Correlations among all measured grain traits were also examined to understand links among traits and between them and SW.

3.2. Methods

3.2.1. Grain Samples

Nine spring barley malting cultivars from the Agriculture and Horticulture Development Board's (AHDB's) Recommended List (RL) 2016/17 were used in this study: KWS Irina, Octavia, Odyssey, Laureate, Origin, Concerto, Olympus, Propino and Sienna (https://cereals.ahdb.org.uk). These cultivars were chosen due to their phenotypic range in SW and varying levels of screenings, according to AHDB's RL 2016/17. The purpose of including multiple cultivars with a range of SWs was to extend the phenotypic variation in SW and its components. These were grown in Docking, Norfolk under natural rainfall conditions during the 2016 season for the AHDB's RL crop trials. Prior to analysis, samples were cleaned by shaking over a 2.50 mm slotted sieve, with 19.05 mm long slots for 20 seconds. Grain retained by the sieve was used for analysis.

3.2.2. Specific Weight

To achieve a detailed grain-level analysis of how differently shaped grains pack within a volume, and influence SW, it is necessary to have a scaled-down procedure for measuring SW which corresponds to the industry standard measurements, similar to that described by Gooding et al. (2003). Therefore, an accurate scaled-down method for measuring SW was developed in this study. Grain was poured from a height of 2 cm into a 25 ml measuring cylinder until it overflowed and superficial grains were removed by striking across the top of the cylinder with a straight edge. The total volume of the cylinder (39.16 ml) was obtained by weighing the amount of water required to fill the cylinder (Kern analytical balance PLJ 750-3N, accuracy ± 0.01 g).

The weight of grain in the cylinder was divided by cylinder volume and multiplied by 100 to give an estimate of SW in kg hl-1. The results from this scaled-down method were highly correlated with an industry standard measurement of SW in a trial ($r^2 = 0.84$, $P < 0.001$). This technique of estimating SW is similar to that described by Gooding et al. (2003) and Walker and Panozzo (2011).

3.2.3. Representative Sampling

Grain samples (350 g) were sieved sequentially into the following size fractions using a stack of slotted 3.25, 3.00, 2.75 mm sieves, with 19.05 mm long slots: large (>3.25 mm), medium (3.25 to 3.00 mm), small (3.00 to 2.75 mm) and very small (<2.75 mm). The weight of grain in each fraction was recorded (Kern analytical balance PLJ 3500-2NM, accuracy ± 0.01 g) and where the fraction size was greater than 25 g, SW was measured in triplicate using the scaled-down SW measurement described above. A 100 grain sample was taken from each fraction, and the mean grain weight from each fraction was used to estimate the total grain number in each size fraction and in the whole sample. A number of grains proportional to the total number of grains from each fraction were chosen at random, to give a 100-grain sample that was representative of the grain size distribution within the larger bulk sample.

3.2.4. Grain Size Parameters and Image Analysis

On the representatively sampled 100 grains from each of the nine cultivars the following measurements were taken. The grain dimensions length (L), width (W) and depth (D) were measured (see Appendix Figure A-1) using a hand-held digital caliper (accuracy ± 0.01 mm). These dimensions were used to calculate grain sphericity which was calculated as the cube root of L × W × D divided by L (Coşkuner and Karababa, 2007). This value was multiplied by 100 to give a percentage, with a value of 100% representing a sphere. The two-dimensional (2- D) area of grains was measured using ImageJ (National Institutes of Health, USA, https://imagej.nih.gov/ij/). All of these measures describe grain “size”, which in this study refers solely to physical dimensions of the grain, whereas

"weight" refers to mass. Individual grain area density is a measure of the mass per unit area (mg mm-2), a combination of size and weight, and was calculated by dividing grain weight by 2-D area.

3.2.5. Packing Efficiency and Grain Density

Grain volume and density were measured on the same 100-grains as above. Grain volume was measured by water displacement, with the weight of water displaced being equal to the volume of the grain (Archimedes' Principle). Grains were individually weighed using a Mettler AE 160 electronic balance (Mettler, Toledo, accuracy ± 0.0001 g) then submerged using a 0.5 mm x 25 mm hypodermic needle (BD Microlance) into a beaker of water using the same balance. Grain density (g cm^{-3}) was calculated by dividing the grain mass by grain volume. Packing efficiency was defined as the proportion of space occupied by the grain in the 25 ml cylinder, and was calculated by multiplying mean grain volume by the mean grain number in the cylinder, divided by the cylinder volume. Mean grain number was calculated from three cylinder re-fills.

3.2.6. Data Analysis

All data analysis was carried out using R software version 3.4.1 (R Core Team, 2017). An analysis of variance ($\alpha = 0.05$) was done to determine whether the choice of different cultivars was successful in achieving significant differences in measured grain traits, thereby extending the phenotypic range within the analysed samples. Cultivar was found to be a significant factor in all grain traits apart from volume. Post hoc Tukey's Honestly Significant Difference ($\alpha = 0.05$) tests were done to determine which cultivars were significantly different from each other to gain insight into whether differences in grain traits among samples corresponded with sample differences in SW. For sequential sieve analysis, the effects of fraction size and cultivar among SW samples were analysed using a multiple linear model. Calculation of 95% confidence intervals using the 'emmeans' package was used to compare the SW between grain fractions both within and between cultivars (Lenth, 2018). The effect of the product of PE and grain density on SW among the three replicated samples measured was analysed using a simple linear regression. For this model, the y-intercept was removed as it can be assumed that when SW is equal to zero the product of PE and grain density is also zero. A two-way ANOVA was done with SW as the dependent variable and PE and grain density as the two independent variables. To determine the relative contribution of both PE and density to the variance in SW, the proportion of the sums of squares (SS) for each variable to total SS was calculated. Principal component analysis (PCA) was carried out using mean individual grain dimensions (L, W and D), plots of scores were created to investigate grain shape among the nine cultivars. The associations among all measured traits describing both individual grains and grain bulks were studied using a correlation matrix of Pearson correlation coefficients, which was produced using the 'corrplot' package (Wei and Simko, 2016).

3.3. Results

3.3.1. Grain Traits

Grain traits were measured on 100 representatively sampled grains from each cultivar; the mean values and standard error of the mean for the 100-grain samples are presented in Table 3-1 for each cultivar as 'Individual Grain Analyses'. Significant differences in traits among grain samples were achieved in this case through use of cultivar selection within this 2016/17 field trial, providing a wide range of grain phenotypes with which to investigate performance of grain bulks. The 'Bulk Analysis' traits were measured on the larger bulk sample of each cultivar as supplied from AHDB, and the mean and standard deviation of these technical repeat measurements are presented in Table 3-1 to give a measure of variation within the bulk for these measurements. Cultivar samples are listed in order of descending bulk SW, from Sienna with the highest (69.40 kg hl^{-1}) to KWS Irina with the lowest (64.53 kg hl^{-1}).

Among the grains sampled, Concerto had the lowest grain weight (47.49 mg) which was significantly lower than grains of Sienna ($P < 0.05$), Propino ($P < 0.05$) and Laureate ($P < 0.001$). Concerto also had the shortest (7.79 mm) and least wide (3.80 mm) grains, which were significantly shorter than grains from all other cultivars and less wide than Origin ($P < 0.0001$), Olympus ($P < 0.0001$), Laureate ($P < 0.01$) and Propino ($P < 0.05$). Grain volume and 2-D area were lowest in Concerto (37.85 mm^3 , 21.71 mm^2), although its volume was not significantly smaller than any other cultivars, its 2-D area was significantly smaller than Laureate ($P < 0.0001$), KWS Irina ($P < 0.0001$), Origin ($P < 0.001$) and Odyssey ($P < 0.05$). Sphericity was significantly higher in Concerto (57.62%) than all other cultivars. In terms of bulk analyses, Concerto had the highest number of grains in the measuring cylinder (555.5). Laureate had the highest grain weight (52.45 mg) which was significantly higher than Octavia ($P < 0.05$), Olympus ($P < 0.01$) and Concerto ($P < 0.001$). Laureate also had the highest volume and density (40.37 mm^3, 1.31 g cm^{-3}), although its volume was not significantly larger than any other cultivars its density was greater than Octavia ($P < 0.01$), Concerto ($P < 0.01$), KWS Irina ($P < 0.001$) and Odyssey ($P < 0.0001$). In terms of bulk analyses, Laureate had the lowest mean grain number in the cylinder (492.2) and packing efficiency (50.7%), compared to all other cultivars. Despite grains within the Laureate and Concerto samples having significantly different dimensions and weight, the SWs of 66.33 kg hl^{-1} and 66.84 kg hl^{-1} of each cultivar sample, respectively, are very similar to one another. These results demonstrate that among grain bulks, the same SW can be achieved through different combinations of grain traits.

Table 3-1 Measured[a] grain traits for the nine spring barley cultivars[b] examined.

	Cultivar								
	Sienna	Propino	Olympus	Concerto	Origin	Laureate	Odyssey	Octavia	KWS Irina
Individual Grain Analysis									
Weight (mg)	51.20 ± 0.79 ab	50.97 ± 0.79 ab	48.32 ± 0.75 bc	47.49 ± 0.78 c	49.36 ± 0.72 abc	52.45 ± 0.81 a	50.01 ± 0.73 abc	48.61 ± 0.85 bc	49.67 ± 0.75 abc
Depth (mm)	2.98 ± 0.02 bc	3.06 ± 0.02 a	2.91 ± 0.02 d	3.03 ± 0.02 ab	2.88 ± 0.02 d	3.03 ± 0.02 ab	2.95 ± 0.02 cd	3.01 ± 0.02 abc	2.91 ± 0.01 d
Length (mm)	8.12 ± 0.06 d	8.22 ± 0.06 cd	8.22 ± 0.06 bcd	7.79 ± 0.07 e	8.56 ± 0.06 a	8.53 ± 0.06 a	8.48 ± 0.05 ab	8.33 ± 0.07 abcd	8.45 ± 0.06 abc
Width (mm)	3.82 ± 0.02 cd	3.90 ± 0.02 abc	3.94 ± 0.02 a	3.80 ± 0.02 d	3.95 ± 0.02 a	3.93 ± 0.02 ab	3.85 ± 0.02 bcd	3.80 ± 0.02 d	3.89 ± 0.02 abcd
Volume (mm^3)	39.61 ± 0.65 a	39.61 ± 0.63 a	38.01 ± 0.62 a	37.85 ± 0.70 a	38.71 ± 0.57 a	40.37 ± 0.70 a	40.17 ± 0.57 a	38.39 ± 0.66 a	39.59 ± 0.66 a
Density ($g\ cm^{-3}$)	1.30 ± 0.01 ab	1.29 ± 0.01 abc	1.27 ± 0.01 abcd	1.26 ± 0.01 cd	1.28 ± 0.01 abcd	1.31 ± 0.01 a	1.25 ± 0.01 d	1.27 ± 0.01 bcd	1.26 ± 0.01 cd
2-D Area (mm^2)	22.26 ± 0.25 cd	22.53 ± 0.26 bcd	22.72 ± 0.27 bcd	21.71 ± 0.28 d	23.37 ± 0.24 ab	24.02 ± 0.25 a	22.94 ± 0.22 abc	22.38 ± 0.26 bcd	23.88 ± 0.26 a
Sphericity (%)	55.77 ± 0.20 bc	56.14 ± 0.21 b	55.44 ± 0.22 bcd	57.62 ± 0.27 a	53.81 ± 0.24 e	54.77 ± 0.20 def	54.07 ± 0.21 ef	54.97 ± 0.28 cde	54.16 ± 0.19 f
Area Density ($mg\ mm^{-2}$)	2.29 ± 0.02 a	2.25 ± 0.02 ab	2.12 ± 0.02 cd	2.18 ± 0.02 bc	2.11 ± 0.02 cd	2.17 ± 0.02 c	2.17 ± 0.02 c	2.16 ± 0.02 c	2.07 ± 0.02 d
Bulk analysis									
Grain Number	544.67 ± 2.08	523.00 ± 4.36	549.50 ± 3.46	555.50 ± 5.63	527.17 ± 3.33	492.17 ± 4.16	522.50 ± 8.79	522.33 ± 0.58	520.33 ± 4.54
PE (%)	55.09 ± 0.21	52.90 ± 0.44	53.34 ± 0.34	53.69 ± 0.54	52.11 ± 0.33	50.73 ± 0.43	53.60 ± 0.90	51.20 ± 0.06	52.60 ± 0.46
SW ($kg\ hl^{-1}$)	69.40 ± 0.38	68.05 ± 0.25	66.95 ± 0.28	66.84 ± 0.38	66.53 ± 0.37	66.33 ± 0.69	65.93 ± 0.24	65.53 ± 0.55	64.53 ± 0.67

[a]Individual grain analysis values are expressed as mean ± standard error of the mean and bulk analyses expressed as ± standard deviation.

[b]Cultivars which do not share a letter for each of the measured traits are significantly different from one another.

3.3.2. The Effect of Grain Fraction Size on Specific Weight

To examine how grain size correlates with specific weight among bulks, samples from each of the cultivars were sequentially sieved into different grain size fractions, creating a total of 25 samples with different grain sizes. Not all fractions were represented within each cultivar since not enough grain was retained of every size fraction for a SW estimate to be measured. Analysis of the SW of grain size fractions produced indicated significant differences between the largest and smallest fractions present for five out of the nine cultivar bulks (Figure 3-1), these were: KWS Irina, Octavia, Laureate, Concerto and Propino. For these five cultivars, the smallest size fraction yielded grain with a higher SW than the largest fraction size. KWS Irina, Origin and Olympus only had the three smallest size fractions, whereas Octavia, Laureate, Concerto and Propino had the three largest size fractions. Both Odyssey and Sienna only had enough grain for estimates to be made on the middle two size fractions. This demonstrates that within these bulk samples, these two cultivars have a more uniform grain size than the other seven when grown in the conditions of this trial. This may vary when cultivars are grown under different environmental conditions during another season or location. Specific weight was not consistent for size fractions among samples from different cultivars. For example, the medium size fraction for Sienna which had a SW of 70.1 kg hl^{-1}, which was significantly greater than the medium size fractions of all other cultivars. These data demonstrate that grain size alone is insufficient to determine SW among bulks, and that density and packing efficiency of the grains must be taken into account.

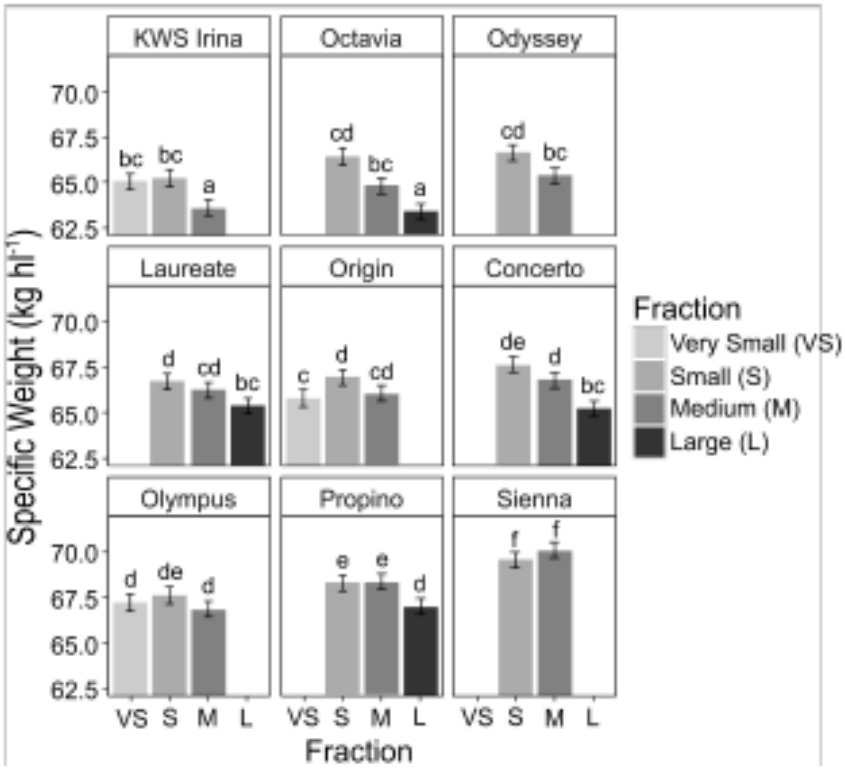

Figure 3-1 Specific weight measured on four size fractions of nine spring barley cultivars. Size fractions are the following: very small (2.50 to 2.75 mm), small (2.75 to 3.00 mm), medium (3.00 to 3.25 mm) and large (> 3.25 mm). Cultivars are ordered from the lowest mean SW from KWS Irina to the highest mean SW, Sienna. When fractions share a letter, the SWs are not significantly different from one another and when a letter is not shared the fractions are significantly different from one another, $P < 0.05$. Bars are the standard error of the means.

3.3.3. Defining Specific Weight by its Components: Packing Efficiency and Grain Density

Regression analysis showed a strong positive correlation between the product of PE and grain density with SW ($r = 0.66$, $P < 0.01$) among the 100-grain samples from each cultivar. The output of the linear regression is shown by the solid black line and the equation SW = 0.988 × (PE × grain density) (Figure 3-2). Seven of the nine cultivars appear close to the y=x line, shown by the dashed line, with four of these almost exactly on this line. This demonstrates that for the vast majority of cultivar samples used, the procedure used to estimate SW through PE and grain density was successful. Two cultivar samples, however, KWS Irina and Sienna, are beneath the linear regression due to PE × grain density being larger than the SW. Through examining the mean grain weight of the 100-grain sample and mean weight of grains in the cylinder, KWS Irina and Sienna had the greatest differences of +1.11 mg and +1.30 mg, respectively. ANOVA showed that both PE and grain density had a statistically significant effect on SW at $P < 0.01$ (Table 3-2). Further analysis using the sum of squares to calculate the proportion of variation contributed by each component showed that PE contributed to 36.5% of the variability in SW, and grain density contributed 48.5%. The contribution of the residual error was small at 15.0% (Table 3-2).

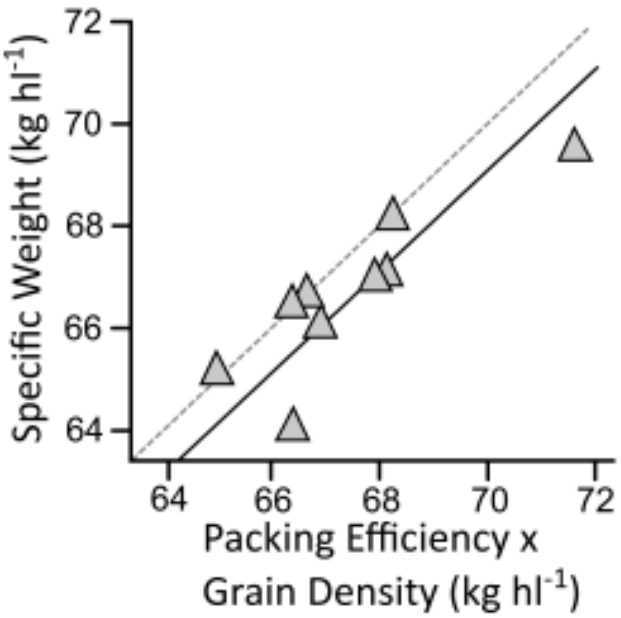

Figure 3-2 The SW of nine barley cultivars plotted against the product of PE and grain density. The linear regression is shown by the solid black line, whereas the dashed line indicates the y=x relationship.

Table 3-2 ANOVA table for specific weight showing the proportional contribution[a] of packing efficiency and density to SW.

Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value	Contribution (%)
Packing efficiency	1	5.85	5.85	14.60	0.0088	36.48
Density	1	7.78	7.78	19.42	0.0045	48.52
Residuals	6	2.40	0.40			14.99
Total	8	16.03				

[a]Calculated as a percentage of the sum of squares for each variable

3.3.4. The Influence of Grain Dimensions on Packing Efficiency

Grain shape was further investigated through principal component analysis (PCA). Principal component 1 (PC1) contributed 91.8% of the total variance, cultivars with a high score in PC1 tended to have shorter grains. Principal component 2 (PC2) contributed 5.3% to the total variance, cultivars with a high PC2 score have deeper grains. The relationship between grain length, width and depth and the PCs are shown in Figure 3-3. A principal component biplot of PC1 against PC2 (Fig. 3-3) shows cultivars with longer grains have a lower PC1 score such as Laureate, Odyssey, KWS Irina and Origin. As cultivars increase in length from Concerto with the shortest grain length to Origin with the longest grain length, they have a higher PC1 score. Further separation occurs by PC2, cultivars with deep grains have a more positive PC2 such as Octavia, Laureate, Propino and Odyssey. Again, this analysis shows the difference in grain size between Laureate and Concerto, which occupy opposite sides of the plot. The plot separates cultivars according to their grain dimensions, which also corresponds to a diagonal gradient of grain number in the cylinder, because a greater number of small grains pack into the cylinder. Therefore, Laureate is positioned in the far top left as it has the largest grains and hence fewest in the cylinder (492.2). The next diagonal portion of the plot is occupied by Origin, KWS Irina, Odyssey Octavia and Propino with similar grain numbers of 527.2, 520.3, 522.5, 522.3 and 523.0, respectively. The final diagonal portion in the bottom right of the plot has cultivars with the highest grain numbers Sienna (544.7), Olympus (549.5) and Concerto (555.5). Grain number is one aspect of PE; therefore, grain dimensions may help to partly explain PE but not the full extent of this component of SW.

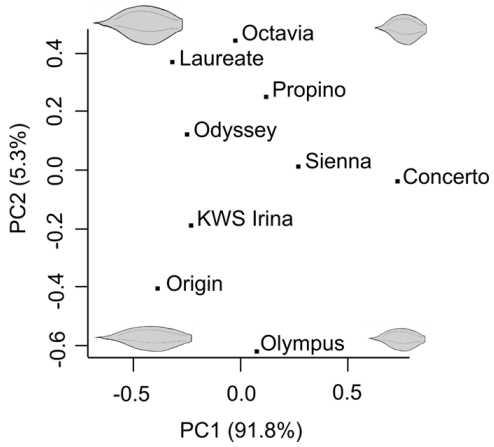

Figure 3-3 Biplot of the principal component analysis of grain shape parameters of nine spring malting barley cultivars. Grain dimensions used in this analysis: L, length; W, width and D, depth. Example grain shapes (not to scale) are shown on the plot to indicate which grain shapes have high or low scores in each of the principal components.

3.3.5. Combined Correlation Analysis on Grain Parameters

The significance of correlations between measured traits was analysed, and a matrix of Pearson correlation coefficients (r) is given in Table 3-3. The significant correlation between sphericity and grain 2-D area ($r = -0.77$, $P < 0.01$) highlights that more between grain number and length, ($r = -0.77$, $P < 0.05$) and confirms the discovery in the previous PCA that fewer longer grains pack into a cylinder. This can also be related to grain volume, since grain number and volume negatively correlate ($r = -0.72$, $P < 0.05$). The sum of grain depth and length in this analysis strengthened the correlation between the dimensions and both grain number and PE than just length alone. Another strong positive correlation was observed between area density and SW ($r = 0.81$, $P < 0.05$). Area density summarises the weight of grain in a given area and SW is a measure of the weight of grain in a given volume, therefore, the strong correlation between these variables was expected.

Table 3-3 Correlation matrix[a] of Pearson correlation coefficients (r) for grain dimensions, shape parameters and components of SW.

	Weight (mg)	Depth (mm)	Length (mm)	Width (mm)	Volume (mm^3)	Density ($g\ cm^{-3}$)	2-D Area (mm^2)	Sphericity (%)	Grain Number	Area Density ($mg\ mm^{-2}$)	SW ($kg\ hl^{-1}$)	PE (%)
Weight (mg)	1	0.26	0.46	0.28	0.89**	-	0.51	-0.20	-0.69	-	0.30	-0.16
Depth (mm)		1	-0.47	-0.47	0.13	0.36	-0.41	-	-0.15	0.68	0.31	-0.11
Length (mm)			1	0.58	0.56	0.02	0.85***	-	-0.77*	-0.44	-0.46	-0.57
Width (mm)				1	0.16	0.31	0.68*	-	-0.35	-0.44	-0.06	-0.36
Volume (mm^3)					1	-	0.58	-0.45	-0.72*	0.27	0.02	-
Density ($g\ cm^{-3}$)						1	0.16	0.17	-0.28	0.50	0.59	-0.15
2-D Area (mm^2)							1	-0.77**	-0.77*	-	-0.50	-0.57
Sphericity (%)								1	0.59	0.52	0.50	0.40
Grain Number									1	0.13	0.40	-
Area Density ($mg\ mm^{-2}$)										1	0.81*	0.45
SW ($kg\ hl^{-1}$)											1	0.59
PE (%)												1

[1] The symbol "-" indicates that one variable was used to calculate the other, therefore no correlation was calculated.

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

4. Increased grain density of spring barley (Hordeum vulgare L.) is associated with an increase in grain nitrogen (Paper 2)

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Authors: Aaron Hoyle, Maree Brennan, Gail Jackson and Steve Hoad.

4.1. Background

The quality of cereal grains is evaluated by different measures. In spring malting barley, specific weight is one important measure. Increased specific weight is thought to be associated with a higher malt output, but this has not yet been proven. Therefore, the value of specific weight as a malt quality indicator is disputed. Specific weight is the product of grain density and packing efficiency. We examined grain composition and density, to understand how specific weight relates to malt output. Our results show that both nitrogen content and the proportional volume of starch B-granules were positively correlated with grain density. An equation was built to predict grain density from grain nitrogen and the proportional volume of starch B-granules describing 47% of the observed variation in grain density. When validating the equation we found that starch B-granules were not as important for predicting density, but a model using nitrogen content alone was sufficient to estimate grain density. There is evidence that different genotypes and environments may require different coefficients for more precise prediction. These data show that nitrogen content is consistently correlated with grain density and, hence, specific weight. Therefore, a high specific weight could be detrimental for some malting end-uses.

4.2. Materials and Methods

4.2.1. Materials

Barley grains of five cultivars (Sienna, Laureate, Concerto, Olympus and Odyssey) from the Agriculture and Horticulture Development Board's (AHDB's) Recommended List (RL) 2016/17 were used in this study. These cultivars were selected due to their phenotypic range in grain size, SW and SGD (Hoyle et al., 2018). All cultivars were grown at AHDB's RL crop trials site in Docking, Norfolk under natural rainfall conditions in the 2016 season. Before analysis, grain samples were cleaned using a 2.50 mm slotted sieve, with 19.05 mm long slots and shaken for 20 s. Barley grains from a separate sample of Sienna were used to validate the equation derived from the original five cultivars. This sample was a commercial bulk provided by Bairds Malt and grown during the 2017 season, which contains spring barley grown across Scotland.

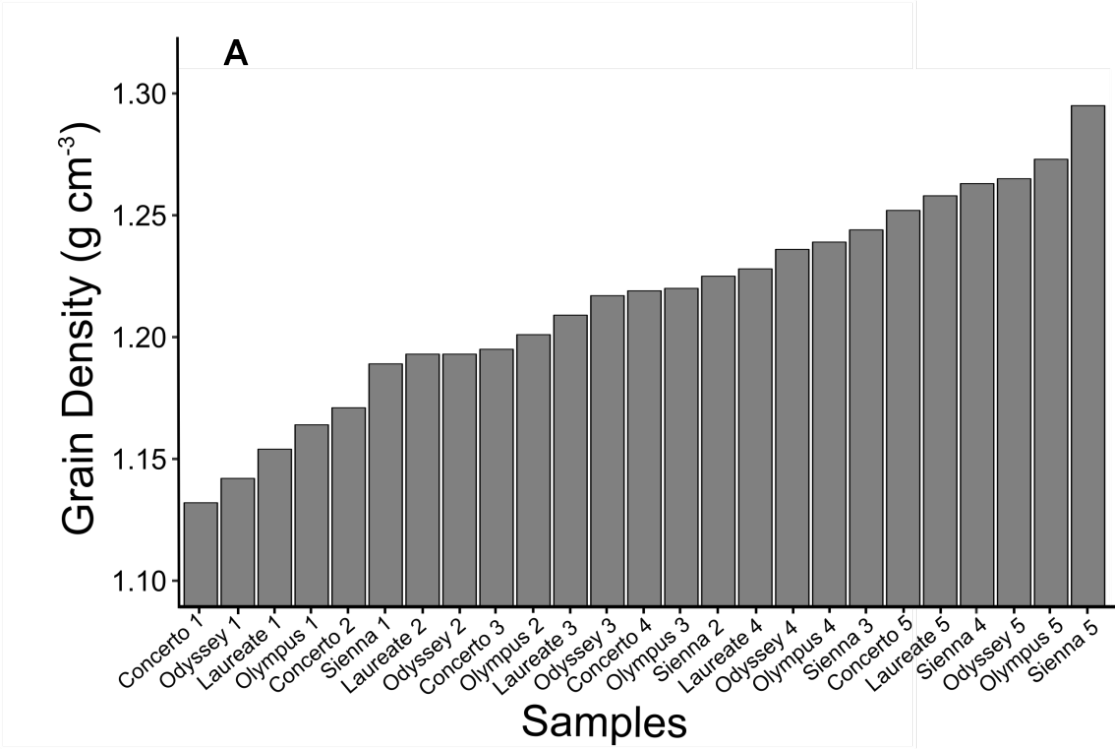
4.2.2. Sampling

In order to obtain a representative sample of grains to analyse, 350 g grain samples were sequentially sieved into a range of size fractions using a stack of slotted 3.25, 3.00 and 2.75 mm

sieves, with 19.05 mm long slots. The weight of grain in each size fraction designated; large (> 3.25 mm), medium (3.25 to 3.00 mm), small (3.00 to 2.75 mm) and very small (< 2.75 mm) was recorded using a Kern analytical balance PLJ 3500-2NM (accuracy ± 0.01 g). Three 100-grain samples were weighed from each size fraction, and the mean grain weight used to estimate the total number of grains in each fraction. A number of grains proportional to the total number of grains from each fraction were chosen at random, to give 300-grain samples which were representative of the total larger bulk sample, for each cultivar used in this study.

4.2.3. Grain Density and Sample Stratification

On each 300-grain sample, grains were individually weighed using a Mettler AE 160 electronic balance (Mettler-Toledo, accuracy ± 0.0001 g). The volume of individual grains was measured by placing them in a submersed, but suspended crucible in a beaker of water. The change in weight on the balance due to the buoyant force acting on the grain is equal to the weight of water displaced and, hence, the volume of the grain (Archimedes' principle). To create five density classes within each cultivar, grains were ordered by density. Density classes were created by grouping the 60 least dense grains and so on until the 60 most dense were left, creating 25 samples in total (Figure 4-1 A). In order to visualise the endosperm, and in particular, the starch granules within endosperms of different densities, scanning electron microscope (SEM) images were taken of five high density and five low density Laureate grains from the 60-grain sample (Figure 4-1 B, C).



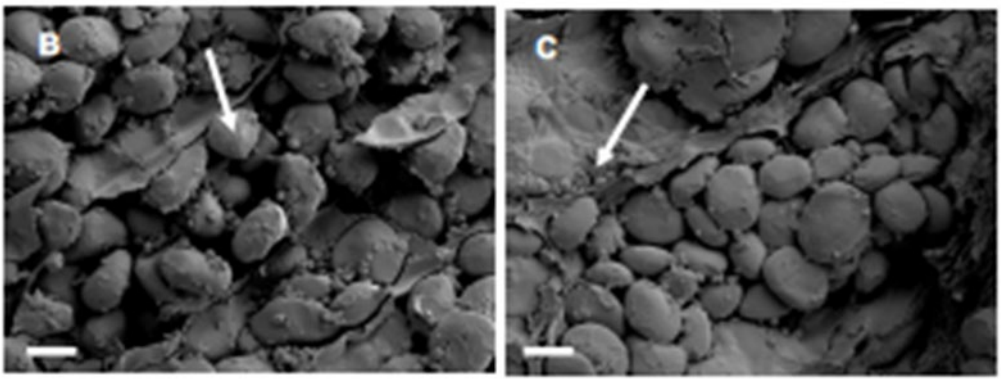


Figure 4-1 Range of grain densities created by stratifying grain samples (A) from five cultivars into five individual classes according to density. Concerto 1 referring to the least dense 60 grains of the 300-grain sample and Concerto 5 referring to the densest 60 grains. Scanning electron micrographs from cracked endosperms of spring barley cultivar Laureate, (B) high density and (C) low density. Scale bar = 10 µm. The arrow in fig 1B points to a large starch A-granule and the arrow in fig 1C points to a small starch B-granule.

4.2.4. Elemental and Starch Analyses

Twenty grains from each 60-grain sample were milled into a fine powder using a ball mill (Mixer Mill MM 200, Retsch, Germany) for compositional analyses. The proportion of carbon and nitrogen in the grain, typically referred to as carbon and nitrogen contents, were determined with a FLASH 2000 Organic Elemental Analyzer (Thermo Scientific). Total starch content and 2116 the ratio of amylose to amylopectin were measured using Megazyme kits: Total Starch Assay Kit (K-TSTA-100A) and Amylose/Amylopectin Assay Kit (K-AMYL) (Megazyme Ltd. Ireland) using the assay procedures provided by the manufacturer. Starch analyses are reported as percentage content for amylose and amylopectin (w/w) and ‘as is’ basis (g/100g) for starch content.

4.2.5. Starch Granule Isolation and Size Distribution Analysis

Starch was purified separately from three 10-grain subsamples of the 60-grain samples according to the “method 1” in Verhoeven et al. (2004) and then freeze-dried using an Alpha 1-4 LSCplus (Christ, Germany) overnight prior to analysis. A known mass of purified starch was dispersed in 100 ml of Isoton II Diluent (Beckman Coulter, United States). The size distribution of starch granules was determined with a Multisizer 4e Coulter Counter (Beckman Coulter) with a 70 µm aperture tube. The Multisizer measures the volume of each starch granule passing through its aperture between two electrodes using the Coulter Principle. In excess of 200,000 particles were measured per sample, and size frequency distributions were recorded in 400 logarithmically spaced bins between the diameter range of 1.4 µm to 42 µm. The number of granules passing through the aperture was counted and the surface area of these estimated by using the surface area of a sphere with the same

measured volume. Therefore, results of starch granule analysis include B-granule: number, volume and surface area. These are all reported as a percentage of the total for all measured granules. Consistent with previous studies (Chmelík et al., 2007), we used a threshold of 8 µm to distinguish between A- and B-type granules, as this threshold effectively approximated the minima between the size distribution curves of the A- and B-type granules.

4.2.6. Statistical Analysis

Data analysis was carried out in R software version 3.4.1 (R Core Team, 2019). Analysis of variance ($\alpha = 0.05$) was used to determine whether grain density class and cultivar had a significant effect on SGD, elemental analyses and starch analyses. Where a significant effect was indicated, a post-hoc Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$) test was conducted to determine which samples differed from one another. This is indicated by different letters in the results table. A stepwise linear regression was performed in R using the 'olsrr' package to determine which variables significantly contributed to predicting SGD and, therefore, should be included in the equation (Hebbali, 2018). The response variable was SGD, and the dependent variables were: nitrogen, carbon, total starch, amylose, and B-granule volume. Independent variables were selected based on p-value, the threshold for a variable to enter the equation was $P < 0.1$ and to exclude a variable from the equation was $P > 0.3$. The correlation between measured grain density and calculated grain density was determined using Pearson's product-moment in the R package "corrplot" (Wei and Simko, 2016).

4.3. Results

Single grain density and compositional variables including: nitrogen (N) content, carbon (C) content, total starch content, amylose/amylopectin ratio and starch B-granule; number, volume and surface area were measured on the 25 samples created by stratifying 300 grains from each cultivar into five density classes.

4.3.1. Effect of Single Grain Density on Grain Composition

Table 4-1 summarises the means and standard deviations of SGDs and compositional aspects of the five different density classes: very low, low, medium, high and very high. Stratifying samples by density created a range of 1.16 g cm^{-3} to 1.27 g cm^{-3}. No differences in C content were observed between the different density classes; this measure only had a small range of 39.85% to 40.23% from the medium and low density classes. Density had a significant effect on grain N content, with N content sequentially increasing with each density class. Nitrogen content of the very low and low class was 1.36% and 1.40%, respectively. These were both significantly ($P < 0.05$) lower than that of the very high class (1.53%). Starch content did not differ significantly among density classes. All

starch contents were within the range from 58.62 g/100 g to 58.78 g/100 g. Amylose content was highest in the very low density class (20.76%) which was significantly greater ($P < 0.05$) than the high density class (16.98%). The inverse was the case for amylopectin content. No significant differences were observed in the three measures of B granule content. However, the values increased sequentially from the very low density class to the very high density class as follows: B granule number 97.21% to 97.56%, B granule volume 20.20% to 23.55% and B granule surface area 54.79% to 59.05%.

Table 4-1 Grain density, elemental analysis and starch analyses on different density groups[a]

Density class	Grain density (g cm^{-3})	Nitrogen (%)	Carbon (%)	Total Starch (%)	Amylose (%)	Amylopectin (%)	B granule number (%)	B granule volume (%)	B granule surface area (%)
Very low	1.16±0.010^{d}	1.36±0.025^{b}	40.03±0.13^{a}	58.64±0.32^{a}	20.76±0.52^{a}	79.24±0.52^{b}	97.21±0.21^{a}	20.20±1.32^{a}	54.79±4.48^{a}
Low	1.20±0.009^{c}	1.40±0.012^{b}	40.23±0.07^{a}	58.69±0.07^{a}	18.57±1.04^{ab}	81.43±1.04^{ab}	97.29±0.26^{a}	22.02±1.44^{a}	56.88±4.81^{a}
Medium	1.22±0.008^{bc}	1.46±0.025^{ab}	39.85±0.20^{a}	58.78±0.26^{a}	18.34±0.96^{ab}	81.66±0.96^{ab}	97.44±0.16^{a}	22.40±1.31^{a}	57.76±3.83^{a}
High	1.24±0.007^{ab}	1.47±0.030^{ab}	40.14±0.13^{a}	58.75±0.62^{a}	16.98±0.45^{b}	83.02±0.45^{a}	97.47±0.22^{a}	23.09±1.52^{a}	58.58±4.64^{a}
Very High	1.27±0.007^{a}	1.53±0.046^{a}	39.92±0.14^{a}	58.62±0.41^{a}	19.40±0.82^{ab}	80.60±0.82^{ab}	97.56±0.12^{a}	23.55±0.98^{a}	59.05±2.60^{a}

[1] Data are reported on a wet weight basis and are means of five different cultivars ± standard error of the mean. When comparing mean values within a column those followed by different letters are significantly different from one another ($p<0.05$).

4.3.2. Effect of Cultivar on Grain Composition

Table 4-2 summarises the means and standard deviations of SGDs and compositional variables of the five spring barley cultivars; Sienna, Laureate, Concerto, Olympus and Odyssey. Mean SGD ranged from 1.24 g cm^{-3} for Sienna to 1.19 g cm^{-3} for Concerto, although no significant differences were observed among cultivars. No significant differences were observed in grain C or N contents among cultivars. Odyssey had both the lowest C and N contents at 39.85% and 1.41%, respectively. Sienna had the highest C content (40.22%), and Laureate the highest N content (1.50%). The total starch content of grains was highest in Sienna and Olympus which had 59.33 g/100 g and 59.17 g/100 g, respectively, both were significantly higher than Odyssey which had the lowest at 57.94 g/100 g ($P < 0.05$). The ratio between amylose and amylopectin did not differ significantly among the cultivars measured. The three measures of starch B granules; number, volume and surface area shown as a percentage of total granules, all showed similar patterns across the cultivars. Starch B granule number was highest in Laureate (97.75%) which was significantly higher ($P < 0.05$) than Odyssey (97.28%). Concerto's B granule number (96.75%) was significantly lower ($P < 0.05$) than

Table 4-2 Grain density, elemental analysis and starch analyses on five spring barley cultivars[b]

Cultivar	Grain density (g cm^{-3})	Nitrogen (%)	Carbon (%)	Total Starch (%)	Amylose (%)	Amylopectin (%)	B granule number (%)	B granule volume (%)	B granule surface area (%)
Sienna	1.24±0.018[a]	1.42±0.030[a]	40.22±0.10[a]	59.33±0.47[a]	19.81±0.64[a]	80.19±0.64[a]	97.67±0.12[ab]	23.28±1.03[a]	59.37±1.36[a]
Laureate	1.21±0.017[a]	1.50±0.038[a]	39.88±0.14[a]	58.73±0.23[ab]	18.49±0.98[a]	81.51±0.98[a]	97.75±0.07[a]	24.27±0.78[a]	60.51±0.79[a]
Concerto	1.19±0.021[a]	1.42±0.017[a]	40.09±0.17[a]	58.30±0.18[ab]	20.65±0.64[a]	79.35±0.64[a]	96.75±0.16[c]	17.86±0.88[b]	51.15±1.39[b]
Olympus	1.22±0.018[a]	1.46±0.064[a]	40.13±0.13[a]	59.17±0.26[a]	17.35±0.88[a]	82.65±0.88[a]	97.53±0.07[ab]	24.16±0.88[a]	59.44±0.99[a]
Odyssey	1.21±0.021[a]	1.41±0.023[a]	39.85±0.14[a]	57.94±0.16[b]	17.74±0.78[a]	82.26±0.78[a]	97.28±0.05[b]	21.69±0.40[a]	56.59±0.53[a]

[b] Data are reported on a wet weight basis and are means of five different density grades per cultivar ± standard error of the mean. When comparing mean values within a column those followed by different letters are significantly different from one another ($p<0.05$).

the other four cultivars. Concerto had significantly lower B granule volume and surface area (17.86% and 51.15%, respectively) ($P < 0.05$) than the other four cultivars. Laureate had the highest B granule volume (24.27%) and surface area (60.51%), but this was only significantly higher than Concerto ($P < 0.05$).

4.3.3. Correlations Between Compositional Traits

The significance of correlations between SGD and different compositional variables were analysed and a matrix of the Pearson correlation coefficients (r) are given in Table 4-3. The highly significant positive correlation between SGD and N content ($r = 0.61$, $P < 0.01$, Figure 4-2A.) highlights the effect of SGD on N content which was observed in 3.2. In addition to this, there is a significant correlation between SGD and B granule volume ($r = 0.55$, $P < 0.01$, Figure 4-2D.). These are the only two variables with which SGD is significantly correlated. Single grain density did not correlate with either C content or starch content (Figure 4-2B, Figure 4-2C). Alongside correlating with SGD, B granule volume positively correlated with N content ($r = 0.44$, $P < 0.05$), starch content ($r = 0.43$, $P < 0.05$) and was negatively correlated with amylose content ($r = -0.57$, $P < 0.01$).

Table 4-3 Correlation matrix of Pearson correlation coefficients (r) for grain density, elemental analysis and starch analyses.

	Grain density (g cm^{-3})	Nitrogen content (%)	Carbon content (%)	Starch content (%)	Amylose content (%)	B granule volume (%)	B granule number (%)	B granule surface area (%)
Grain density (g cm^{-3})	1	0.61**	-0.01	0.2	-0.31	0.55*	0.51**	0.55**
Nitrogen content (%)		1	0.1	0.09	-0.37	0.44*	0.34	0.41*
Carbon content (%)			1	0.19	0.05	-0.17	-0.19	-0.18
Starch content (%)				1	-0.12	0.43*	0.34	0.39
Amylose content (%)					1	-0.57**	0.37	-0.52**
B granule volume (%)						1	0.94***	0.99***
B granule number (%)							1	0.98***
B granule surface area (%)								1

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

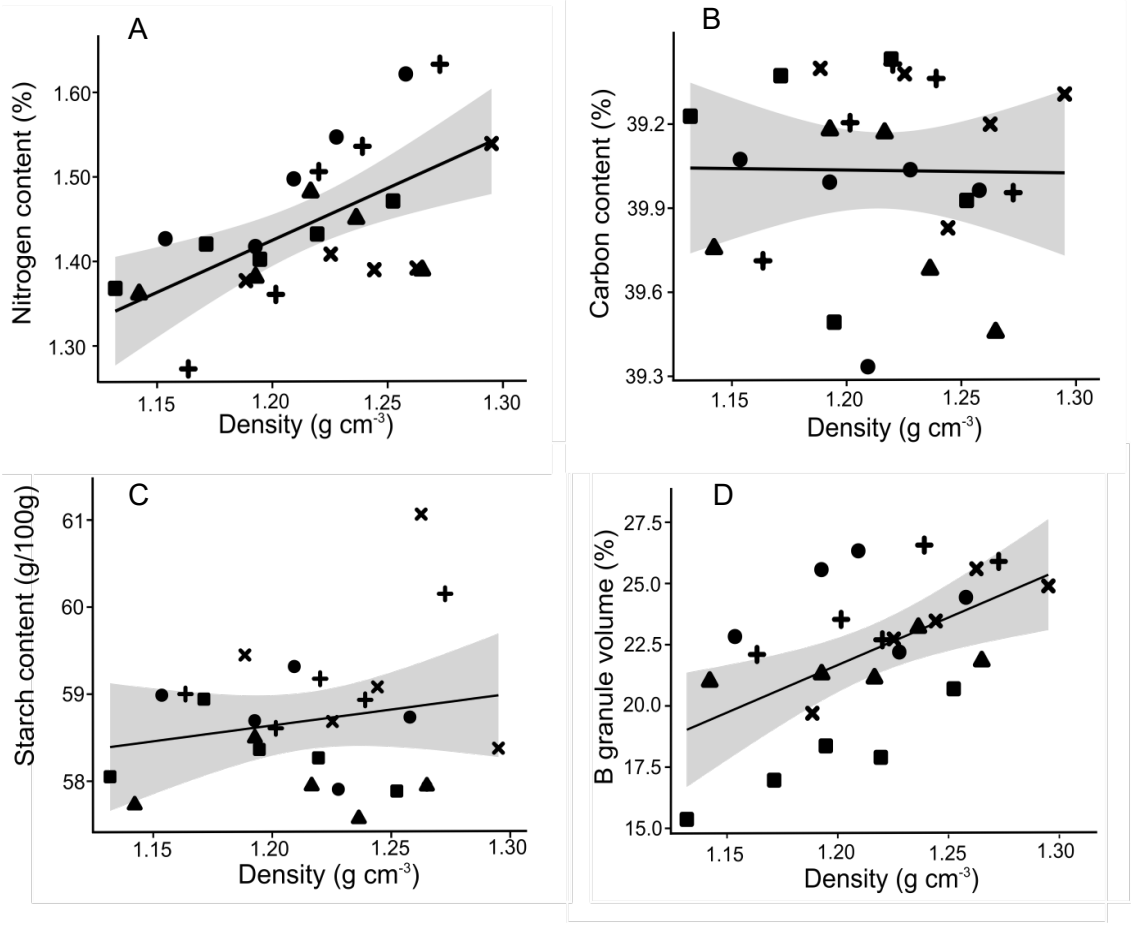


Figure 4-2. Regression analysis of grain density against grain constituents; (A) nitrogen content (r = 0.61, *P* = 0.001), (B) carbon content (r = 0.21, *P* = 0.948), (C) starch content (r = 0.06, *P* = 0.348) and (D) B granule volume (r = 0.53, *P* = 0.004). ■, Concerto; ●, Laureate; ▲, Odyssey; +, Olympus; ×, Sienna. Shaded areas represent the 95% confidence interval of the regression.

4.3.4. Predicting Single Grain Density from Compositional Traits

In order to determine the cumulative contribution of the independent variables to density (the dependent variable), a stepwise linear regression including all 25 grain samples was used. Independent variables which were calculated from one another (amylose/amylopectin) and those which displayed high levels of collinearity (B granule; volume, number and surface area) are represented only once by amylose and B granule volume, respectively. Stepwise regression analysis removed all independent variables apart from N content (%) and B granule volume (%). The independent variables removed were C content (%), amylose (%) and total starch (g/100 g). The predictive equation derived from this analysis was:

Density (g cm-3) = 0.779 + 0.224*N + 0.005*B

N - Nitrogen Content (%)

B - Starch B granule volume (%)

Nitrogen content alone described 37.1% of the variation in SGD. The addition of B granule volume to the equation resulted in the r^2 value increasing from 0.371 to 0.473, with the final equation describing 47.3% of the variation in SGD. The relationship between measured grain density and the predicted grain density using this predictive equation on the original 25 samples was highly significant (r^2 = 0.473, P < 0.001, Figure 4-3A). Each cultivar is likely to have a slightly different slope, therefore, this predictive equation may need to be altered for highly accurate predictions to account for different genotypes.

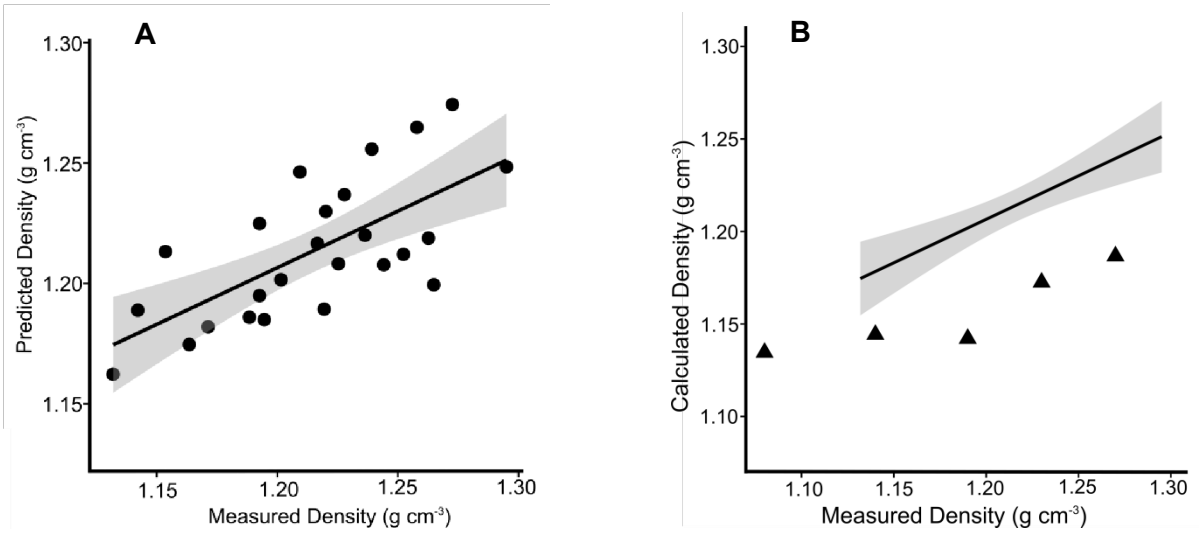


Figure 4-3 Scatter plots of measured grain density using Archimedes' Principle and predicted grain density using the predictive equation built in 3.3.4 for (A) the original 25 samples from five cultivars and (B) using N alone to predict the density of the validation five samples. The regression line (black) in both parts if formed from the original dataset, with the confidence interval of 95% shown by the grey shaded area.

4.3.5. Validation of the Density Equation

A separate sample of commercial barley grains from the cultivar Sienna was stratified in the same way to create five samples of differing densities to provide samples for equation validation. These were analysed for N content and starch B granule volume. The relationship between measured grain density and the predicted grain density (using the predictive model built in section 4.3.4) of the validation sample was not significant ($r = 0.83$, $P = 0.085$). However, when a model was built from the original data set using N content alone to predict density and applied to this validation set, a significant positive correlation with measured grain density and predicted grain density was observed ($r = 0.91$, $P < 0.05$, Figure 4-3B). When comparing grain density with B granule volume and measured grain density, no significant correlations were observed.

5. Relationship Between Specific Weight of Spring Barley and Malt Quality (Paper 3)

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5.1. Background

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 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 (PSY) ($r = 0.84$, $P < 0.01$), 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.

5.2. Materials and Methods

5.2.1. 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 as 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, 3348 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 based on whether their weight was above or below the mean weight of the corresponding fraction. 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 ± 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 5-1.

Table 5-1 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	
Concerto	D	3.00 to 3.25		35.5
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

5.2.2. 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 to 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).

5.2.3. Micromalting

Laboratory micromalting and malt analyses were performed using equipment at the Scotch Whisky Research Institute (SWRI, Roberston 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 were measured. 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 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 two minutes. This created a total of 30 malt samples for malt analyses. Prior to analysis, samples were stored in sealed bags to preserve their integrity.

5.2.4. Malt Analyses

5.2.4.1. Moisture and Nitrogen Analysis

Malt samples were first analysed by NIR using an Infratec 1241 Grain Analyser instrument (Foss Analytics, 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.

5.2.4.2. Friability and Homogeneity

A subsample of malt (50 g) was loaded into a Friabilimeter (Pfeuffer, Germany) and the machine ran for 8 minutes. 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.

5.2.4.3. 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 minute 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.

5.2.4.4. 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 minute period to 20°C and held at this temperature for 10 minutes. Samples were then made up to 450 g with water and shaken for 4 to 5 minutes, 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 yeast, and the 44 hour 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.

5.2.5. 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.

5.3. Results

5.3.1. 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 5-2.

Table 5-2 Mean values[a] for grain parameters measured on the ten grain 3450 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±2.66ab	60.82±0.18d	39.86±0.28a	1.32±0.02ab	30.19±0.49a
B2	39.08±0.62f	34.18±0.44f	1.15±0.03a	56.57±1.16ab	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±0.71ab	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±1.20ab	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±0.89ab	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±0.60ab	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±1.09ab	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±0.98ab	39.32±0.35a	1.23±0.08b	32.02±1.50a

[a] Mean values are expressed as mean ± standard deviation. [b] Fractions which do not share a letter for each of the measured parameters are significantly different from one another.

As expected, in fractions with increasing grain size, grain weight and grain volume increased from 29.06 mg and 26.66 mm^3 in fraction A to 57.94 mg and 50.87 mm^3 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 $mm^{3,}$ 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 (Figure 5-1A). 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.

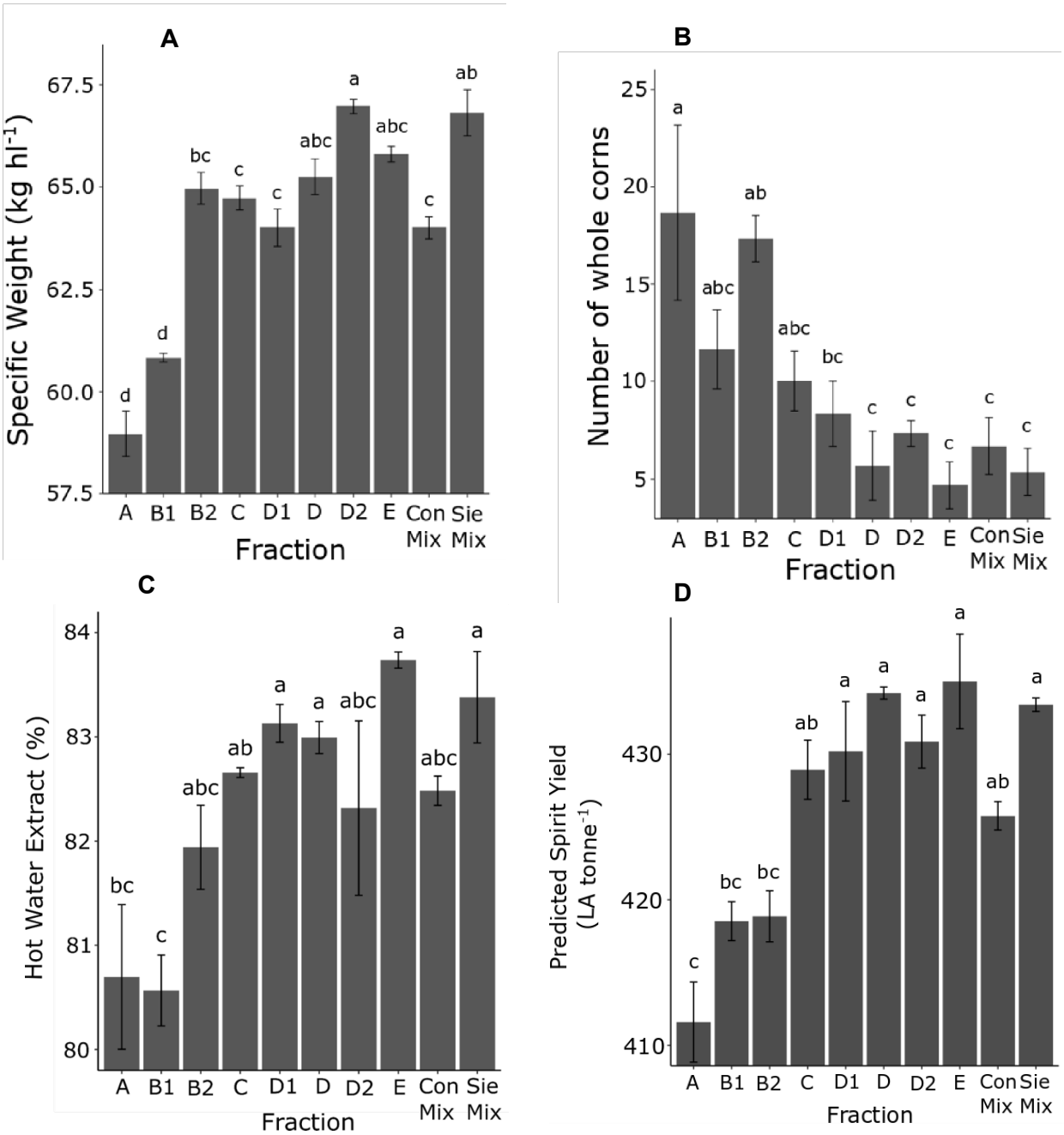


Figure 5-1 Mean values of (A) whole corns, (B) specific weight, (C) hot water extract and (D) predicted spirit yield. Error bars represent ± standard error of the mean (n = 3). G rain fractions with different letters are significantly different at P < 0.05.

5.3.2. Malt Quality Parameters

Malt quality parameters including PSY, HWE, friability, homogeneity and nitrogen 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 5-3.

Table 5-3 Mean values[a] for malt and starch quality parameters measured on the ten grain fractions[b] used in this study.

	Malt						Starch			
Fraction	Soluble Nitrogen (%)	Total Nitrogen (%)	Soluble Nitrogen Ratio (%)	Friability (%)	Homogeneity (%)	Number of Whole Corns	Moisture (%)	Predicted Spirit Yield (LA/tonne)	Hot Water Extract (%)	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±2.1ab	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±3.52ab	82.99±0.27ab	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. [b] Fractions which do not share a letter for each of the measured parameters are significantly different from one another.

All measures of malt N content which included soluble N, total N and the soluble N:total N ratio showed no significant differences between fractions. Friability was lowest in the smallest fraction, fraction A with 89.17% and highest in fraction D with 94.89%. Homogeneity was lowest in fraction B2 with 98.43% and highest in Sienna Mix with 99.35%. 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 (Figure 5-1B). 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% (Figure 5-1C). No significant differences were observed between malt moisture contents. Predicted spirit yield showed interesting differences across the fractions created in this study (Figure 5-1D), fraction A had the lowest PSY with 411 litres of alcohol per tonne (LA tonne^{-1}) which 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.

5.3.3. Correlations Between Grain and Malt Quality Parameters

Table 5-4 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:total 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) 3504 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).

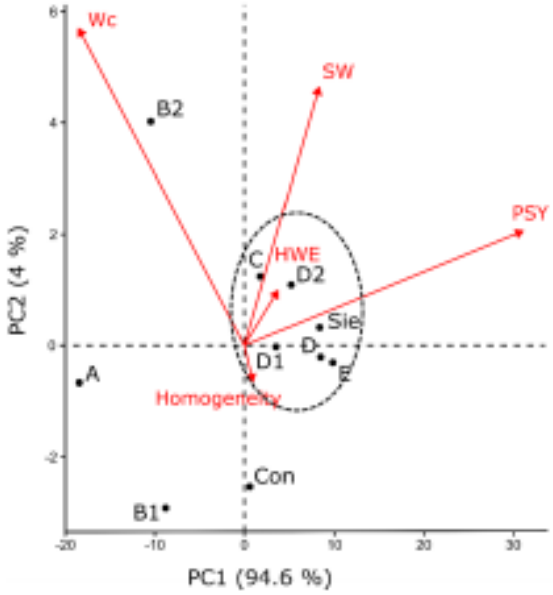
Table 5-4 Correlation matrix of Pearson's correlation coefficients (r) for grain and malt parameters.

		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.

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 (Figure 5-2) displays how grain fractions differ according to the aforementioned parameters. Figure 5-2 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.



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

Figure 5-2 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.

6. Discussion

6.1. Overview

The grain quality trait – specific weight (SW) – was the central focus of this study. The primary reason for investigating SW was to understand its physical and biochemical components and investigate association between this trait and the malting process, which to date had not been established. Furthermore, there was no quantitative information about how individual grain or bulk level parameters determine SW. The main aims of this research have been addressed through the:

i) identification of grain attributes of spring barley which contribute to SW,
ii) uncovering what grain compositional characteristics contribute to the density of barley grains,
iii) enhancement of the current understanding of how environmental conditions influence plant development and consequently, SW and iv) examination of the effects of SW and its components on the malting process.

The results of experimental papers 1 to 3 (sections 3 to 5) show how SW is determined by GD and PE, describe how grain composition (N content and starch B-type granules) is associated with GD, demonstrate how under a moderate but prolonged water stress, SW can be maintained and finally, established the impact of changing SW and its components on the malting process. In this final discussion, the findings and limitations of these are collated and discussed as a whole. Finally, avenues of future work on SW and related topics will be discussed, to suggest how to build on this current progress in understanding this grain quality measure.

Specific weight had previously been thought of as a singular grain quality trait, however, initial work demonstrated that this is not the case (Hoyle et al., 2018). Specific weight is, in fact, a product of two components: the mean GD of a sample and the PE of this sample. Each of these components are in turn, determined by many additional grain characteristics. ***Figure 0.1*** is not an exhaustive list of the grain traits that have the potential to influence SW but summarises the main traits to help portray the complexity of this measure. All of the measures outlines are a result of the interaction of barley genotype and environmental conditions, in this case, environmental conditions also including post-harvest grain handling. This is what differentiates SW from many other grain quality traits, which are a measure of solely one characteristic, for example N content. In terms of SW's relevance to the malting industry, it is unknown whether these two components of SW are beneficial for malting, deleterious, or if their effects change according to what has contributed to them.

Specific weight is quick to measure, however, GD at the grain level and PE at the bulk level are not, which may contribute to that fact that little research has been done on these components (Walker et al., 2013). Both of these components involve measuring the volume of irregular shaped barley grains,

which is in itself a science with many years of research dedicated to it (Walker and Panozzo, 2012). However, the difficulties in measuring these components does not undermine the important role they

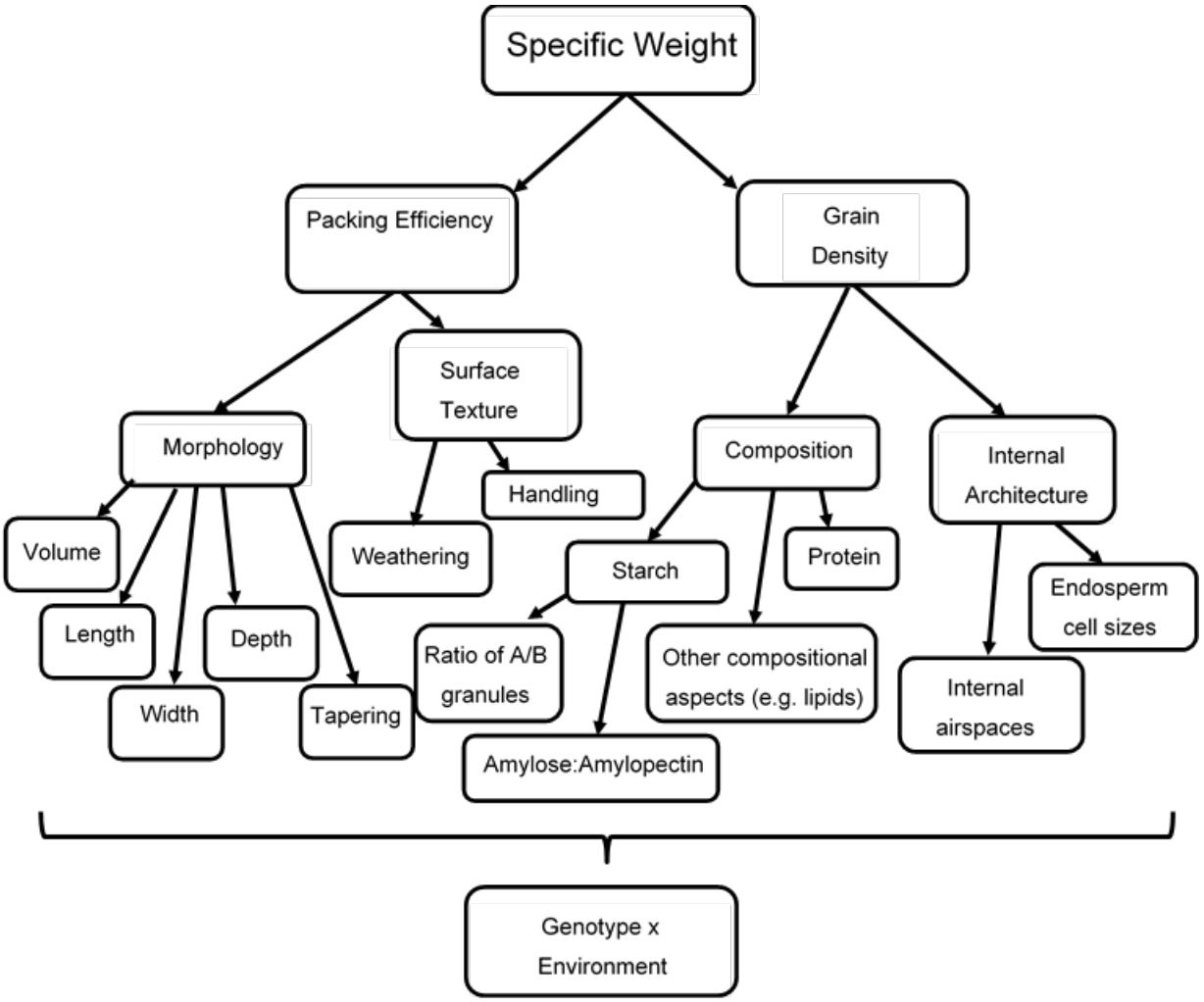


Figure 0.1 Specific weight and its components packing efficiency and grain density, with additional potential grain characteristics which contribute to SW.

could have upon malting. Initial work also established that both GD and PE contribute significantly to the variation in SW, i.e. it is not only one component that causes changes in SW. Therefore, both needed to be addressed in this research, which can inform future work on SW.

6.2. Packing Efficiency

6.2.1. Specific Weight is Determined by Packing Efficiency at the Grain Bulk Scale

As previously mentioned, research on the PE of barley grains has been deficient, however, some research exists on the PE of oat and wheat grains. It could be hypothesised that principles of PE are similar across the majority of cereal species. For example, if smaller wheat grains have increased PEs, it would be assumed that smaller barley grains also would. However, this may not be the case when comparing barley and millet due to their divergent morphology. Comparative study among

species was not tested in this thesis but could be an extremely useful approach in terms of integrating grain quality research in PE in future. The PE of oats has been shown to be influenced by genotype, but appears not to be by environment (Doehlert and McMullen, 2008). In addition to this, an increased PE in oats was associated with smaller grains (Doehlert and McMullen, 2008). Recent work on wheat has shown that long and narrow grains result in an increased PE, in comparison to more spherically shaped grains (Yabwalo et al., 2018). Small, needle-like grains are considered detrimental for malting, as a result of them having lower proportions of starch and higher proportions of protein in comparison to larger grains. Therefore, if smaller, or more needle-like barley grains increase PE, SW may not be the best indicator for an efficient malting process or indicative of a high quality malt.

The PE of grains within a volume (chondrometer) can be dissected further into: the number of grains in the given volume, and the mean volume of these (Hoyle et al., 2018). Therefore, variation in either of these, can result in a change in PE. Work from Section 3 demonstrated that individual grain dimensions and other measures of grain size negatively correlated with PE, but none of them significantly so. However, when investigating the effect of these on the number of grains in a given volume, grain length exhibited a significant negative correlation. In addition to this, when grain dimensions were combined, the sum of grain length and depth significantly and negatively correlated with both: the number of grains in a given volume and the PE of these. These observations are akin to those from previous studies on different cereal grains, that smaller grains can increase PE. This highlights a concern for end-users of grain for how PE and SW has been increased, if it was attributed to smaller grain alone.

Work in Section 5 suggests that not only mean grain dimensions, but the variation in grain sizes within a sample may influence PE. This hypothesis was a result of the two mixed fractions, containing grain from all size fractions having the highest PEs. Therefore, future studies could investigate not only the effect of grain dimensions on PE, but also the manipulation of the variation of these dimensions. A similar study has been performed on oats. Oat grain size distribution is different to other cereal species. Oats exhibit a distribution similar to a bimodal distribution rather than normal because of the presence of secondary grains (Doehlert et al., 2006). However, oats do not product a perfect bimodal distribution because of triple-grain spikelets. Therefore, mixed distributions of different size fractions in oats can be produced with relative ease, and it has been hypothesised that increasing the ratio of smaller grains will increase PE in oats by filling in those gaps left between larger grains. This was tested in a different study, however, the data did not support this hypothesis (Doehlert et al., 2004).

6.2.2. Packing Efficiency: Genotype and the Environment

Specific weight is a complex grain trait, influenced by many different grain characteristics, each of which can be influenced by environmental change. Additional work in this thesis indicated that a prolonged, but moderate water stress treatment did not alter SW or PE. However, water stress had

significant effects on plant development, which consequently influenced other aspects of grain quality. It was also demonstrated that SW is a product of GD and PE, even under water stressed conditions.

Despite water stress significantly reducing ear number, grain number, plant biomass, grain yield, harvest index and the length of grain filling, grain weight and morphology was maintained. Furthermore, PE was similar across all cultivars and growing conditions whilst a multitude of traits were being changed by water stress. These findings do no discount different effects of water stress upon PE that might occur in the field. For example, differences in the field could be a result of physical weathering of the grain, rainfall has been shown to result in the loosening and swelling of the seed coat in wheat (Gan et al., 2000). When this seed coat dries it is shrivelled which negatively influences SW.

6.2.3. Packing Efficiency and Malting

The water stress study in this thesis examined only the physiological effect of a water stress and did not include potential physical effects on the grain. The effect of misting on the grain quality trait, skinning has previously been studied, however, a similar experiment to investigate the effects of this on SW and malting quality would be of interest (Brennan et al., 2017). This would come with logistical problems of ensuring the misting treatment does not increase the moisture content of the misted pots. Also, enough grain would need to be produced to be used in a micromalting study, so the effect of this physical damage on malting can be investigated.

In future work, it would be interesting to investigate how PE influences steeping. This could determine if PE influences malting efficiency through another mechanism, rather than just the quantity of grain which can be included in a steeping vessel. Theoretically, if PE was increased to the extreme, difficulties would arise during steeping because grains would be in contact with less steep water.

6.3. Grain Density

6.3.1. Specific Weight is Determined by Grain Density at the Grain Level

The GD of barley grains was investigated in Section 4. The primary finding was that N content and the number of starch B-type granules explain roughly half of the observed variation in GD (Hoyle et al., 2019). Both of these positively contribute to GD. Endosperm texture was not examined in this study, but these findings seem consistent with the aforementioned research. Endosperm texture is not to be confused with surface texture. Surface texture refers to the roughness of the barley husk, whereas endosperm texture describes the hardness or susceptibility to crumbling. Barley grains are either classified as mealy or steely, mealy gains crumble easily, however, steely grains tend to fracture cleanly.

Nitrogen is a proxy for protein content in cereal grains, with a range of conversion factors recommended depending on the cereal species (Mariotti et al., 2008). Therefore, with denser grains having a higher N content, it is presumed that these are steely, having a more compacted endosperm

as a result of an increased protein content. However, this is just speculation, because in this study endosperm texture was not assessed alongside composition. This additional information would be useful to hypothesise further about the effect of a changing GD on the malting process through links with endosperm texture. Additional questions to be asked in future work include: are higher density grains always steely? Do lower density grains have increased volume of airspaces in the endosperm? Can higher density grains be created without increased protein content and steeliness? These are key questions which were not addressed in the thesis which would contribute great value to the SW debate.

Other researchers, have shown that lower density grains have a mealy endosperm texture, containing loosely packed starch granules in a patchy protein matrix (Walker et al., 2013), whereas steely grains have higher levels of C hordein, a glycoprotein commonly referred to as gluten (Ferrari et al., 2010). Mealiness and steeliness are measures of endosperm texture and have been shown to be intercorrelated with GD. However, whether composition is linked to GD remained unknown. In turn, grains with a mealy endosperm texture in comparison to steely textured grains are associated with increased modification rates in malting (Ferrari et al., 2010). Therefore, it may be a lower GD and increased mealiness which are beneficial for malting through achieving a more uniform modification. This could result in low SW samples with a low GD, malting efficiently due to having a mealy grain texture.

6.3.2. Grain Density: Genotype and the Environment

Grain density was not significantly affected by the water stress, but was so by cultivar, suggesting a strong genotypic effect on GD. Grain density had the same rank order as SW in this study for all cultivars, providing more evidence for the importance of GD in contributing to SW. In oats, it has also been demonstrated that across different genotypes, GD is more important than PE at accounting for observed variation in SW (Doehlert et al., 2009). In both the water stress study and in Section 4, Sienna had the highest GD of the cultivars examined. Sienna is marketed as a high SW cultivar, this thesis indicates this is a result of its high GD rather than a high PE, so in effect, it is a high GD cultivar. Despite GD not being affected by water stress, composition was affected by this stress. Nitrogen content increased in all cultivars as a result of the stress, this was not statistically significant, but the reduction in the C:N ratio was. This is an important finding, demonstrating that this change in composition and consequently grain quality is masked by a stable SW. Starch content and the composition of this starch in terms of amylose/amylopectin ratios were the same under water stressed and control conditions. One aspect of starch composition which was not analysed in this study was the ratio of A-type and B-type starch granules. This could have implications for downstream processing so would be a good trait to measure in relation to this environmental stress and other stress in the future.

6.3.3. Grain Density and Malting

Section 5 examined the effect of a changing SW on the malting process. In terms of its application to industry, this section is the most important, aiming to provide stakeholders with information on how SW affects the malting process. This is of particular importance with the lack of studies which have attempted to link SW with malting.

Specific weight was manipulated within one cultivar through changing both grain size and grain weight. This primarily resulted in variation in the GD component of SW, rather than PE. This approach provided a means to manipulate SW, with variation in GD, but without changing many other traits that may have impacted upon malt quality. Although as previously mentioned in Section5, PE was highest in the two mixed fractions used, which may be of interest to future work investigating if a mix of grain sizes is beneficial for PE. In general, SW was a good predictor at assessing malting output, correlating strongly with both HWE and PSY. Of the components of SW, GD also correlated with HWE and PSY, but PE did not.

In each micromalting run, samples consisted of 500 g of grain, and when malt analyses were performed in the laboratory 50 g of grist were used. This is all standard procedure for assessing malt quality. However, 500 g of grain will occupy a different volume depending upon its SW. Therefore, if assessing the output of each malting batch it may be worth changing micromalting protocols to requiring a volume of grain rather than weight of grain. This would be a more accurate reflection of industry because it is the volume of tanks that dictate how much can be malted, as opposed to how much weight can be held by them. This may be a reason why PE appears to show no correlation with malt output, whereas in maltings where tanks are filled by volume, it is likely that it could result in a higher throughput of malt.

This study manipulated SW within one genotype, Concerto; this aspect needs to be taken into consideration when relationships are observed between SW and malt quality parameters in this study. It is believed that this was the best way in manipulating SW, without changing many other traits that may have impacted upon malt quality. For example, if cultivars were used to create variation in SW, additional parameters would have been altered which may have impacted malt quality. This is demonstrated by the significantly reduced N content in the Sienna sample included in the micromalting in comparison to all Concerto fractions apart from Concerto B1.

6.4. Future Work on Specific Weight

The work done throughout this study has increased the current understanding of SW, but has also identified the areas for more research on SW. Although the contributing factors to PE have been elaborated on in this study, there is scope for more investigation on this topic. When investigating PE it is important to highlight whether changes in PE are between genotypes or within genotypes. Data

from Section 5 suggested that the variation in grain sizes may influence PE. This could be tested by the sequential sieving of more cultivars and measuring the PE of each fraction and the PE of the natural mix. This would confirm whether the pattern of increased PE in samples with a higher variation in grain size is consistent across cultivars.

The stratification and grouping of grains by GD were useful methods to examine differences between groups of grains of varying GD. This work could be developed further by including more measures. Due to the fact that grain texture, in terms of mealiness and steeliness, is known to affect the modification of grains, this would be an interesting trait to look into. Particularly as the initial work demonstrated that higher density grains had increased levels of protein which is typically associated with a steely grain texture. Additionally, the proportion of internal airspaces within the grain was not measured in this study, these would negatively contribute to density.

Exploring the relationships between environmental conditions and SW is a challenging task, with the multitude of different conditions that barley can be exposed to and the differing magnitudes of these. This work highlighted that not only SW and its components that need to be measured when investigating the potential effects of environmental conditions on malt quality, but also that other quality traits show SW can mask other changes. A useful further study would also include the physical effects of rainfall on SW. In recent years, increased rainfall at the harvest time for spring barley has become more common causing numerous harvesting issues. The effect of this on SW has not been investigated, neither has the downstream effects of this on malting. Therefore, a controlled environment experiment could effectively examine how wetting of mature grains close to harvest affects SW and malt quality.

As a result of recent progress on barley genetics, particularly the sequencing of the barley genome, possibilities in barley genetics have expanded greatly (Mayer et al., 2012). Quantitative trait loci (QTLs) have been identified for many malt quality parameters such as: malt extract, diastatic power, free amino acid content, protein content and soluble protein content (Fang et al., 2019). Numerous QTLs have been identified for GD on chromosomes 2H and 6H (Walker et al., 2013). However, no research has uncovered QTLs for PE, although it would be expected that these would be similar to those previously identified QTLs for grain dimensions. Identification of these, and potential SW QTLs would allow for marker assisted selection for SW, or either of its components. If SW could be manipulated in this way it would allow for further malting studies, to investigate how extremes of SW affect malting.

6.5. Conclusions

Specific weight is the product of two components; GD and PE; these are in turn, influenced by numerous other grain characteristics. Specific weight can be influenced simultaneously, both

positively and negatively by many of these characteristics (***Figure 0.1***). Despite SW being contributed to by many important grain traits which are indicative of grain quality for malting uses, it does not capture details of them all at once. For example, a high SW is presumed to often be beneficial for malting through conferring higher levels of starch, large and plump uniform grains within the sample. However, if GD has been increased through a high protein content within the grain, the increase in SW may not necessarily result in higher quality grain for malting. Similarly, if PE has been increased through an altered grain morphology to more needle-like grains, the higher SW from this may not convey higher quality. Nevertheless, SW is a useful and rapidly measurable indicator, which is generally indicative of barley grain quality. However, due to the complexity of this measure and its multifarious nature, other important grain quality traits can be masked. Therefore, it is important to measure this trait in tandem with other well established and understood grain quality parameters, in order to gain a reliable understanding of how a sample of grain will perform in a maltings and downstream uses.

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