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Understanding and predicting alcohol yield from wheat

by

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1. ABSTRACT

Bioethanol is a promising renewable biofuel and wheat is currently the main candidate as the feedstock for its production in the UK context. In general, the end-use quality determination of wheat in terms of alcohol yield has not been thoroughly investigated; breeders and growers are unclear about which characteristics of wheat are relevant to alcohol production and how to breed and cultivate for these characteristics.

This work focused on understanding and predicting the alcohol yield from wheat according to its physical, physicochemical and chemical characteristics. The research used the wheat samples of the *GREEN Grain* project, which consisted of a range of wheat varieties, agronomic regimes and growing sites from the four harvests years 2005-2008.

High alcohol-yielding wheats typically have high starch, mealiness and albumin+globulin fraction, and also low protein, gliadin fraction and hardness. They also have larger and more spherical kernels. The application of N fertiliser increases the protein components and yields smaller and more elongated kernels. High alcohol-yielding varieties tend to be softer with lower protein and larger and more spherical kernels. Alcohol yield could be predicted based on SKCS-reported values of hardness and diameter plus protein.

It is frequently hypothesised that larger and more rounded kernels produce more alcohol because they have a smaller relative amount of the unfermentable outer layers. To test this hypothesis, the pericarp thicknesses and crease characteristics of the wheat samples were measured. It appears that larger kernels tend to have thicker pericarp which largely eliminates the hypothesised benefit.

The Perten Single Kernel Characterisation System (SKCS) fundamental crushing force data (rather than the reported hardness values) were further analysed in an effort to improve alcohol yield predictions. It was found that the initial peak in the averaged Crush Response Profiles (aCRP) does not occur because of breakage of the “shell” (*i.e.* the bran layers), but is principally influenced by the crease; a deep crease gives kernels that are easier to break and that therefore exhibit a lower peak force. The aCRP parameters could improve the alcohol yield predictability of the *GREEN Grain* wheats to an R^2 of about 82% with a standard error of the regression of 6.3 l/dry ton.

2. SUMMARY

Bioethanol is a promising renewable biofuel and wheat is currently the main candidate as the feedstock for its production in the UK context. The quality of the numerous varieties of wheat developed in the past by plant breeders has been well examined in terms of bread, biscuit and pasta producing industries; similarly, farmers know how to grow wheats suited to these end uses. In general, the end-use quality determination of wheat in terms of alcohol yield has been less thoroughly investigated; both breeders and growers are less clear about which characteristics of wheat are relevant to alcohol production and how to breed and cultivate for these characteristics.

This work focused on understanding and predicting the alcohol yield from wheat according to its physical, physicochemical and chemical characteristics. The research ran alongside the *GREEN Grain* project and utilised its wheat samples, which consisted of a range of wheat varieties, agronomic regimes and growing sites from the four harvests years 2005-2008. The combined *GREEN Grain* dataset encompassed a diverse range of chemical, physicochemical and physical characteristics.

An initial multivariate analysis indicated that the first principal component, which explains most of the variability of the wheat characteristics, is related with the classification of wheat as hard or soft. High alcohol yielding wheats typically have high starch, mealiness and albumin+globulin fraction, and also low protein, gliadin fraction and hardness. They also have larger and more spherical kernels.

Analysis of Variance was applied in order to identify differences between the varieties, the sites and the application or not of N fertiliser. The ANOVA showed that the application of N fertiliser increases all the protein components, particularly the gliadin and the LMW glutenins. Application of N fertiliser also yields smaller and more elongated kernels. High alcohol yielding varieties tend to be softer with lower protein and larger and more spherical kernels. The following model, based on the SKCS-reported values of hardness and diameter plus protein, could predict the alcohol yield with an R^2 of about 78%:

$$\textit{Alcohol yield} = 466.62 - 5.07\times\textit{Protein} - 0.21\times\textit{Hardness} + 11.6\times\textit{Diameter} \pm 6.94 \text{ l/dry ton}$$

It is frequently hypothesised that larger and more rounded kernels produce more alcohol because they have a smaller relative amount of the unfermentable outer layers. To test this hypothesis, the pericarp thicknesses and crease characteristics of the wheat samples were measured. It was found that pericarp thickness and crease dimensions vary with kernel size,

with significant differences between varieties. A physical model was developed that accounted for these differences to allow calculation of the endosperm to non-endosperm ratio. None of the variables obtained by the physical model could be related to alcohol yield. It appears that larger kernels tend to have thicker pericarp which counteracts the smaller surface to volume ratio and eliminates the hypothesised benefit.

The Perten Single Kernel Characterisation System (SKCS) calculates kernel hardness and other parameters from the raw data and reports these calculated values. However, modern versions of the SKCS allow the raw crushing force data to be recovered and analysed directly, potentially allowing more meaningful interpretation and more useful predictions. The SKCS fundamental data were therefore further analysed in an effort to improve alcohol yield predictions. It was found that the averaged Crush Response Profiles are more reproducible than the hardness index itself. It was shown that the initial peak does not occur because of breakage of the "shell" (*i.e.* the bran layers) as suggested in the literature, but is principally influenced by the crease; a deep crease gives kernels that are easier to break and that therefore exhibit a lower peak force. Examination of the effects of moisture content on the aCRPs showed that their first quarter is equivalent to the stress-strain plots of dedicated rheological tests. The remaining parts of the curve relate to the post-failure behaviour of the kernels and to hardness, as used in cereal science. The aCRP parameters could improve the alcohol yield predictability of the *GREEN Grain* wheats to an R^2 of about 82% with a standard error of the regression of 6.3 l/dry ton.

Textural testing of cereals is constrained by the complexity of the wheat kernel structure and exacerbated by between-kernel variation. The current work has demonstrated how SKCS data can be interpreted more insightfully in order to improve end-use quality predictions. The aCRP parameters clearly contain rheological information about wheats. Further research to establish their examination by more standardised methodologies will allow effective investigation of connections between the rheological properties, chemical characteristics, processing behaviour and end-use quality prediction of wheat, helping breeders and growers to produce wheats suitable for biofuel production.

3. INTRODUCTION

Throughout the last century oil provided cheap energy. Economies, technology and human civilization generally developed rapidly, taking advantage of the low price and the abundance of fossil fuels. However, during the final third of the last century, both of these advantages of fossil fuels were challenged. It is now commonly accepted that oil stocks are finite, while their price currently follows an upward trend unlikely to change in the foreseeable future.

Moreover the environmental impact of fossil fuels has also been of great concern during the final decades of the last century. During the combustion of oil and its derivatives, CO_2 is emitted and accumulated in the atmosphere; this is generally believed by scientists to cause warming of the planet and climate change. These serious concerns led the governments of many countries to establish the Kyoto protocol in 1997, which provided a strategy for the reduction of the CO_2 emissions (Kyoto Protocol, United Nations Framework Convention on Climate Change (UNFCCC), http://unfccc.int).

Renewable sources of energy are needed in order to meet the protocol's targets. In the case of transport fuels, which account for about 20% of total global CO_2 emissions (Childs and Bradley, 2008), bioethanol and biodiesel are providing an immediate partial solution. These two biofuels are produced using crops rich in starch or sugar (cereals or sugar crops) or vegetable oil, respectively. The CO_2 emitted into the atmosphere during their combustion has been previously consumed for the growth of the plants, such that in principle, at least to a first approximation, biofuels could be carbon-neutral.

Throughout history cereals have been used principally to feed people and livestock. The environmental concerns caused by the overuse of fossil fuels prompted the beginnings of a solution through the use of cereals to produce transport fuels. This transition had the potential to take place quickly, since no major technological advances were required. This advantage, in combination with certain desirable geopolitical consequences, has led governments to subsidise biofuels from crops, in order to be more energy independent and to make progress towards the new energy standards.

The general consensus is that sufficient cereals can be produced to feed people and livestock and also to partially cover the needs of the transportation energy in the short term. The consumption of cereals for fuel use was less than 1% of total EU cereal production in 2007/2008. The estimated consumption of cereals for use in biofuels in the EU will be 4.3%

and 6.4% in 2010 and 2014, respectively. The equivalent percentage globally is currently about 4% (Anonymous, 2010).

Bioethanol can also be produced from lignocellulosic raw materials (such as wood residues, agricultural residues (wheat straw) and dedicated energy crops), abundant feedstocks with very low prices. These are the second generation bioethanol technologies. It is estimated that around 80% of total biomass is in the form of lignin and cellulose, and that today's transportation fuels consumption (10^{20} Joules per year), could be met from about 10% of the global arable land (Legge, 2008). However, the lignocellulosic to ethanol processes are not yet viable and there are major technological challenges that need to be met. Until then, bioethanol can be produced from cereals, replacing fossil fuels gradually while in parallel introducing the new infrastructures required for their complete replacement. Regardless of any macro economical environment and of any political or geopolitical interest, cereal scientists have to adjust their knowledge to include the novel uses of cereals (Legge, 2008).

The bioethanol industry favours starch-rich wheat varieties to improve the yield, since starch is the component that is actually converted to ethanol, while the food industry, and in particular the bread-making industry, prefers protein-rich varieties. When the starch content of wheat increases, protein in principle decreases (Schellenberger, 1964), therefore the protein content of wheat itself allows for an initial estimation of its end uses.

Although starch is the main component for bioconversion to ethanol, it is difficult to quantify it accurately and routinely, so is not capable itself to provide an accurate model for predicting the alcohol yield. Other factors affect the yield and the fermentation performance directly or indirectly, including protein content, hardness, non-starch non-protein components, residue viscosity and the size and shape of the kernel (Swanston *et al*., 2005, 2007; Agu *et al.*, 2006). Growers and industry favour reproducible and standardised processes for the accurate prediction of alcohol yield of a particular variety, field or delivery of wheat.

Moreover, quantifying the alcohol yield with respect to several other characteristics will also provide a target to the breeders. Breeding practices principally aim for the improvement of wheat yield, and secondarily to serve the needs of the food industry, by focussing on varieties that are appropriate for the breadmaking industry in particular. The complete investigation of the determination of alcohol yield and its correlations with other characteristics will result in improvements in breeding, growing and processing targets and practices in the light of the new demand for wheat for bioethanol production (Smith *et al.*, 2006).

The alcohol yield from wheat is an important but novel and less studied aspect of cereal end-use determination and quality prediction; what are the characteristics of a wheat that is suitable for distilling, how can breeding produce wheats with these characteristics, how can growers maximise desirable characteristics through cultivation practices, and how can processors effectively evaluate their quality relative to end-use at the biorefinery intake? More generally, our broader knowledge of end-use quality prediction needs to be updated with novel and effective methods, in order to allow better wheat management.

Therefore the objective of the current research was to understand the relationships between the chemical, physicochemical and physical characteristics of wheat and the resulting alcohol yield, in order to develop statistical- and mechanistically-based models that would deliver improved predictions of alcohol yield from wheat.

The current PhD project ran alongside the "*GREEN Grain*" project and utilized its wheat samples. *GREEN Grain* is a large LINK project sponsored by Defra and SEERAD in collaboration with HGCA, ADAS, Syngenta, Scottish Crop Research Institute, Scotch Whisky Research Institute, Wessex Grain, Grampian Country Foods Group, FOSS UK Ltd and Nottingham University. The project has the combined aims of genetically reducing the nitrogen emissions and growing costs of wheat production whilst enhancing the value of wheat grain for the bioethanol and grain distilling industries, for pigs and poultry feed and for other markets. The project seeks to achieve these goals by identifying wheat genotypes with minimal nitrogen storage in the stems, and reduced gliadin protein in the grain. (http://www.adas.co.uk/Home/Projects/Greengrain/tabid/277/Default.aspx).

4. THE CHEMICAL, PHYSICOCHEMICAL AND PHYSICAL CHARACTERISTICS OF WHEAT AND THEIR RELEVANCE TO END-USE QUALITY PREDICTION

The chemical, physicochemical and physical characteristics of wheat define in principle its end-uses. Therefore this Chapter surveys the current state of knowledge regarding the characteristics of wheat that are relevant to alcohol yield, in order to identify opportunities to undertake research that would enhance our understanding of the origins of alcohol yield and our ability to predict it from easily measured parameters.

4.1. Physical characteristics of wheat

It is known that the physical characteristics of wheat play a role in many aspects of cereal processing. It is frequently stated that larger kernels have a higher relative quantity of endosperm (endosperm to non-endosperm ratio). This has been used to speculate on variations of milling extraction rates and of alcohol yield (Scott, 1938; Pace 1959; Pence *et al.*, 1964; Smith *et al.*, 2006). Large wheat kernels, for example, resulted in better flour extraction than small kernels in a fixed milling system (Gwirtz *et al.*, 1999, cited in Osborne and Anderssen, 2003). In agreement with this finding in the context of alcohol yield, Smith *et al.* (2006) speculate that large well filled grains contain more endosperm and therefore more starch than poorly-filled shrivelled grains. Swanston *et al.* (2007) provided a model for the prediction of alcohol yield based on protein, Thousand Grain Weight (TGW) and length-to-width ratio. The partial coefficients of TGW, a measure of size, was positive, thus it adds to alcohol yield. Conversely the partial coefficient of length-to-width ratio, a measure of how well-filled the kernel is, was negative, thus well filled kernels add to alcohol yield. Evidently, the physical characteristics of the kernels influence their end-use quality. The current work aims to build on this work in order to provide a more detailed, mechanistic basis for understanding the observed relationships.

4.2. Chemical characteristics of wheat

4.2.1. Proteins

Protein content and quality are arguably the most crucial chemical parameters of wheat that define its quality and its end uses (Lynn, 2000). The proteins of wheat can be classified into soluble and storage proteins. The latter proteins when mixed with water form a network called gluten. The breadmaking industry favours in principal high protein wheats with good gluten quality.

The soluble (non-gluten) proteins, albumin and globulin, are highly heterogeneous (Pace, 1959), especially if it is considered that the germ protein consists mainly of soluble protein (Pence *et al.*, 1964). In any such active metabolic and genetic tissue, many enzymes, nucleoproteins *etc.* are undoubtedly present (Pence *et al.*, 1964). The first two fractions account only for about 1.5% of the grain weight and about 15-20% of the total wheat proteins. They are mainly derived from the germ and the outer layers of the kernel (Pace, 1959; Pence *et al.*, 1964). Albumins and globulins are mainly located in the outer layers of the wheat kernel with much lower concentrations in the endosperm (Goesaert *et al.*, 2005).

Gliadin and Glutenin (which together are the gluten proteins) form 40-50% and ~40% of the total protein of the grain, respectively (Lookhart and Bean, 1995). They are both mainly located in the endosperm. The protein concentration increases from the centre to the outer layers of the endosperm (Pace, 1959; Pomeranz and Schellenberger, 1961).

The protein fractions and their effects on dough and on bread have been extensively studied (Orth and Bushuk, 1972; Hamer *et al.*, 2009). The baking behaviour of flours has been correlated statistically with the albumin/globulin ratio, and it was concluded that a high ratio is indicative of good baking (Pace, 1959).

In general gliadins, low and high molecular weight (LMW and HMW) glutenins and their fractions in the total wheat protein play an important role on the breadmaking performance of flours (Uthayakumaran *et al.*, 1999); firstly, the gliadin to glutenin ratio and secondly, the quantity, the quality, the size distribution and the structure of the LMW and more importantly the HMW glutenins (Goesaert *et al.*, 2005).

4.2.2. Starch

Starch is the predominant chemical component of wheat. Together with a small quantity of free sugars it is bioconverted to ethanol in fermentations. For this reason starch was an important factor in the *GREEN Grain* project. Starch consists of amylose and amylopectin. Both fractions are almost fully degraded to glucose in a typical bioconversion to ethanol. The starch granules in the endosperm could be categorised as large or small. Brosnan *et al.* (1998) showed that the total amount of starch is more important for alcohol yield than the relative amounts of large and small granules in UK wheats. The starch digestibility was shown to be influenced by the fineness of the milled particles in the context of poultry feeding; it is negatively correlated with the hardness and the particle size of flour. A similar argument however could not be used in the context of alcohol yield since the process involves gelatinisation (Smith *et al.*, 2006). No other starch related characteristics were examined in the current study.

4.3. Physicochemical characteristics of wheat

Hardness is a commonly used parameter that describes the texture of wheat kernels. In particular it describes how difficult it is to penetrate the kernels or to reduce them to smaller particles (Mikulikova, 2007). Therefore the hardness of the grains is of major importance for the behaviour of wheat during milling (Campbell, 2007). Soft (mealy) wheats break easily into small particles but it is more difficult to separate them by sifting, while hard (vitreous) wheats break in general into larger particles that can be sifted more easily, but have a higher percentage of starch damage. Typically the end-use utilisation of wheat is dependent on the character of the texture of its kernels, as an indirect result of the variation of its chemical composition (Lillemo *et al.*, 2006). Soft wheats are preferred for making biscuits, cakes and pastries, hard wheats are preferred for the bread-making industry and yeast-raised products while durum wheat is used for making pasta (Tipples *et al.,* 1994; Turnbull *et al.*, 2003). Varieties preferred for distilling are in principle soft.

Pomeranz and Williams (1990), in a comprehensive review of wheat hardness research up until that time, observed "Kernel texture is the most important single characteristic that affects the functionality of a common wheat… a parameter of great significance in both the wheat and flour industry and in domestic and world trade [that] affects every aspect of wheat functionality except gluten strength and its associated factors.", and noted that over 100 different methods for measuring wheat hardness have been documented.

A vitreous (translucent) appearance is generally associated with hardness and high protein content, and opaqueness (mealiness or flouriness) with softness and low protein content (Carson and Edwards, 2009). Hard wheats in general have high protein and tend to be vitreous, although the causes for hardness and vitreousness are different (Anjum and Walker, 1991). Vitreous character is the result of a lack of air spaces within the kernel. Air spaces make the opaque grain less dense and it is believed that they are formed during grain drying. In general, hardness increases with protein between the classes but also between varieties of a particular class. The environmental conditions during growth influence hardness (Weightman *et al.*, 2008).

4.4. Objectives of the current work

Clearly the chemical, physicochemical and physical characteristics of wheat influence its end-use character in general. The use of wheat for alcohol production in particular has been examined less comprehensively than its more conventional uses. Therefore the objective of the current work was, in the context of the *GREEN Grain* project, to understand the origins of alcohol yield from wheat more fully, in terms of the characteristics of the wheat kernels, in order to be able to guide breeders and growers in selecting and growing wheats well suited for alcohol production, and to be able to predict alcohol yield more reliably.

5. THE *GREEN GRAIN* DATASET

The PhD studentship that led to this Thesis is part of the *GREEN Grain* project; all the wheat samples that were examined in this work were provided by the *GREEN Grain* project.

The wheat samples cover a range of varieties, grown with different levels of nitrogen fertilizer at several sites across the UK. Figure 1 shows the location of the major sites of the *GREEN Grain* project wheats. Table 1 provides the frequencies of the various subgroups. All the wheat samples represent four harvest years from 2005-2008. Most wheat varieties are soft and high alcohol yielding as used traditionally by the Scottish distilling industry, but there are also a small number of samples of hard wheats. The nitrogen fertiliser level for each year and site was usually different, representing the optimum according to some agricultural criteria. Complete details on the strategy that was followed for the selection of genotypes, nitrogen nutrition and breeding techniques of the *GREEN Grain* project wheats will be published in the future (Weightman R, 2009, personal communication). Table 1 also indicates that the data set is not balanced; the subgroups have different number of samples.

The *GREEN Grain* project partners (ADAS, SCRI, SWRI, FOSS) determined between them a range of parameters: the grain yield of the wheat samples (tonnes per hectare), the chemical composition (protein, starch, moisture, non-protein non-starch (by difference), various protein fractions expressed both as a percentage of dry material and as a fraction of total protein), the fermentation performance (residue viscosity and alcohol yield) and also physical characteristics (Thousand Grain Weight (TGW), length, width and length-to-width ratio). All the protein-related variables were measured both by High Performance Liquid Chromatography (HPLC) and by Near Infrared Reflectance (NIR). The physical characteristics were measured by Marvin Image Analyser (MIA). A few other variables were also measured on particular subgroups of the dataset including mealiness, percentage of grains smaller than 2.5 mm, α-amylase units. The latter variables, however, were not extensively analysed because they were measured only on a limited number of samples.

Figure 1. The locations of the major sites of the *GREEN Grain* project wheats

Table 1. The *GREEN Grain* project wheat frequencies

	Levels	**Frequencies**	**%**
Year	2005	132	25.43
	2006	124	23.89
	2007	134	25.82
	2008	129	24.86
N fertiliser	With	345	66.47
	Without	174	33.53
Site	HM	109	21.00
	Morley	3	0.58
	Rosemaund	7	1.35
	SCRI	176	33.91
	TT	202	38.92
	WH	22	4.24

HM: High Mowthorpe, TT: Terrington,

SCRI: Scottish Crop Research Institute, WH: Whittlesford

The contribution of the current PhD programme to the *GREEN Grain* project dataset was the determination of the dimensions of the kernels (length, width, depth) by the Rice Image Analyser (RIA) and of the mass, diameter, moisture content and hardness by the Single Kernel Characterisation System (SKCS).

Clearly, in the current PhD programme, only part of the experimental work was conducted by the author. The requirements of the current study involved the statistical analysis of the whole *GREEN Grain* project dataset. Full details of the *GREEN Grain* project wheats, including the details of these analyses, will be published by the collaborating institutes in due course.

The chemical, physicochemical and physical characteristics of UK wheats measured in the *GREEN Grain* project dataset provide an opportunity to study the inter-correlations between them, and in the context of this study, their correlations with alcohol yield. The following Chapter provides the results of the statistical analysis of the dataset.

6. STATISTICAL ANALYSIS OF RELATIONSHIPS BETWEEN WHEAT KERNEL CHARACTERISTICS AND ALCOHOL YIELD

For the complete statistical analysis of the dataset three statistical methods are used in this Chapter (Petrides, 1997):

1. Principle Component Analysis (PCA), in order to identify and visualise correlations between variables in a two-dimensional graph when the wheat samples are treated pooled.
2. Analysis of Variance (ANOVA), in order to examine these trends with respect to basic differences between the wheats (factors of variance): varietal, growing site and Nitrogen fertiliser (application or not).
3. Multiple Linear Regression (MLR), in order to quantify the observed trends and to provide a model that predicts alcohol yield.

6.1. Principle Component Analysis (PCA) of the dataset

The first statistical analysis conducted on the data set was a PCA, in order to identify how the variables relate with each other. The variables included in the PCA were the following:

- Physical characteristics:
    1. TGW adjusted to 15% moisture content
    2. SKCS diameter

3. Length
4. Width
5. Depth
6. Length-to-width ratio
7. Sphericity: $sphericity = \frac{\sqrt[3]{length \times width \times depth}}{length}$ (Equation 1)
8. Grain volume (volume of ellipsoid, Knud Thomsen, 2004):

$$volume = \pi \times \frac{length \times width \times depth}{6} \qquad \text{(Equation 2)}$$

- Chemical characteristics:
  1. Starch % (db)
  2. Protein % (db)
  3. High molecular weight glutenin protein fraction (HMW fraction)
  4. Low molecular weight glutenin protein fraction (LMW fraction)
  5. Gliadin fraction
  6. Albumin and Globulin fraction (ALB&GLO fraction)
  7. Moisture %
- Physicochemical characteristics:
  1. Hardness
  2. Residue viscosity
  3. Mealiness

The total number of variables is 19 including alcohol yield.

Table 2 provides the correlations between the variables. Many of the correlations are significantly different from zero. These are shown in bold and correspond to correlation values larger than (±)0.19, this being statistically significant (for a significance level $\alpha = 0.05$ and number of samples $n = 101$).

Table 2. The correlation matrix of the variables for the 2005 samples (*n*=101)

Variables	**Starch %DM**	**Res.Visc mPa**	**HMW fraction**	**LMW fraction**	**GLIA fraction**	**ALB&GLO fraction**	**Mealiness**	**Protein%**	**SKCS TGW**	**SKCS MC**	**RIA Width**	**RIA Length**	**RIA Depth**	**SKCS Diameter**	**SKCS Hardness**	**Length/Width**	**Grain Volume**	**Sphericity**
Starch %DM	1																	
Res.Visc mPa	-0.439	1																
HMW fraction	0.235	-0.191	1															
LMW fraction	0.294	-0.546	0.603	1														
GLIA fraction	-0.607	0.561	-0.613	-0.540	1													
ALB&GLO fraction	0.552	-0.380	0.122	-0.031	-0.793	1												
Mealiness	0.574	-0.599	0.475	0.492	-0.873	0.697	1											
Protein%	-0.651	0.481	-0.147	-0.084	0.807	-0.946	-0.771	1										
SKCS TGW	0.289	-0.367	-0.187	0.044	-0.205	0.314	0.302	-0.394	1									
SKCS MC	0.400	-0.210	0.186	0.161	-0.482	0.487	0.397	-0.424	0.305	1								
RIA Width	0.420	-0.540	-0.003	0.193	-0.489	0.538	0.510	-0.592	0.816	0.403	1							
RIA Length	-0.087	0.155	-0.635	-0.272	0.520	-0.299	-0.430	0.236	0.344	-0.072	0.028	1						
RIA Depth	0.206	-0.396	-0.098	0.123	-0.290	0.355	0.322	-0.396	0.743	0.343	0.736	0.096	1					
SKCS Diameter	0.218	-0.344	0.039	0.097	-0.299	0.333	0.353	-0.366	0.826	0.376	0.740	0.026	0.836	1				
SKCS Hardness	-0.432	0.624	-0.215	-0.542	0.636	-0.463	-0.726	0.578	-0.271	-0.201	-0.445	0.104	-0.251	-0.113	1			
Length/Width	-0.377	0.498	-0.449	-0.332	0.724	-0.602	-0.680	0.603	-0.361	-0.335	-0.716	0.675	-0.471	-0.526	0.397	1		
Grain Volume	0.255	-0.379	-0.309	0.037	-0.159	0.306	0.220	-0.373	0.880	0.323	0.834	0.463	0.869	0.765	-0.282	-0.289	1	
Sphericity	0.305	-0.467	0.420	0.309	-0.678	0.568	0.628	-0.558	0.364	0.345	0.660	-0.676	0.622	0.609	-0.332	-0.956	0.337	1
Alcohol yield l/dry t	0.678	-0.526	0.262	0.330	-0.804	0.776	0.766	-0.869	0.409	0.393	0.604	-0.275	0.425	0.399	-0.620	-0.642	0.380	0.601

Figure 2 shows the biplot of the two principal components. The variables that are projected closely to each other have a high positive correlation. The variables that are located in completely opposite directions have a high negative correlation. The variables that are located orthogonally in the biplot are not correlated. The first two principal components explain 66.27% of the total variability.

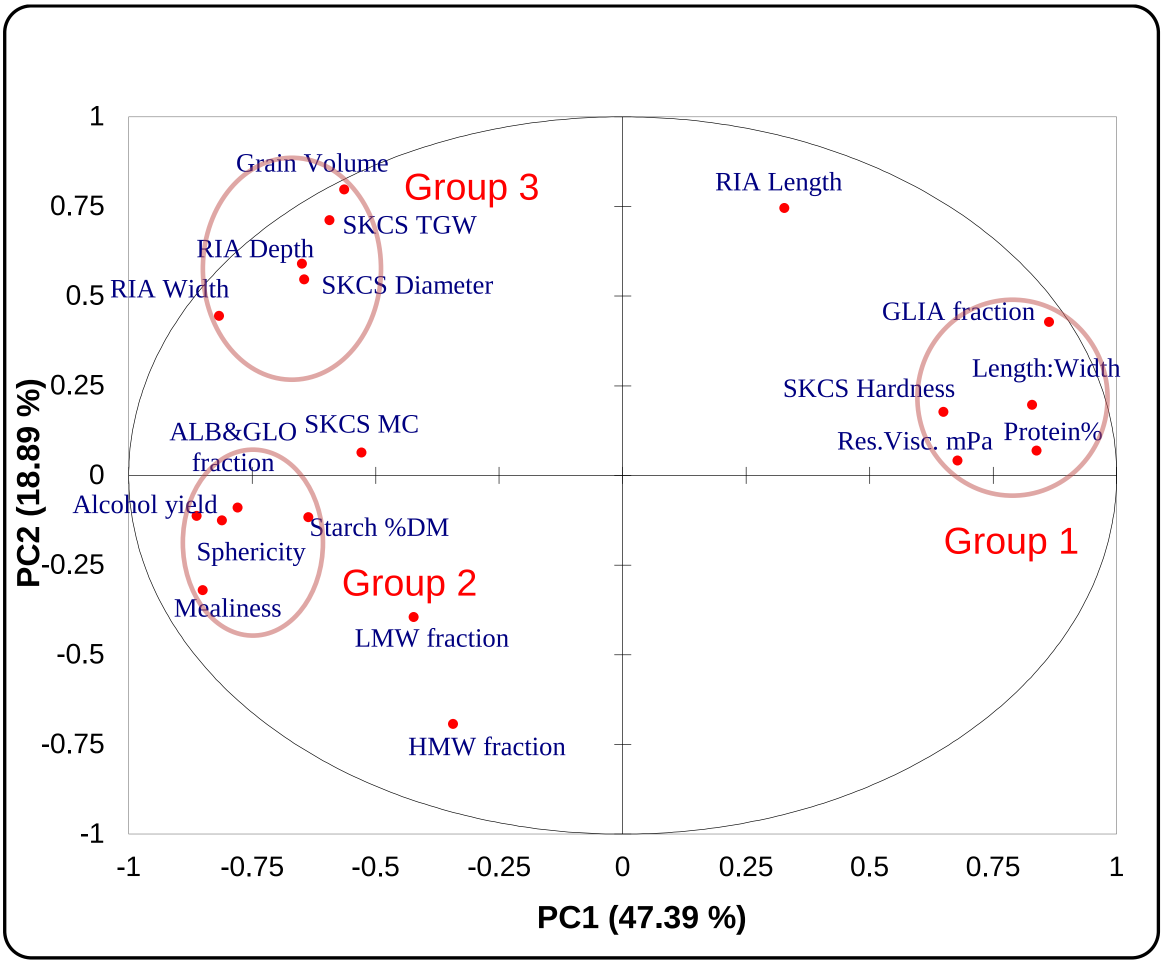

Figure 2. Biplot of the first two principal components (2005 data)

Many conclusions can be drawn by carefully examining the above map. Initially the variables can be categorised into three groups that lie closely together :

- Group 1: Protein, hardness, residue viscosity, length-to-width ratio and gliadin fraction
- Group 2: Alcohol yield, mealiness, sphericity, starch, and albumin and globulin fraction
- Group 3: Depth, width, volume, TGW and SKCS diameter

The variables of each group are highly correlated with each other. The variables between Groups 1 and 2 are highly negatively correlated, while Group 3, which is related to the size of the kernels, is somewhat positively correlated with Group 2 and negatively with Group 1.

In particular, Figure 2 in combination with Table 2 indicates that the alcohol yield is highly positively correlated with the albumin and globulin fraction, mealiness, starch, kernel width and sphericity. It is also highly negatively correlated with protein, gliadin fraction, kernel length-to-width ratio, and hardness (in agreement with Swanston *et al.*, 2005, 2007; Smith *et al.*, 2006). All of these variables score highly with respect to the first Principal Component, while the second PC seems to be mainly related with grain size (grain volume, TGW, length, width, depth, SKCS diameter) and HMW gluten fraction.

The first PC (47% of the total variability) is related with alcohol yield which is the focus of this research. As expected the PCA shows a high positive correlation between alcohol yield and starch, and high negative correlation between alcohol yield and protein. It is known that protein and starch content are also negatively correlated, since when protein increases, starch decreases. Thus preliminary observation of the PCA yields patterns that agree with expectations.

In fact there is a stronger correlation between alcohol yield and protein than between alcohol yield and starch, in agreement with Smith *et al.* (2006). This confirms the conclusion of Swanston *et al*. (2005) that the protein variation explains alcohol yield variation consistently better than starch. This is probably because the analytical measurements for the determination of protein are in general more accurate than the equivalent methods for determining the starch content (Smith *et al.*, 2006).

The albumin and globulin fraction has the highest positive correlation with alcohol yield, which may indicate a subtle relationship of these components with starch. Gliadin fraction correlation with alcohol yield is also very high. Clearly the expected variance of the protein fractions is evident in the particular dataset. Moreover they have very high correlations with alcohol yield. Therefore they are analysed in more detail later using ANOVA.

In addition, there is a high correlation between hardness and protein content (in agreement with Anjum and Walker, 1991). Mealiness on the other hand is negatively correlated with both variables (in agreement with Dobraszczyk, 1994). A preferred wheat variety for alcohol production also has low residue viscosity for minimizing processing problems (Smith *et al.*, 2006). The PCA shows that high alcohol yielding wheats have low residue viscosity. Clearly the first Principal Component is related to the chemical composition of wheat and its classification as hard or soft.

Width, depth, SKCS diameter, TGW and estimated grain volume are all highly correlated (Group 3), as expected as these all relate to kernel size. It is broadly believed that larger wheat kernels have higher ratio of starch-rich endosperm to non-endosperm components (Pence *et al.*, 1964). Therefore it is expected that the size of the kernels might affect the alcohol yield (Swanston *et al.*, 2007). The length-to-width ratio is negatively correlated with the width, the depth and the TGW; slim kernels weigh less than plumper ones of equivalent length. In addition it is broadly accepted that the size of the kernels is negatively correlated with protein and therefore with hardness as well (Ohm *et al.*, 1998; Muhamad, 2004). The PCA identifies all of the above correlations.

In addition, it can be shown that the correlations between alcohol yield and shape are higher than the correlations between alcohol yield and size. Shape characteristics belong to Groups 1 and 2, which are related to the chemical characteristics. On the other hand size characteristics belong to Group 3, which is indeed significantly correlated with the other Groups, however with smaller correlations. In any case the general trends of the PCA indicate that size and shape vary similarly with other characteristics. Therefore their variability is statistically analysed below with ANOVA.

6.2. Analysis of variance of the protein fractions and the size and shape of the kernels

The multivariate analysis of the data set indicated that alcohol yield is highly correlated with the chemical composition and the physical and physicochemical characteristics of the wheat. The statistical analysis so far examined only the general trends based on pooled wheat samples, with no consideration of factors such as growing site, growing conditions or variety that gave rise to sample characteristics and variability. It is now necessary to explore these trends in respect of the several distinct factors that add variance. Nitrogen (N) fertiliser, for example, is the single most important factor to be considered when growing wheat for bioethanol, due to its large effects on grain yield, protein content and therefore alcohol yield (Smith *et al.*, 2006). Other factors contributing to wheat characteristics include genetic (wheat variety) and environmental conditions (including soil quality and weather conditions; summarised as growing site). The most important variables are analysed in this section with respect to the levels of each factor.

Unfortunately the data set is not balanced; the total number of wheat samples of each subgroup is not the same. The imbalances are mainly on the genotypes; the data set includes many genotypes that were included only once or twice. There are however adequate numbers of samples for 12 varieties, most of which have traditionally high alcohol yield. The remaining factors of variance are better nested.

At this point it should also be clarified that the amount of N fertiliser was not the same for all sites. Depending on the quality of the soil, the three main sites examined in this research had different amount of applied N fertiliser. Details of the N nutrition strategy of the *GREEN Grain* project wheats will be published in the future. In the current study it is necessary to examine only two different levels; “with” and “without” N fertiliser.

6.2.1. Analysis of variance of the protein fractions

The total protein and the protein components were determined by NIR and HPLC, respectively. Table 3 summarises the ANOVAs of alcohol yield, total protein content, and the protein subgroups expressed both as DM% concentration and as fractions of total extracted protein. The F value of the ANOVA was larger than the limiting F value in all cases; therefore the means are not all equal. The analysis used the Fischer method to examine the multiple pairwise comparisons (Petrides, 1997).

Table 3. ANOVA of protein and its subgroups expressed both as dry material percentage basis and as fractions of total protein

	Protein DM%	**Alcohol yield (l/dry ton)**	**HMW DM%**	**LMW DM%**	**Gliadin DM%**	**Alb& Glob DM%**	**HMW fraction**	**LMW fraction**	**Gliadin fraction**	**Alb& Glob fraction**
Fertiliser										
Without	8.227^{a}	459.48 ^{b}	0.922^{a}	2.032^{a}	3.974^{a}	2.880^{a}	0.094^{a}	0.205^{a}	0.396^{a}	0.299 ^{b}
With	10.235^{b}	447.69 ^{a}	1.130^{b}	2.637^{b}	5.370^{b}	3.067^{b}	0.093^{a}	0.216^{b}	0.430^{b}	0.257 ^{a}
Genotype										
Consort	8.232^{a}	456.69 ^{c}	0.918 ^{bc}	2.066 ^{ab}	3.944 ^{a}	2.786 ^{ab}	0.095 ^{cde}	0.210 ^{bcd}	0.395 ^{ab}	0.290 ^{bc}
Zebedee	8.455 ^{a}	459.88 ^{c}	0.858 ^{ab}	2.144 ^{ab}	4.305 ^{abc}	2.925 ^{abc}	0.086 ^{bc}	0.210 ^{bcd}	0.407 ^{abc}	0.289 ^{bc}
Glasgow	8.477 ^{a}	458.85 ^{c}	0.984 ^{bcd}	2.089 ^{ab}	4.045 ^{ab}	2.673 ^{a}	0.103 ^{e}	0.215 ^{cde}	0.396 ^{ab}	0.280 ^{b}
Istabraq	8.519 ^{a}	454.62 ^{bc}	0.866 ^{b}	2.003 ^{a}	4.226 ^{abc}	2.822 ^{ab}	0.089 ^{bcd}	0.201 ^{b}	0.419 ^{cd}	0.286 ^{bc}
Wizard	8.603 ^{a}	455.10 ^{bc}	1.090 ^{cd}	2.361 ^{bc}	4.225 ^{abc}	3.041 ^{bc}	0.101 ^{de}	0.217 ^{cde}	0.394 ^{a}	0.289 ^{bc}
Riband	8.757 ^{ab}	456.83 ^{c}	1.112 ^{cd}	2.069 ^{ab}	4.048 ^{abc}	2.659 ^{a}	0.112 ^{e}	0.209 ^{bcd}	0.407 ^{abc}	0.275 ^{b}
Eclipse	8.800 ^{ab}	454.30 ^{abc}	1.056 ^{cd}	2.389 ^{bc}	4.544 ^{abcd}	2.994 ^{abc}	0.095 ^{bcde}	0.215 ^{cde}	0.414 ^{bcd}	0.279 ^{b}
Kipling	9.093 ^{ab}	442.82 ^{a}	0.699 ^{a}	1.837 ^{a}	4.731 ^{bcd}	3.190 ^{c}	0.069 ^{a}	0.178 ^{a}	0.443 ^{ef}	0.307 ^{c}
Atlanta	9.697 ^{bc}	456.73 ^{c}	1.116 ^{d}	2.660 ^{c}	5.444 ^{e}	3.167 ^{c}	0.091 ^{bcd}	0.212 ^{cd}	0.431 ^{de}	0.270 ^{b}
Soissons	10.568 ^{cd}	445.77 ^{ab}	1.297 ^{e}	2.453 ^{bc}	5.187 ^{de}	3.160 ^{c}	0.109 ^{e}	0.204 ^{bc}	0.410 ^{abc}	0.266 ^{b}
Buster	10.647 ^{cd}	439.37 ^{a}	0.905 ^{bc}	2.709 ^{c}	5.022 ^{cde}	3.318 ^{c}	0.075 ^{ab}	0.224 ^{de}	0.394 ^{ab}	0.279 ^{b}
Claire	10.926 ^{d}	439.28 ^{a}	1.413 ^{e}	3.238 ^{d}	6.343 ^{f}	2.946 ^{abc}	0.100 ^{cde}	0.227 ^{e}	0.449 ^{f}	0.225 ^{a}
Site										
SCRI	9.213 ^{a}	452.10 ^{a}	0.956 ^{a}	2.336 ^{a}	4.724 ^{a}	3.004 ^{a}	0.087 ^{a}	0.211 ^{a}	0.411 ^{a}	0.280 ^{a}
TT	9.449 ^{a}	453.85 ^{a}	1.092 ^{b}	2.424 ^{a}	4.733 ^{a}	2.995 ^{a}	0.098 ^{b}	0.212 ^{a}	0.412 ^{a}	0.273 ^{a}
HM	9.589 ^{a}	456.43 ^{a}	1.034 ^{ab}	2.385 ^{a}	4.871 ^{a}	3.204 ^{b}	0.091 ^{ab}	0.206 ^{a}	0.409 ^{a}	0.283^{a}

$^{a, b, c, d, e, f}$ indicate the differences of the means as obtained by the Fischer's pairwise comparisons at a significance level of 0.05

HM= High Mowthorpe, SCRI= Scottish Crop Research Institute, TT= Terrington

The alcohol yield of the *GREEN Grain* project wheats varied from 392-483 l/dry ton, a range of about 90 l/dry ton. Table 3 shows that alcohol yield is significantly reduced with the application of Nitrogen fertiliser (for this particular dataset by about 12 l/dry ton). There are significant differences between the varieties; the maximum was about 20 l/dry ton between Zebedee and Claire. Table 3 also shows that the application of N fertiliser increased the protein content by about 2% (from 8.2% to 10.2%). All the protein components expressed on a dry material basis also increase significantly with the application of nitrogen fertiliser (in agreement with Pence *et al.*, 1964). It also shows that although the gliadin and the LMW glutenin fractions increase, the HMW glutenins remain practically unchanged (selective increase of the storage proteins), while the albumin and globulin fraction decreases.

The means above are in agreement with the literature. They initially confirm that N nutrition is one of the most important factors influencing protein concentration and quality (Kindred *et al.,* 2008). The LMW glutenins and the gliadins appear to increase more than the HMW glutenins and the albumins and globulins (Pechanek *et al.,* 1997).

There were no significant differences of the total protein between the several sites. The gliadins and the LMW (expressed both as dry material percentage and as a fraction of total protein) are unchanged between the three sites. The albumins and globulins (DM%) appear increased in the HM site. However, if their fraction is examined there is no significant difference. The between-sites variance indicates that the only consistent difference is in the HMW glutenins fractions, which appear slightly increased in the TT site.

Genotype is a factor of variance that clearly influences protein and its fractions. Genotypes with the lowest protein content (Consort, Zebedee, Glasgow, Istabraq, Wizard, Riband) are actually the ones that are suitable for distilling according to Smith *et al*. (2006) and HGCA. The most consistent trend is that high protein varieties have higher gliadin and lower albumin and globulin concentrations and fractions (note the letters in Table 3). The between genotype variation is in line with the general trends obtained by the multivariate analysis.

Regarding the relationship between the protein fractions and alcohol yield, there is a remarkable consistency: high alcohol yielding wheats have low gliadins and high albumin and globulin fractions. This was obtained when the wheat samples were treated pooled (PCA) and also when subgroups of N fertiliser and genotypes were considered (ANOVA). The question that immediately arises is whether this is a direct or an indirect effect. This question cannot be answered by the approach of this study. It can be hypothesised that a direct relationship might indicate either: (i) increased albumins and globulins assist the fermentations due to their solubility and the nitrogen availability to the yeast; or (ii) the gliadins (and LMW to a lesser extent) somehow inhibit the fermentations or the starch availability to the yeast and its enzymes. A more simple indirect relationship would probably indicate that the concentration of albumins and globulins varies a little in wheat kernels therefore only gliadins (and LMW to a lesser extent) replace starch and practically reduce alcohol yield.

6.2.2. Analysis of variance of the shape and the size of the kernels

Similarly with the variance of the protein fractions, this section analyses the size and shape characteristics. The PCA indicated that, in general, high alcohol yielding wheats tend to have larger and more rounded kernels. The correlations between the shape characteristics and alcohol yield were higher than those between size characteristics and alcohol yield.

The shape and size characteristics in the current study were determined by the Rice Image Analyser (for the 2005 dataset), the Marvin Image Analyser (MIA) (for all years) and the SKCS (2005-2007). Table 4 summarises the ANOVAs of some basic size and shape characteristics. The sphericity was calculated according to Equation 1; combining the length and width as measured by the MIA and the depth as measured by the SKCS (measurements for these two devices are available for all harvest years).

Table 4. ANOVA of the basic size and shape characteristics

	MIA			**SKCS**		**MIA & SKCS**
	Length-to-width	**Length**	**Width**	**TGW**	**Diameter**	**Sphericity**
Fertiliser*						
**p*	*< 0.0001*	*0.275*	*0.021*	*0.112*	*<0.001*	*< 0.0001*
Without	1.749^{a}	6.651^{a}	3.803^{b}	48.838^{a}	3.120^{b}	0.645^{b}
With	1.799^{b}	6.695^{a}	3.727^{a}	47.849^{a}	3.021^{a}	0.630^{a}
Genotype						
Riband	1.666^{a}	6.499^{abc}	3.905^{e}	52.197^{ef}	3.200^{cd}	0.666^{f}
Consort	1.682^{a}	6.404^{a}	3.808^{de}	46.62^{bc}	3.102^{bcd}	0.659^{f}
Atlanta	1.734^{b}	6.425^{ab}	3.707^{bcd}	45.18^{ab}	3.042^{b}	0.649^{e}
Eclipse	1.762^{bc}	6.604^{bcd}	3.749^{bcde}	48.266^{cd}	3.109^{bcd}	0.644^{de}
Savannah	1.782^{bc}	6.906^{ef}	3.877^{de}	54.844^{f}	3.224^{d}	0.64^{cde}
Glasgow	1.787^{c}	6.536^{abc}	3.659^{b}	44.323^{a}	2.898^{a}	0.629^{bc}
Istabraq	1.790^{c}	6.760^{de}	3.779^{cde}	48.236^{cd}	3.033^{b}	0.630^{bc}
Wizard	1.794^{cd}	6.766^{de}	3.776^{bcde}	48.323^{cd}	3.001^{ab}	0.627^{ab}
Soissons	1.804^{cd}	6.607^{cd}	3.665^{bc}	46.159^{abc}	3.066^{bc}	0.636^{bcd}
Kipling	1.812^{cd}	6.853^{ef}	3.784^{cde}	48.282^{cd}	3.063^{bc}	0.627^{ab}
Zebedee	1.829^{d}	6.994^{f}	3.828^{de}	50.169^{de}	3.063^{bc}	0.620^{a}

Claire	1.900^{e}	6.757^{cde}	3.57^{a}	45.810^{abc}	2.975^{ab}	0.620^{a}
Site						
SCRI	1.75^{a}	6.500^{a}	3.700^{a}	48.552^{a}	3.082^{b}	0.645^{c}
HM	1.773^{a}	6.683^{a}	3.772^{a}	47.771^{a}	2.943^{a}	0.627^{a}
TT	1.788^{a}	6.785^{a}	3.798^{a}	48.708^{a}	3.082^{b}	0.635^{b}

$^{a, b, c, d, e, f}$ indicate the differences of the means as obtained by the Fischer's pairwise comparisons at a significance level of 0.05

* the probability that the two means of the two levels of N fertiliser are different

Genotype influences the size and shape of the kernels. In the current study, Claire and Glasgow have the smallest kernels while Savannah and Riband have the largest kernels. In parallel Zebedee and Claire have the most elongated kernels while Riband and Consort have the most rounded ones.

Table 4 also shows that size differences with respect to site are largely insignificant. Only the depth (SKCS diameter) of the HM samples appears significantly smaller than the other sites. The remaining differences are insignificant. However, when they are combined for the calculation of the sphericity they provide significant differences.

The application of nitrogen fertiliser reduces the width and the depth significantly ($p \approx 2.1\%$ and <0.1%, respectively), while it appears to increase the length, but not significantly. By combining these influences for the calculations of the shape characteristics, the differences become more evident (further reduction of p at <0.0001 for length-to-width ratio and sphericity). The application of nitrogen fertiliser results in more elongated kernels; increased length-to-width ratio and reduced sphericity (p<0.0001).

Figure 3 provides the interaction of the genotype and the nitrogen fertiliser on the a) MIA width (a typical size parameter); b) MIA length-to-width ratio; and c) sphericity (MIA and SKCS combined). Eclipse and Istabraq are the two varieties for which their width appears to be increased, Atlanta and Wizard are practically unchanged, while all the remaining varieties have reduced width with nitrogen fertiliser (Figure 3a). The latter is the predominant effect. The fact that not all varieties exhibit a consistent response to N slightly reduces the probability that the means are different (Table 4; p=0.021), although it remains well below the significance level of α=0.05. On the other hand the trends are much clearer regarding the shape characteristics (Figure 3b and c); the length-to-width ratio is increased and the sphericity is reduced with nitrogen fertiliser in all varieties (p<0.0001).

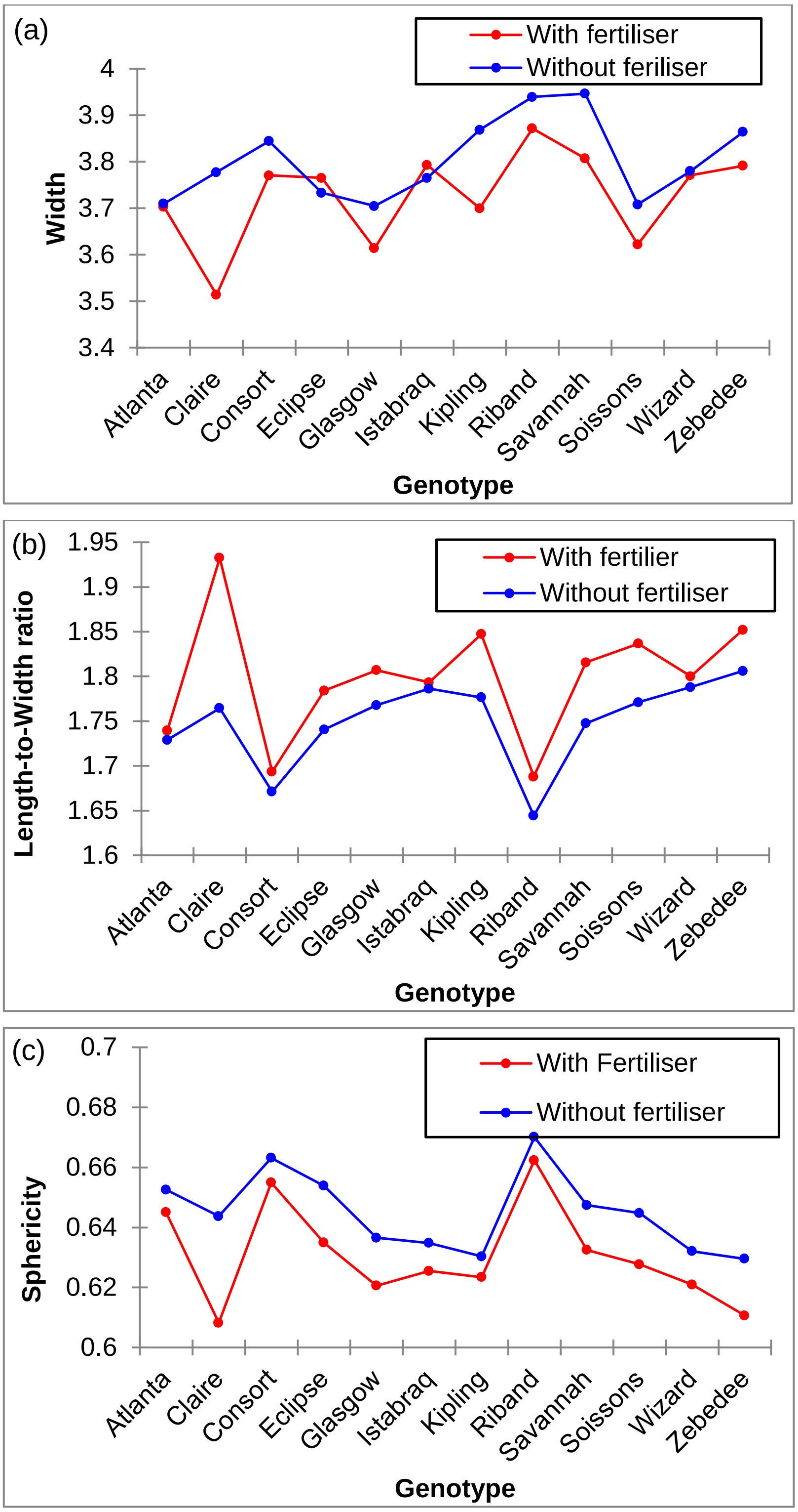

Figure 3. The interactions of genotype and nitrogen fertiliser with respect to a) MIA width b) MIA length-to-width and c) Sphericity

The general trends of the PCA indicated that the physical characteristics of the UK wheats relate with the chemical and the physicochemical. The ANOVAs clearly indicate that these trends are consistent between genotypes and between the N nutrition levels. Figure 4 shows the genotype averages of protein (a typical chemical characteristic) against width (a typical physical characteristic). Clearly, the PCA trends are consistent even between the genotypes. Glasgow is an outlier variety (in accordance with Swanston *et al.*, 2007). It is a high alcohol yielding wheat with soft kernels and low protein (Table 3). However, its kernels are small (Table 4). This contradicts the general trends identified so far. In fact Glasgow is the main outlier variety.

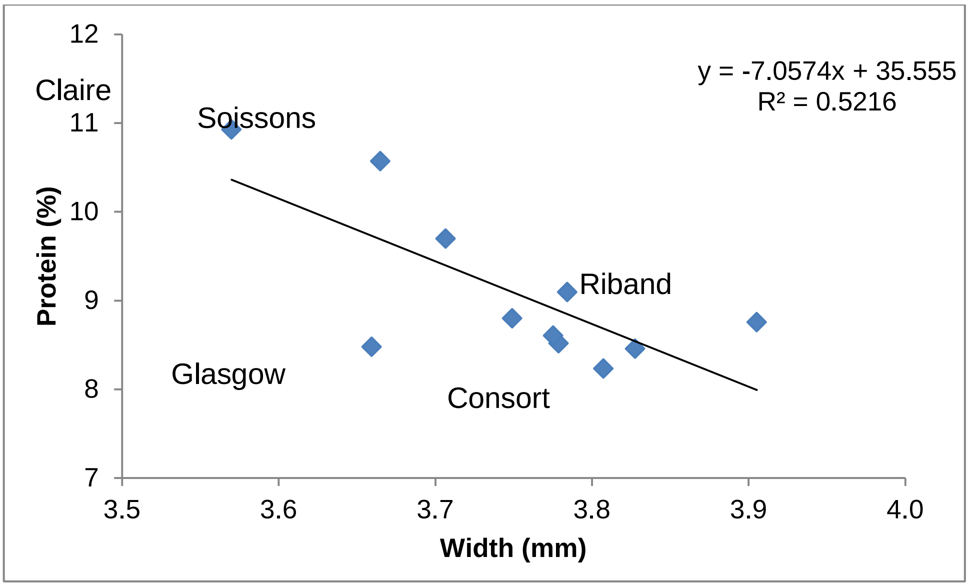

Figure 4. Genotype means of protein (%) against width

For each genotype the application of N fertiliser increases its protein content. In line with the general trends this increase results in smaller, more elongated kernels.

Other important variables like hardness were also examined by ANOVA. It was found that nitrogen fertiliser increased hardness significantly, in agreement with the close location of these variables on the PCA biplot. Harder varieties tend to have higher protein and smaller, more elongated kernels.

High alcohol yielding soft wheats with low protein tend to have more spherical kernels and, to a lesser extent, larger kernels. Clearly these are the predominant trends obtained by both the PCA (in which the wheat samples were treated pooled), and the ANOVAs (in which samples were divided into subgroups in terms of site, genotype and N nutrition). The PCA biplot captures a concise summary of these relationships.

6.2.3. Effects of nitrogen fertiliser on the size and shape of kernels

The current study focused on the variance of the size and shape of the wheat kernels and demonstrated statistically significant differences. The significant differences between the genotypes indicate that this variance occurs because of genetic differences of the several varieties. The significant differences between the several sites and N nutrition levels indicate that this variance is also affected by agronomic factors. One of the questions that immediately arises is why does the nitrogen fertiliser influence the size and shape of the kernels? The answer to this question requires dedicated experiments and knowledge derived from the combined agricultural, biological and plant physiology literature. Although it is not central in the current study, the following paragraphs present an attempt to explain this variance.

The controlled application of N fertiliser at the several stages of plant development increases both the number of tiller spikes and the number of grains per spike (Oscarson, 2000). This increases the yield of grain on a per hectare of land basis. In another experiment, the removal of a few grains per spike resulted in the enlargement of the remaining grains (Radley, 1978). This indicates that the grains act competitively in the spike and if this competition is reduced, they grow and fill more. The enlarged remaining grains had higher levels of auxins and gibberillines, the basic growth hormones of the plant development (Radley, 1978). Therefore it appears that the application of N fertiliser increases the total amount of grains, but the grains are smaller due to the increased competition. This mechanistic relationship is in agreement with the statistical relationships observed in the current work.

Another question that arises from the statistical analysis is: why is the depth consistently significantly reduced while the length appears to increase (albeit insignificantly) with nitrogen fertiliser? Considering that the number of grains per spike increases (Oscarson, 2000) and also the way that the kernels are attached on the spike then possibly their depth is practically and mechanically limited during growth. The depth is therefore smaller at maturity. That could also explain the combined slight increase of the kernel’s length, since the development of the grains could take place freely in that direction.

6.3. Application of Multiple Linear Regression for the prediction of alcohol yield

The previous sections established statistically the covariance of alcohol yield with other basic characteristics of wheat. This section examines which combination of characteristics (variables) can predict alcohol yield without performing costly and time consuming fermentations. The most appropriate statistical tool for this is Multiple Linear Regressions (MLR).

This section creates an MLR model based on wheat kernel measurements that are available for all the harvest years of the *GREEN Grain* project. Consideration is also given to the practical requirement that the predictors of the alcohol yield ideally need to be measured efficiently at the biorefinery uptake. For this purpose, the SKCS stands out as its measurements can be obtained in 5 minutes; meaningful predictions based solely on SKCS measurements would therefore be particularly valuable. However, wheat is typically traded on its protein content, so it could be assumed that this might also be known at the biorefinery intake. Therefore this section applies best subsets regression for the creation of a model based on the SKCS parameters plus protein.

The variables that were considered were protein, TGW, SKCS diameter and SKCS hardness. Moisture content was excluded. Table 5 shows the best subset regression results of the 2005 samples. The best combination of variables according to several statistical criteria (the R^2_{adj}, Mallows C_p and the S), which have common optima, were: Protein, SKCS Hardness and SKCS Diameter. The other two harvest years also yield the same combination. These variables together can provide an R^2_{2005} of about 78.7%.

Table 5. Best subsets regression for the 2005 dataset, based on protein content and SKCS measurements

Variables	**R^2**	**R^2_{adj}**	**Mallows C_p**	**S (l/dry ton)**	**Protein**	**TGW**	**SKCS diameter**	**Hardness**
1	75.3	75.1	15.6	6.17	✓			
1	38.4	37.9	191.3	9.75				✓
2	77.4	77.0	7.5	5.93	✓			✓
2	76.2	75.7	13.6	6.09	✓		✓	
3	**78.7**	**78.0**	**3.7**	**5.80**	✓		✓	✓
3	77.9	77.2	7.4	5.90	✓	✓		✓
4	78.8	78	5	5.80	✓	✓	✓	✓

The model based on the SKCS is the following:

$\mathit{Alcohol\ yield} = 478.14 - 4.984 \times \mathit{Protein} + 8.256 \times \mathit{diameter} - 0.118 \times \mathit{hardness} \pm 5.8$ l/dry ton ($R^2_{2005} \approx 78.7\%$)

As expected protein and hardness reduce alcohol yield, while diameter increases it. The variables use completely different scales, therefore the coefficients of the model do not actually correspond to the contribution of each predictor to the alcohol yield variation. For this reason the standardised coefficients are used. Figure 5 shows the standardised coefficients of the model with their 95% CI spaces. Protein contributes most to the model (a chemical characteristic), followed by hardness (a physicochemical characteristic) and then diameter (a physical characteristic).

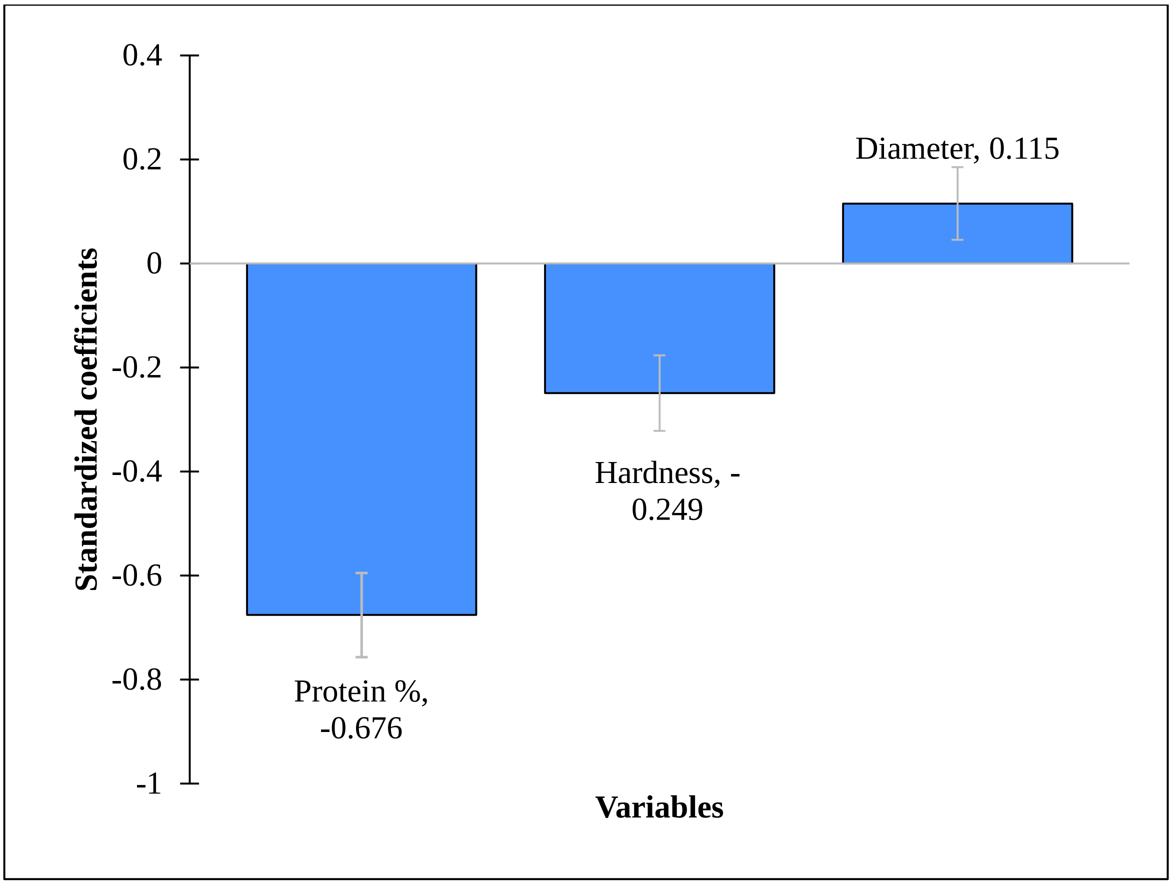

Figure 5. The standardised coefficients of the alcohol yield model based on SKCS measurements and protein content

This model was generated by the 2005 wheat samples. Two aspects should be raised at this point: i) the optimum partial coefficients of the predictors and the intercept vary slightly when different harvests are used; and (ii) the maximum R^2 achieved is slightly different for each harvest year. This probably reflects other differences between the years that are not taken into consideration (weather conditions, slightly different levels of N fertiliser). For this reason it is more appropriate to perform a regression using the data of all three years. Such a model averages the differences and therefore it is more applicable for future harvests. The final SKCS alcohol predicting model proposed is the following:

Alcohol yield = 466.62 – 5.07 × protein – 0.21 × hardness + 11.6 × diameter ± 6.94 l/ dry ton (R^2 ≈78.2%)

This model, like the others, assumes protein to be measured as %db, hardness in the arbitrary units reported by the SKCS, and diameter in mm as reported by the SKCS. Using these units, the equation predicts alcohol yield in units of litres per dry ton.

By considering all years, the R^2 of the model is slightly reduced to 78.2%. If the basic outliers of the regression are excluded then the R^2 is improved to about 82%. Clearly, protein is the predominant predictor, while hardness and diameter together add about 5% to the R^2. Although their contribution is relatively small, they reduce the standard error of the regression, as can be seen in Table 5.

6.4. Summary

This Chapter presented a detailed statistical analysis of the *GREEN Grain* project dataset, drawing together existing data with new data added from the current PhD project. The PCA identified the general patterns of variation of the chemical, physicochemical and physical characteristics of UK wheats. High alcohol yielding wheats with low protein content and hardness typically have more spherical kernels and, to a lesser extent, larger kernels.

The ANOVA has demonstrated that these trends are consistent between the N fertiliser levels and between the genotypes and growing sites. N fertiliser increases the protein content, and appears to increase the gliadins and the LMW glutenins more than the HMW glutenins and the albumins and globulins. In parallel it makes the kernels thinner. Similarly, genotypes that have rounded and larger kernels tend to be softer and have lower protein content. Glasgow is an outlier variety, being soft with low protein but with small kernels.

This consistent variability allows the prediction of alcohol yield without performing costly and time consuming fermentations. The SKCS outputs together with protein can predict alcohol yield with an R^2 of about 80%. The remaining variability cannot be explained by the variables considered so far.

7. PERICARP THICKNESS, SKCS FUNDAMENTAL DATA AND THEIR RELATIONSHIPS WITH ALCOHOL YIELD

Two central questions were raised by the analysis of the previous Chapter: (i) why do wheats with larger kernels tend to yield more alcohol? and (ii) can the SKCS raw data (rather than the reported parameters that the SKCS calculates from the fundamental data) improve the predictability of alcohol yield further? Within this studentship, an effort was made to answer these questions which are detailed in the complete thesis. In this report only the results of the investigation are presented.

7.1. Pericarp thickness and the development of a physical model

Regarding the first question, it is frequently hypothesised that larger kernels have a higher endosperm to non-endosperm ratio (Scott, 1938; Pace 1959; Pence *et al.*, 1964; Smith *et al.*, 2006). This hypothesis has mainly been used to speculate on variations of milling yield, but also on variations of alcohol yield. It makes sense to hypothesise that kernels with higher (starch-rich) endosperm to non-endosperm ratios produce more alcohol. However, no article has ever been published systematically analysing this hypothesis. The key measurements required to test this hypothesis are bran thickness and kernel dimensions.

This hypothesis could explain the relationship between size and shape characteristics with the chemical characteristics and in the context of this study with alcohol yield. It was further hypothesised that different wheats may exhibit different bran thicknesses, which would also affect their alcohol yield potential; thicker bran implies less endosperm and hence less fermentable material. These hypotheses, which are related (bran thickness may vary with kernel size and shape), have not previously been tested. The most appropriate way to test these hypotheses is to measure the outer layers of the kernels and to create a physical model, in order to estimate their variances, their contributions to the kernel's weight, hence the endosperm contribution and therefore the variances of these quantities with respect to alcohol yield.

The pericarp thickness and the crease characteristics were determined by scanning cut kernels across the crease in a high resolution commercial scanner and measuring them using image processing and analysis software. An ANOVA indicated that the pericarp thickness increases together with the size of the kernels. There were also differences in pericarp thickness between wheat varieties. These were in line with the kernel size differences, but if the pericarp thickness was divided by the width or the depth to create dimensionless ratios, the ratios also differed between the varieties. Similar results were obtained for the crease characteristics, showing that variations in pericarp thickness and in crease morphology could contribute to variations in the ratio of endosperm to non-endosperm material in different wheats and hence to variations in their alcohol yield potential.

Combining the pericarp thickness measurements with kernel dimensions, using fitting of ellipsoids to mimic the complex kernel shape, a physical model of the kernel was constructed. The pericarp thickness was multiplied by the surface area of the kernel to estimate the pericarp volume. The physical model could thus estimate the relative amount of endosperm of each wheat sample. The ranges of the variables are within the ranges of equivalent variables obtained from the literature. Unfortunately the variation of the endosperm to non-endosperm ratio, or of any other variable obtained through the physical model, could not improve alcohol yield predictability. The pericarp thickness measurements successfully indicated that there are differences between varieties and that larger kernels tend to have larger pericarp thicknesses. The physical model however failed to demonstrate that this variance is related to alcohol yield. It could possibly be proved useful for explaining variations of milling yield between varieties. In any case, the small variation of the outer layers thickness, in combination with the limited ability to measure it accurately and effectively, reduces the potential of the practical use of such an approach in a relevant cereal processing industry.

It had been hoped, based on literature reports, that a practical measure of bran thickness could be derived from the fundamental Crush Response Profile data reported from modern versions of the SKCS but, as the next section explains, these literature reports were mistaken and a new interpretation of the SKCS results and their relation to alcohol yield prediction was required.

7.2. Re-examination of the SKCS raw data

It is broadly accepted in cereal science that the rheological properties of wheat, in particular hardness, indicate many relevant aspects of its end-uses. Fundamental rheological testing, however, cannot be practically applied due to the between-kernel variation which obliges a large number of kernels to be examined. In the SKCS, the kernels are crushed in the declining gap between a crescent and a rotor and their resistance profile is stored as “raw” data. This profile is called Crush Response Profile (CRP).

Osborne and Anderssen and their co-workers argue that the average Crush Response Profile (aCRP) of the kernels in the SKCS can be considered as a pseudo stress-strain plot that effectively averages the individual kernel responses. In light of the current research, this general foundational argument was strengthened, although their detailed interpretation of the aCRP curves was demonstrated to be erroneous and a new interpretation was formulated. Figure 6 shows the aCRPs of representative soft and hard wheats. The aCRPs of wheat typically have an initial small peak followed by a trough, then a predominant peak after which the resistance reaches zero again as the crushed kernels exit the device. The aCRPs can therefore be divided in four quarters; (1) from the initial force until the initial peak, (2) from the initial peak until the trough, (3) from the trough until the predominant peak and (4) from the maximum force until the collapse to zero.

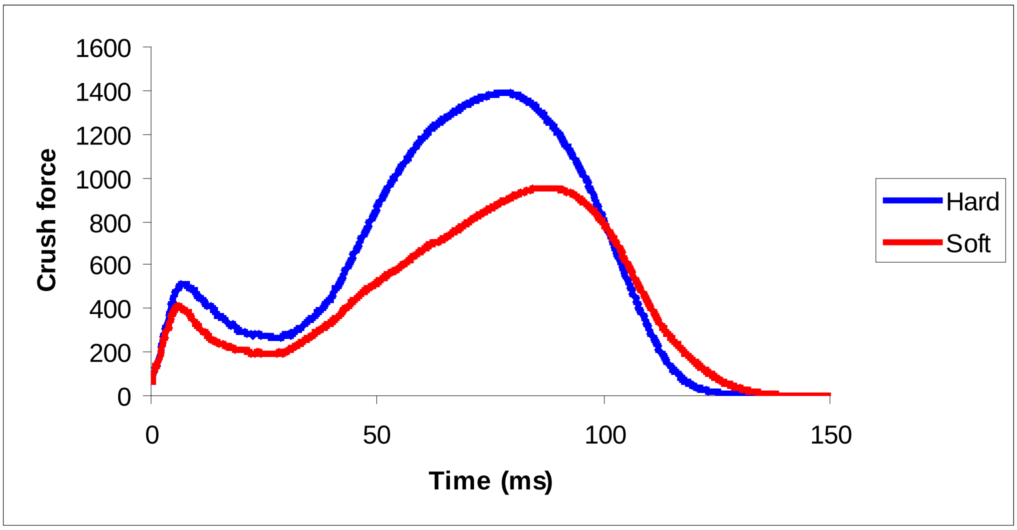

Figure 6. Average Crush Response Profiles of a hard and a soft wheat

The approach followed here was firstly to demonstrate that the two uptrends of the aCRPs give representative averaging of the 300 kernels. The two downtrends are actually a measure of the number of kernels that approach or reach zero crushing force at these particular time intervals, as a result of the initial fracture in the first case (the broken fragments for a time are too small to traverse the rotor crescent gap and to transmit resistance) or due to crushed kernels exiting the crushing mechanism in the second case. When ten batches of a particular wheat sample were measured, it was shown that the reproducibility of most parts of the curve was better than the reproducibility of the calculated hardness index itself. Therefore, even if the first downtrend is not essentially a representative averaging, it is still more precise than the hardness index itself. In fact only the final time intervals of the curve are less reproducible than the hardness index. Having established this, the obvious notable aCRP parameters of each wheat are the four extreme Crushing Forces (CF) (*i.e.* the initial force, those occurring at the first peak, the first trough, the second peak), the four time intervals at which these CFs occurs, the four slopes (two upward and two downward), the area corresponding to the four quarters of the curve, and the several ratios between these various parameters.

The initial peak does not occur because of the “shell” breakage as proposed by Osborne and Anderssen (2003). This was shown through three separate analyses that combined to demonstrate this conclusively. Firstly, drawing on the quantification of crease and kernel dimensions from the earlier work described in Section 5.1, it was shown that this initial force was highly correlated with crease morphology, implying that the initial breakage was influenced principally by the crease rather than by the kernel’s “shell”. This was confirmed by breaking or cutting kernels along the crease and by cutting kernels across the crease, and passing these half-kernels through the SKCS. The half-kernels broken along the crease (hence removing the crease) essentially eliminated the first peak in the aCRP, while those cut across the crease (hence retaining the crease) continued to exhibit the initial peak in their aCRPs. Finally, the influence of the “shell” on the initial peak was dismissed by pearling kernels to different levels and testing the pearled kernels in the SKCS. Even after 16% pearling, a level at which most of the outer layers have been removed, the initial peak was still evident. Therefore it is concluded that the initial peak of the aCRP arises not as result of initial breakage of the “shell”, but rather because of the geometry of the kernels and in particular because of the crease.

By examining the effects of moisture on the aCRPs it was identified that the first stage of the aCRPs behaves very similarly with conventional stress-strain graphs. When moisture was increased the brittle kernels gradually became ductile. This transition is reflected in stress-strain graphs of standard textural testing by the increase in failure strain and by the reduction of the compressive strength and the modulus of elasticity. Similarly in an aCRP, the initial peak takes place after a later time interval (increased strain) and the initial slope (modulus of elasticity) is reduced when moisture is increased. The reduction of the compressive strength was also evident when the kernels were wetted. However the dried kernels had also a smaller force at the initial peak (maximum compressive strength) than the untreated kernels, although the opposite might have been expected. It was discussed that this inconsistency may have been due to the drying method used, resulting in extensive internal cracking that weakened the endosperm, and it was speculated that if a more moderate drying method was used then the force at the initial peak of the dried kernels would be larger than that of the untreated kernels.

Clearly the first stage of an aCRP is equivalent to the stress-strain response observed in a conventional textural test. The third stage of an aCRP, which is the main uptrend, represents the post-failure rheological behaviour of the collection of broken particles. It makes sense to assume that larger kernels of similar texture require more force in order to be crushed than smaller kernels. By dividing the crushing force with the kernel's weight, this proportionality is reduced. The slope of the post-failure uptrend divided by the average reported weight of the kernels is a parameter that correlates very strongly with the SKCS hardness index. The aCRPs thus contain fundamental rheological information of a wheat sample, although only the initial slope can be interpreted as a conventional elastic modulus.

The *GREEN Grain* project wheats (2006 and 2007 samples) were tested in the newer SKCS model, their fundamental crushing force data were extracted and their aCRP parameters were calculated. The new parameters, together with the original wheat characteristics of the dataset, were re-examined in best subset regressions. From this analysis the aCRPs parameters were selected as predictors for alcohol yield.

The subgroups of the *GREEN Grain* wheat samples had different moisture levels and in some cases different agricultural and post-harvest treatments. A model based on all the 2006 and 2007 samples improved the predictability of alcohol yield to give an R^2 of about 82.3% with a standard error of 6.3 l/dry ton. The moisture content was selected as a predictor in this regression. Particular subgroups with similar moisture contents had much higher R^2 values at about 92%; the moisture content was not selected as a predictor in these runs. Clearly the aCRP parameters can improve alcohol yield predictability. Further standardisation of the method might improve prediction even further, although ultimately this is limited by the accuracy of the alcohol yield determination itself.

7.3. Summary

A physical model of the wheat kernel that accounted for pericarp thickness and kernel shape allowed calculation of the endosperm to non-endosperm ratio. There were significant differences between wheat varieties, but these could not be related to alcohol yield in order to improve predictions. Larger kernels tend to have thicker pericarp, such that they don't have a greater proportion of endosperm as has been hypothesised.

The Crush Response Profiles generated by the SKCS contain fundamental information about the rheological properties of wheat kernels which the SKCS interprets as the reported hardness value. Re-examination of these Crush Response Profiles revealed that the initial peak does not reflect breakage of the outer layers of the kernel, as previously thought, but rather is related to the crease morphology. The initial stage of the aCRP is equivalent to conventional stress-strain testing up to initial failure, while the remaining parts of the curve relate to the post-failure behaviour of the broken particles. Parameters derived from the aCRP could be used instead of the reported hardness to improve alcohol yield predictions.

8. CONCLUSIONS

8.1. Progress made in the current work

Bioethanol is a promising renewable transportation fuel that could contribute towards solutions to the sustainability and environmental concerns of fossil fuels, as well as creating a new market for wheat growers and breeders to address. The end product quality determination and prediction of wheat has been well studied for use in the food industry. However, this is not the case for its developing use for bioethanol production. Starch by itself cannot adequately define the alcohol yield potential of a wheat as its measurement is too inaccurate. Protein, which typically varies inversely with starch, can broadly quantify the alcohol yield potential. Within this context this study investigated the covariance of the chemical, physicochemical and physical characteristics of wheat, the alcohol yield prediction, the development of a physical model to investigate the variability of the unfermentable outer layers of the kernels, and finally an SKCS-based end product quality prediction methodology.

8.1.1. The chemical, physicochemical and physical characteristics of wheat and their relationships with alcohol yield

A PCA investigated the covariances of a diverse array of wheat characteristics. It was concluded that a high alcohol yielding UK wheat is typically soft and mealy with low protein, high starch, high albumin and globulin ratio, rounded kernels and larger kernels. A hard vitreous character (a physicochemical characteristic) is associated with high protein and thus with low starch content (a chemical characteristic). The PCA indicated that it is also associated with smaller kernels and more elongated kernels (physical characteristics). These trends were in accordance with previous work and with the established understanding of the mechanistic relationships between these wheat characteristics and the resulting alcohol yield; however, the PCA and subsequent analyses also revealed more subtle relationships.

It is known that N fertiliser increases the protein content of the grains. An ANOVA concluded that the N fertiliser impacts most of the characteristics in agreement with the general trends obtained by the PCA. The additional protein is mainly in the form of gliadins and LMW glutenins; thus their fractions increase significantly, while the albumin and globulin fraction is reduced. Together with the increase in protein, the kernels have significantly reduced width, depth and TGW. They also have (insignificantly) increased length; thus the kernels become more elongated with reduced sphericity as a result of the application of N fertiliser.

There were also significant varietal differences. This variance was again in agreement with the trends of the PCA. High alcohol yielding varieties with low protein content tend to have larger kernels. (In this case the Glasgow variety was an outlier.) They also tend to have more spherical kernels. The growing site also had small impacts on most of these characteristics.

Based on these established relationships, the alcohol yield could be predicted without performing costly and time consuming fermentations. The SKCS reported outputs together with the protein content can consistently predict the alcohol yield with an R^2 of about 78% and a standard error of about ±6.2 l/dry ton.

Two issues were raised at this point of the analysis that structured the remaining research. Firstly, the approach at that stage had examined the covariance of kernel characteristics solely from a statistical perspective. A simple mechanistic approach, in an effort to explain this, would suggest that larger and plumper kernels contain a larger relative amount of starch-rich endosperm, therefore they have higher starch and thus result in more alcohol. This statement has been used previously to speculate on differences in the milling yield and in the alcohol yield between varieties. Secondly, the SKCS is clearly useful for alcohol yield prediction, but the reported data are derived from the raw data. The latter can be directly examined with the new SKCS model, and may offer more powerful mechanistic understandings of the relationships between wheat kernel characteristics and processing behaviour, as well as more powerful predictors of end-use quality.

8.1.2. Pericarp thickness and the development of a physical model

It was hypothesised that larger and plumper kernels contain more starch-rich endosperm. This statement could explain the relationship between size and shape characteristics and chemical characteristics and, in the context of this study, with alcohol yield. It was further hypothesised that different wheats may exhibit different bran thicknesses, which would also affect their alcohol yield potential; thicker bran implies less endosperm and hence less fermentable material. These hypotheses were tested by measuring the outer layers of the kernels and creating a physical model, in order to estimate their variances, their contributions to the kernel's weight, hence the endosperm contribution and therefore the variances of these quantities with respect to alcohol yield.

Combining the pericarp thickness measurements with kernel dimensions, using fitting of ellipsoids to mimic the complex kernel shape, allowed construction of a physical model of the kernel that could estimate the relative amount of endosperm of each wheat sample. Unfortunately the variation of the endosperm to non-endosperm ratio, or of any other variable obtained through the physical model, could not improve alcohol yield predictability. The pericarp thickness measurements successfully indicated that there are differences between varieties and that larger kernels tend to have larger pericarp thicknesses. The physical model however failed to demonstrate that this variance is related with alcohol yield. It could possibly prove useful for explaining variations of milling yield between varieties. However, the small variation of the outer layer thickness, in combination with limited ability to measure it accurately and effectively, reduces the potential of the practical use of such an approach in a relevant cereal processing industry.

It had been hoped, based on literature reports, that a practical measure of bran thickness could be derived from the fundamental Crush Response Profile data reported from modern versions of the SKCS but, as the next section explains, these literature reports were mistaken and a new interpretation of the SKCS results and their relation to alcohol yield prediction was required.

8.1.3. The SKCS raw data and their interpretation

It is broadly accepted in cereal science that the rheological properties of wheat, in particular hardness, indicate many relevant aspects of its end uses. Proper rheological testing, however, cannot be practically applied in cereal processing industries due to the between-kernel variation, which obliges a large number of kernels to be examined in order to have a representative average. Osborne and Anderssen and their co-workers argue that the aCRPs of the SKCS can be considered as a pseudo stress-strain plot that effectively averages the individual kernel responses. In light of the current research, this general foundational argument was strengthened, although their detailed interpretation of the aCRP curves was demonstrated to be erroneous and a new interpretation was formulated.

The initial peak does not occur because of the “shell” breakage as proposed by Osborne and Anderssen (2003). This was shown through three separate analyses that combined to demonstrate this conclusively. Firstly, it was shown that this initial force was highly correlated with crease morphology, implying that the initial breakage was influenced principally by the crease rather than by the kernel’s “shell”. This was confirmed by breaking or cutting kernels along the crease and by cutting kernels across the crease, and passing these half-kernels through the SKCS. The half-kernels broken along the crease essentially eliminated the first peak in the aCRP, while those cut across the crease (hence retaining the crease) continued to exhibit the initial peak in their aCRPs. Finally, the influence of the “shell” on the initial peak was dismissed by pearling kernels to different levels and showing that even after 16% pearling, a level at which most of the outer layers have been removed, the initial peak was still evident in the SKCS aCRP. Therefore it is concluded that the initial peak of the aCRP arises not as result of initial breakage of the “shell”, but rather because of the geometry of the kernels and in particular because of the crease.

It was identified that the first stage of the aCRP is equivalent to the stress-strain response observed in a conventional textural test. The third stage, which is the main uptrend, represents the post-failure rheological behaviour of the collection of broken particles. The slope of the post-failure uptrend divided by the average reported weight of the kernels correlates strongly with the SKCS hardness index. The aCRPs thus contain fundamental rheological information of a wheat sample, although only the initial slope can be interpreted as a conventional elastic modulus.

Parameters derived from the aCRPs, rather than the reported parameters calculated by the SKCS, were used to develop improved predictions of alcohol yield with an R^2 of about 82% and a standard error of 6.3 l/dry ton.

8.2. Recommendations for future work

Processing of wheat for non-food uses such as fuel alcohol production is increasing around the world and particularly in the UK, and understanding the genetic, agricultural and physicochemical influences on alcohol yield is a new and important challenge for wheat breeders, growers and processors. The current work has drawn on a wide range of wheat varieties, grown under different agronomic practices and at different sites, and extensively characterised using a range of physical, chemical and physicochemical tests. It has employed detailed statistical and physical analyses to confirm expected relationships and reveal new subtleties, leading to new understandings of the relationships between wheat kernel characteristics and processing behaviour. This better understanding will benefit breeders and growers aiming to provide wheats most suited to alcohol production. These analyses have also allowed formulation of more accurate and powerful equations for predicting alcohol yield, benefitting processors aiming to select and process wheats to optimise productivity and profit.

There are several areas in which the work reported in this thesis could be extended in order to maximise the benefits and reveal further knowledge and insights, both for alcohol production from wheat but also for cereal science and engineering more generally. In terms of alcohol yield predictability, the current research investigated only UK wheats. It is not known if the models generated by the UK wheats are applicable to wheats grown under broadly different climatic conditions and up to what extent. It is speculated that they are applicable with slightly reduced accuracy and that by adjusting the partial coefficients and the intercepts, similar predictability could be achieved.

This study encouraged the view that the SKCS aCRPs contain fundamental rheological information about wheat. It was shown that moisture content influences the aCRPs. In line with the calibrations of the hardness index conducted by the inventors of the SKCS, the next contribution on this aspect should be the calibration of the aCRP parameters with respect to moisture content. This would allow their examination to take place on a comparable and more standardised basis, by eliminating the effects of moisture.

Further research is also required in order to convert the aCRPs to actual stress-strain plots. This could be achieved by statistical calibrations of the aCRPs parameters of representative wheats, having in parallel data from their accurate rheological testing. Moreover by knowing the geometrical characteristics of the rotor crescent system it will allow better transformations of the data, and it will enable the estimation of the textural properties from an engineering perspective. The current study did not extensively investigate more complicated transformations. However it was underlined that the crushing force is necessarily to an extent proportional with the size of the kernels. If the entire aCRP curve is divided by the weight of the kernels then this proportionality is reduced and it is more likely that the new parameters correlate better with the actual textural properties. This hypothesis needs to be tested by an approach that will examine the aCRPs and the actual stress-strain plots of different wheats together.

If the results are still encouraging, then the aCRP parameters or their transformations need to be tested against chemical characteristics. The rheological and chemical characteristics of wheat are related, and the end-use quality of wheat is also related with its chemistry. Therefore the aCRP parameters will allow the efficient examination of the relationships between the rheological characteristics, the chemistry of wheat and the end-use quality prediction.

The current study has contributed to the understanding of the alcohol yield from wheat and the variability of its characteristics. The recent and ongoing construction of wheat-to-ethanol plants in the UK makes this work timely, with potential to benefit significantly the development and effectiveness of this new industry and the ability of wheat breeders and growers to serve it.

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