Research Review No. 61

December 2006

Price: £5.00

Wheat as a feedstock for alcohol production

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This is the final report of a twelve month project, which started in July 2004. The project was funded by a contract of £26,000 from HGCA (Project No. 3018), £14,200 from Wessex Grain Ltd., £10,200 from Monsanto UK Ltd and £2,550 from International Centre for Brewing and Distilling, making a total of £63,160.

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Abbreviations

CO_2	Carbon Dioxide
DDGS	Distillers Dried Grains with Solubles (alternatively Distillers Dark Grains with Solubles)
Defra	Department for Environment Food and Rural Affairs
DM	Dry Matter
ETBE	Ethyl tertiary butyl ether
GHG	Greenhouse Gas
GJ	Giga Joules
GLC	Gas Liquid Chromatography
ha	hectare
HCl	Hydrochloric Acid
HFN	Hagberg Falling Number
HGCA	Home Grown Cereals Authority
HPLC	High Performance Liquid Chromatography
l	litre
N	Nitrogen
N_2O	Nitrous Oxide
NABIM	National Association of British and Irish Millers
NIR	Near Infrared Reflectance
NL1, NL2	National List 1, National List 2
NSP	Non-Starch Polysaccharides
RL	Recommended List
RTFO	Renewable Transport Fuels Obligation
SEERAD	Scottish Executive Environment and Rural Affairs Department
SWRI	Scotch Whisky Research Institute
t	tonne(s)

Acknowledgements

This research review has built on on-going work from the GREEN grain project (HGCA Project 2979). The GREEN grain project is supported by Defra and SEERAD through the Sustainable Arable LINK programme, with HGCA, Syngenta, FOSS UK, Scotch Whisky Research Institute, ADAS, Scottish Crops Research Institute, Grampian Country Foods and Wessex Grain/ Green Spirit Fuels as project partners.

The authors acknowledge the use of data from the HGCA-funded Recommended Lists (http://www.hgca.com/varieties) Table 7 and Figure 8.

The authors would like to thank HGCA and the GREEN grain collaborators for their useful comments on drafts of this report.

Abstract

UK fuel-alcohol production from wheat is expected to begin in 2008. If current plans are realised, this requirement will soon add at least 2.5 million tonnes of grain to the 0.7 million tonnes already required for potable alcohol. Other fuel-alcohol markets are based on sugar cane or maize, so wheat-based production methods are not well-developed.

Information is reviewed here to support industry development and to identify R&D requirements on growing wheat for alcohol production. Initially, growing and processing will be based on feed wheat standards, but as expertise develops and as carbon accreditation is introduced, criteria will be applied to maximise alcohol yields and processing efficiency.

'Benchmarks' proposed for current production of fuel-alcohol from UK feed wheat (dry basis) are 7.4 t/ha grain, 11.5% protein, 69% starch, 3% sugar to yield 435 litres alcohol per tonne or 3,220 litres per hectare. Variation in alcohol production per hectare largely arises in the field through differences in grain yield and starch content; new plant breeding initiatives and better use of N fertilisers should improve these parameters and reduce variation in alcohol yield. Efficiency of fuel alcohol processing can also be enhanced. It is expected that best practice will soon exceed 4,000 litres alcohol per hectare, and that this will continue to increase through new R&D.

Executive Summary

Rationale

There is an increasing consensus word-wide that biofuels can help to mitigate climate change, and also improve security of fuel supplies; EU and UK legislation now reflects this. Wheat has the credentials to become the principal feedstock for the EU's emerging fuel-alcohol market. Wheat produces more harvestable starch than any other UK crop and UK wheat yields are amongst the highest in the world. Just as the UK is an important producer of potable alcohol, the UK could become a major fuel-alcohol producer, and even an exporter.

Major fuel alcohol markets are not based on wheat: Brazil uses sugar cane and the US uses maize, so the technologies for fuel alcohol production are not yet well-tailored to wheat. This review considers evidence from biofuels industries elsewhere and from the potable-alcohol industry in the UK, and suggests how wheat may best be grown and processed into fuel-alcohol in the UK, highlighting the key uncertainties for which R&D should prove beneficial.

Alcohol production

More than 90% of the UK's neutral spirit and grain whisky production is from wheat. Specific varieties with soft grain are sourced from northern Britain, where conditions maximise grain starch content. Milled grain is cooked and the gelatinised starch is hydrolysed to sugars by amylases from barley malt; then the sugars are fermented to alcohol and carbon dioxide, and the alcohol is distilled. Processing takes about 100 hours and has an apparent efficiency of substrate-alcohol conversion of about 84% although approximately half of this under-recovery is due to loss of sugars in yeast growth during fermentation.

The fuel-alcohol market will be larger; cost-efficiency will be more crucial; and environmental constraints may apply, particularly to maximise greenhouse gas (GHG) savings with respect to petrol. Thus feedstocks giving higher alcohol yields and increased processing efficiency are beneficial. Grain processing for bioethanol differs from whisky production in that exogenous nutrients and fungal enzymes may be used to improve processing efficiency, and the distillate must be dried further, often by molecular sieves. The remaining material, once dried, forms 'DDGS', used as a high protein feed for ruminants. Current fuel alcohol processes take about 60 hours, but their efficiency is not known.

Feedstock demand and specification

Fuel alcohol production will begin in the UK late in 2007, firstly from sugar beet. It is anticipated that fuel alcohol production from wheat will begin early in 2008. At present new plants are planned in Somerset, Northants, Humberside and Teeside to process about 2.5 M tonnes grain into 0.66 M tonnes bioethanol, so wheat will be sourced throughout the UK. Initially, quality standards are likely to be similar to feed wheat, but as expertise develops and as carbon accreditation is introduced, specifications are likely to include criteria that indicate alcohol processing yield and efficiency. Because methods for starch analysis are unreliable, these specifications are likely to be based on near-infrared (NIR) spectroscopy, possibly referenced against protein content. NIR calibrations are currently being developed. Data from the potable-alcohol industry indicate that protein content accounts for much of the variation in alcohol processing yield. Protein content is largely associated with variation in growing conditions, alcohol decreasing by about 7 litres per dry tonne for every 1% increase in grain protein. Taking a 'benchmark' UK feed wheat (on a dry basis) as having 11.5% protein, 69% starch and 3% sugar, the benchmark alcohol yield (at 92% efficiency) can be taken as 435 litres per tonne. Processing yields from recent laboratory tests (using potable methodology) vary between 410 and 480 litres ethanol per tonne. Feedstock quality also affects processing rate and efficiency, particularly by changing the viscosity of intermediaries and residues, but efficiency is rarely estimated.

Feedstock supply

Wheat grain best suited to biofuel production has large well-filled grains with low protein content, low residue viscosity, and no fungal contamination. Soft wheats, and varieties without the 1BL/1RS rye translocation, have been preferred for potable alcohol production, but whilst these varieties are likely to be easier for bioethanol producers to process, the use of chemicals and enzymes may make this preference less important. Some of the highest yielding varieties also happen to be best suited to alcohol processing: Glasgow, Alchemy and Istabraq. However, high growing costs (per tonne) of low yielding varieties such as Riband, despite good suitability to alcohol processing, render them poorly suited to fuel-alcohol production.

Alcohol production from the best varieties grown in the best UK conditions is likely to exceed 4,000 litres alcohol per hectare. This compares favourably with other cereal-based biofuel production systems in other parts of the world. Initially, growing wheat for the UK fuel alcohol market is likely to be very similar to that for other markets, productivity being crucial. Best

conditions will be on moisture-retentive soils, following a break crop. However, as the fuel alcohol industry develops and as carbon accreditation is applied, premiums and/or other economic instruments will probably serve to maximise feedstock quality, particularly processing yields, and to minimise carbon emissions. On farm, the main ways that crop managers can influence feedstock quality are through variety choice and nitrogen management. Further research is seeking to optimise production strategies for GHG saving.

Recommendations

The industry urgently needs:

(i) wider testing of recommended and candidate varieties,

(ii) research on crop management, especially rotations and use of N fertilisers,

(iii) laboratory-scale processing facilities that can test feedstocks using fuel-alcohol methodology,

(iv) research to define the fermentable constituents of wheat grain, and their interactions with unfermentable constituents (mainly non-starch polysaccharides),

(v) methods to maximise rate and efficiency of processing, especially with regard to energy use,

(vi) investigation of maximising existing and novel uses for co-products, and

(vii) an economic appraisal of how the supply chain could best be optimised to maximise alcohol production, financial returns for growers and processors, and GHG emission savings.

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1.0 Introduction

Wheat is expected to become a major biofuel crop in the UK over the next few years. This review aims to support the rapid development of expertise that will be required by summarising information on the production process, best types of grain, and the varieties and agronomic practices that are likely to provide the best wheats for alcohol production and point to the research and development that is needed.

1.1 Potable, Industrial and Fuel Markets

Wheat markets have traditionally been those for milling (principally for bread and biscuit making), and those used for feed and for brewing and distilling. Research effort has been primarily targeted towards identifying varietal traits and agronomic practices that maximise yield and desirable characteristics for the bread making industry, such as high grain protein content. Characteristics desirable for bread making are not necessarily beneficial for the bioalcohol industry where high grain starch content is advantageous (Loyce and Meynard, 1997).

The potable alcohol industry (which produces whisky, gin and vodka spirits) provides a stable market for around 700,000 tonnes of UK wheat per annum and is a significant contributor to UK tax and export revenue. Alcohol production for transport (fuel alcohol) is a growth industry throughout the world, and UK fuel alcohol production is expected to begin in 2007. Bioalcohol may also be potentially used in industrial applications as a solvent and in cosmetics and toiletries market, although the potential size of this market is unclear (Batchelor *et al.,* 1993).

Wheat is expected to be the major feedstock for the UK alcohol industry. It is therefore important to determine the varietal traits and agronomic practices influencing alcohol production. This review principally considers wheat as a feedstock from the perspective of fuel alcohol, but make full use of information developed by the potable alcohol industry and so will also be useful for growers for the existing industry.

1.2 Why Biofuels?

The world is facing an energy crisis. In the past fossil fuels such as gas, oil and coal were both cheap and readily available and were the preferred global energy source. Fossil fuels are finite; taking millions of years to form and, with increasing global energy demands, supplies of fossil fuels, which made up 80% of global energy usage in 2001, are rapidly declining (World Resources Institute, 2005). At present, fossil fuel prices and supplies are increasingly unstable. At the time of

writing, prices of crude oil are at an all time high and supplies often come from politically unstable countries, compromising security of supply. On top of this, climate change is now recognised as one of the most serious issues affecting the world, and mitigation is essential to avoid the worst social, economic and environmental consequences (HM Government, 2006). Burning fossil fuels releases into the atmosphere the carbon dioxide (CO_2) originally sequestered through photosynthesis by plants. Since CO_2 is a greenhouse gas (GHG), this significantly contributes to climate change. Alternative sources of energy are necessary which do not harm the environment and are renewable. It is likely that a comprehensive approach, utilising a number of energy sources such as biomass, nuclear, wind and solar power will be required. Transport currently accounts for more than 30% of total EU energy expenditure, and 98% of the transport sector is dependent upon oil (EU, 2003). Biofuels are substances produced from crops that can be used as fuels. Increasing use of liquid biofuels such as bioethanol and biodiesel is a key measure to reduce the environmental costs of conventional fuels and to improve security of supply.

1.3 Alternative Fuels

There are several renewable fuels that could potentially replace or be used in addition to conventional fossil fuels. The most economically viable at present are bioethanol and biodiesel. Bioethanol is produced by fermentation of sugars by yeast and can be made from any sugar or starch rich feedstock. Biodiesel is produced from oil-rich crops such as oilseed rape, palm oil and soya oil. Both bioethanol and biodiesel can be utilised in existing engines without modification as low blends, typically 5% biofuel to 95% conventional fuel. Specialised cars, known as flexible fuel vehicles, can utilise blends of up to 85% ethanol to 15% petrol. Because ethanol is hygroscopic care is needed to prevent water from entering the supply chain. Distribution of petrol-ethanol blends can therefore be problematic, particularly in the UK where the major oil companies share an integrated supply system. These issues however are not insurmountable, as evidenced by the use of ethanol blends in other countries, and their supply by independent fuel companies in the UK.

ETBE (ethyl tertiary butyl ether) is derived from ethanol (47% v/v) by reaction with isobutylene (a petroleum by-product) (European Fuel Oxygenates Association, 2006a). Because it is almost half bioethanol, ETBE qualifies for the same tax incentives as other biofuels (European Fuels Oxygenates Association, 2006b) and it can be blended up to 15% with petrol without modification of either the supply chain or engines (European Fuels Oxygenates Association, 2006a) NOT IN REFS. An estimated 2 million tonnes of ETBE were produced in the EU in 2005 (Eur'Observ'ER, 2006).

1.4 World Biofuels

Bioethanol has been a major fuel source in Brazil and the USA for decades. The ProAlcool programme was introduced in Brazil in 1975 in response to the energy crises of the 1970s and as a market for surplus sugar cane. All fuel in Brazil contains at least 25% biofuel and approximately 2.4 million cars in Brazil are able to utilise pure alcohol (Szwarc, 2004). A similar programme was initiated in the USA in 1979 (Wheals *et al.,* 1999). In the USA, bioethanol is largely produced from corn (maize) due to its abundance and low cost (Bothast and Schlicher, 2005). Brazil and the USA together accounted for nearly 90% of global bioethanol production in 2005, whilst European production was estimated at 720,927 tonnes (Eur'Observ'ER, 2006) accounting for only 2.8% of global production (BP, 2006).

The biofuels industry has been developing rapidly in the EU. The EU Biofuel Directive (2003/30/EC) set a target for 2% by energy of biofuel in transport fuels by 2005, 5.75% by 2010 and up-to 20% substitution of conventional fuels by biofuels by 2020 and has provided a major driver for biofuel expansion in the EU (EU, 2003). Tax exemption policies have further encouraged this sector in many EU states; Sweden and Spain have total exemption and France and the UK have partial exemption (Eur'Observ'ER, 2006). The bioethanol sector is developing rapidly in Europe; production grew by 70.5% between 2004 and 2005 (Eur'Observ'ER, 2006). Spain is Europe's largest bioethanol producer accounting for 0.9% of global production in 2005, followed by Germany (0.5%), Sweden (0.5%) and France (0.4%) (BP, 2006).

1.5 The UK Biofuels Market

Biofuels made up only a negligible amount of total fuel sales in the UK until recently and to date, UK production has centred exclusively on biodiesel production. Growth of the biofuels sector has been promoted by relatively high crude oil prices, government policy and tax incentives.

A renewable transport fuels obligation (RTFO) has been announced by the UK government as a method of ensuring the long-term promotion of biofuels supply and usage. The RTFO will place a legal obligation on transport fuel suppliers to acquire a specified proportion of their fuel from renewable sources – from 2008 transport fuel should contain a renewable component, 2.5% by 2008, 3.75% by 2009 and by 2010/11 all transport fuels should contain at least a 5% biofuel component by volume. A 5% biofuel blend is estimated to save 1 million tonnes per annum in CO_2 and is equivalent to taking 1 million cars from the road (Department of Trade and Industry, 2006). Biofuels are usually more expensive to produce than their fossil fuel counterparts and require support to make them competitive. Since 2002 a 20p / litre (l) duty exemption has been in place for biodiesel fuels and

an equivalent incentive was introduced in December 2005 for bioethanol (Department for Transport, 2005).

The standard EN228 unleaded petrol specification permits up to 5% ethanol inclusion (either bio or fossil derived) and so, with blends up to this level there are no vehicle warranty issues as it is still classed as standard petrol. Tesco have been using ethanol at up to 5% blends when profitable to do so at over 185 forecourts in the UK (Tesco, 2006). Morrisons sell an 85% ethanol blend for flexi-fuel cars, such as the Ford Focus and Saab 9-5, at a limited number of sites in Somerset and East Anglia (Morrisons, 2006). Independent fuel blenders such as Greenergy, Futura (now called Harvest Energy) and Mabenaft tend to rely on imported petrol components (presently all from Brazil) that they blend within import terminals and then distribute by road.

The major oil companies are less eager to incorporate ethanol into fuel because it would require alterations to both the supply chain and storage facilities. The fuel used by the major oil companies is distributed via a shared pipeline to 42 storage terminals across the UK. They then collect fuel by road tanker for local distribution. The same pipeline is used to carry several oil products. Because ethanol is a solvent that cleans accumulated residues from the pipes (causing fuel contamination), as well as picking up any water accumulations, the oil majors are reluctant to use ethanol in their petrol. Furthermore, when changing over to an ethanol blend it is desirable to clean out the water from petrol storage tanks. The problem of water build up in petrol tanks is usually associated with older distribution facilities with low fuel turnover. Therefore supermarkets with modern storage tanks and very high throughputs are less likely to suffer from water build up problems.

No bioethanol is presently produced in the UK so it is imported from abroad. However, several bioethanol plants are at various stages of development and UK bioethanol production is expected to begin in late 2007. If all of these come to fruition, around least 2.5 million tonnes of wheat will be needed for bioethanol production and UK production will provide at least 2.6% of predicted petrol demand by 2010.

1.6 Feedstock Types

Bioethanol is a colourless alcohol produced from the fermentation of sugar substrates to ethanol by yeast via pyruvate and acetylaldehyde intermediates. Sugars are derived from sugar crops such as sugar cane and sugar beet and starch crops such as barley, wheat and maize. Cellulose is also a potential source of glucose for alcohol production. In the future, lignocellulosic materials such as

forestry residues, straw or woody perennials such as miscanthus and short rotation coppice (willow or poplar) may be used in ethanol production. Although technology exists, there are no commercial plants for ethanol production from lignocellulosic materials at present anywhere in the world because current processes are uneconomic.

Feedstocks used for alcohol production vary throughout the world depending upon the climatic conditions and prices. Cereal grains are an attractive feedstock because grains contain a high proportion of starch and can be stored dry for many months, allowing year round processing. Maize (corn) is used extensively in the USA with lesser amounts of wheat and sorghum. In 2003, 10% of the US maize crop was utilised by the bioethanol industry and this provided 2% of US transport fuels. Rye is used extensively in German and Polish bioethanol plants, while substantial amounts of triticale, a hybrid of rye and wheat, are used in Sweden (Senn and Pieper, 2000).

Sugar cane is available year round in Brazil, so the Brazilian bioethanol industry almost exclusively utilises sugar cane. Despite their relatively high starch and sugar contents, crops such as potatoes and sugar beet are less viable at current prices and growing costs for large-scale alcohol production in the UK. Potatoes contain 75% water and 25% dry matter comprising 12-21% starch (Senn and Pieper, 2000). The high water content of potatoes relative to dry matter makes them bulky and therefore expensive to transport and store. Potatoes would require long storage for year round supply. Unlike cereals, the starch content of potatoes decreases with storage time, with an 8% decrease in starch after 6 months and a 16.5% decrease after 8 months (Senn and Pieper, 2000). Sugar beet contains approximately 16% sugar but as for potatoes, it has a high water content leading to expense in transportation and storage. Harvested crops must be kept below 10°C or respiration will utilise some of the sugars. British Sugar plan to utilise sugar beet for bioethanol production from 2007 at their Wissington site in Norfolk (Tony Sidwell, British Sugar, *personal communication*; British Sugar, 2006), but production will be seasonal because at present it appears that they do not plan to augment beet supplies with wheat.

With the exception of the British Sugar plant at Wissington, all of the planned UK bioethanol production facilities plan to use wheat as their primary feedstock (Table 1). Wheat is the most economically viable feedstock for UK bioethanol production at present, although alternatives such as triticale or imported maize may also be used in the future. Wheat made up 1,868 thousand hectare (ha) out of 4,427 thousand ha (or 42%) of arable crops in 2005 (Department for Environment Food and Rural Affairs (Defra, 2006). An estimated requirement for 25 million tonnes of petrol in 2010 and the RTFO of 5% renewable component into fuels would require 1.25M tonnes of bioethanol,

assuming the obligation is equally split between petrol and diesel. One tonne of wheat produces 0.29 tonnes of bioethanol. Therefore approximately 3 million tonnes of wheat would be needed per annum to meet the requirements of the RTFO from UK production. The UK currently has an export surplus of approximately 2 million tonnes of wheat per annum (Home-Grown Cereals Authority (HGCA), 2006a) which therefore could make up much of the requirement. In reality, at least some of the bioethanol required to meet the RTFO will be imported.

1.7 Environmental Impacts and Carbon Assurance of Biofuels

There has been considerable debate around the issue of environmental impacts of fuel alcohol production, both in terms of potential GHG savings and broader sustainability issues. The carbon released from combustion of biofuels is equivalent to that taken up by the plant in its growth and hence is carbon neutral. However, much energy can be used in the growing of crops, transport of feedstocks and the processing of the biofuel, with associated GHG emissions. In addition, further effects on factors such as carbon stocks and nitrous oxide (N_2O) emissions from soils can be important in determining the overall GHG benefits of biofuels, as can the fate of co-products from growing the crop and processing the feedstock. Life cycle analysis has therefore been used to quantify the energy costs and GHG emissions associated with biofuels from 'cradle to grave'. Most studies have concluded that biofuels can deliver savings in GHG emissions relative to fossil fuels, though these savings are sometimes found to be small and are obviously dependent on how the crop is grown, the processing technology used and the fate of co-products (Elsayed *et al*., 2003; Mortimer *et al*., 2004; Punter *et al.,* 2004; Billins *et al*., 2005; Farrell *et al*., 2006; Hill *et al*., 2006) although the benefits of biofuels have been questioned by some (Patzek, 2006). Studies on wheat for bioethanol production in the UK have suggested that CO_2 emissions could be reduced by more than 77% relative to petrol, or less than 7% relative to petrol (Punter *et al.,* 2004, Woods *et al.,* 2005). In order to help quantify the GHG emissions associated with the production of bioethanol from wheat, and to optimise crop management practices, the HGCA has developed a GHG calculator (www.hgca.com) and is developing methods for carbon assurance schemes that could work operationally (Billins *et al.,* 2005; Woods *et al.,* 2005). This work is being continued under the HGCA project "Facilitating Carbon Accreditation Schemes for Biofuels: Feedstock Production".

HGCA-funded research has shown that growing wheat for bioethanol is unlikely to have negative environmental impacts compared to existing food farming systems in the UK, and, if growing for biofuels is associated with lower inputs of fertiliser, there could be environmental benefits relative to conventional cropping (Turley *et al*., 2005).

1.8 Conclusion

Bioethanol production in the UK is expected to begin in 2007. The majority of planned bioethanol plants will utilise wheat as their primary feedstock and this will significantly increase the market for wheat in the UK. Bioethanol is widely used world wide as a renewable component of fuels; however, few world regions currently use wheat as a feedstock. Therefore technology needs to be developed from the potable alcohol expertise and expertise from other feedstocks in other parts of the world and applied to wheat.

2.0 The Alcohol Production Process

Ethanol can be derived from any substance yielding fermentable sugars. The nature of the feedstock affects how sugars are obtained. Sugars can be obtained directly from crops such as sugar cane, sugar beet and fruits simply by crushing the material and extracting the juice. Feedstocks containing starch such as wheat and maize must first be treated with the enzymes α-amylase and amyloglucosidase to break down the starch to glucose. Lignocellulosic materials such as wood, paper and straw require extensive pre-treatment using chemicals and / or high pressure and high temperature treatments; cellulases are then added to break down the cellulose biopolymer to its constituent sugars.

A schematic overview of the process from grain to fuel alcohol is shown in Figure 1. The exact production process may vary depending upon individual circumstances; typical modifications are described in the following sections. The process for potable alcohol is broadly similar to that for fuel alcohol but differs in additives that can be used. Fuel alcohol can use commercial enzymes and chemicals, neutral alcohol can use commercial enzymes for saccharification but no chemicals whereas grain whisky production is constrained to using only grains, yeast and water so no chemicals and commercial enzymes are used.

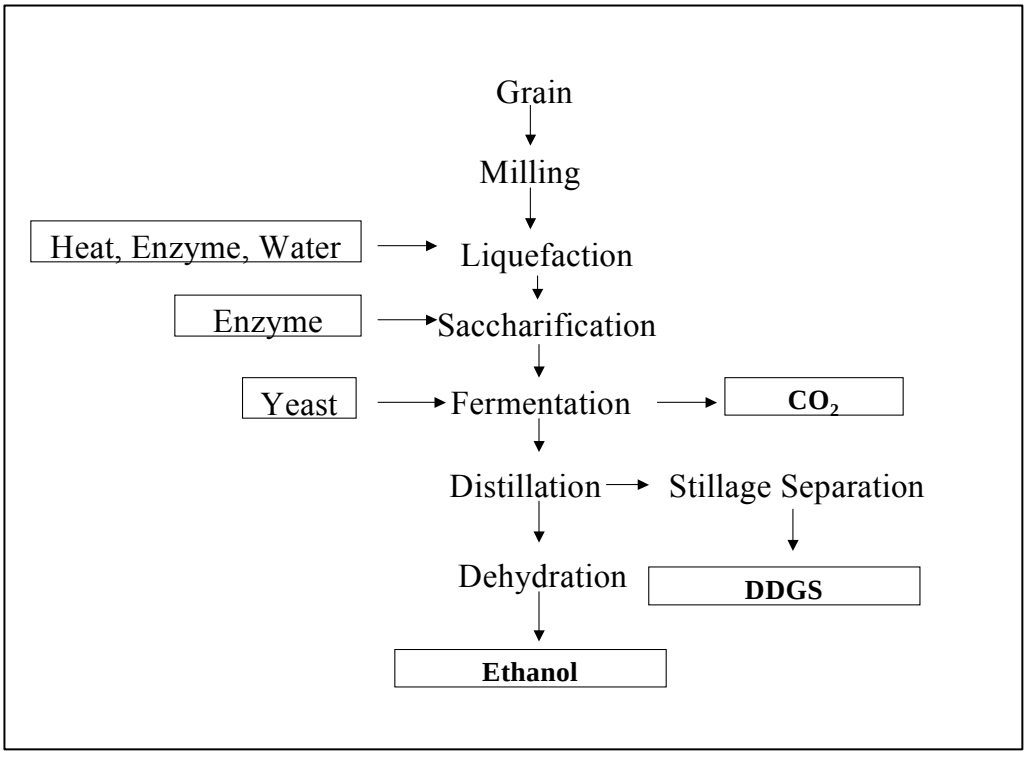

Figure 1 - Overview of the basic "dry grind" process of fuel alcohol production. The products ethanol, DDGS and CO_2 are produced in approximately equal amounts by weight.

The following subsections briefly describe each step outlined in Figure 1, from the perspective of possible effects of feedstock on the process. For a detailed description of the process of alcohol production the reader is referred to "The Alcohol Textbook" edited by Jaques KA, Lyons, TP and Kelsall, DR (2003).

2.1 Milling

The milling process increases the grain surface area, allowing more effective slurrying, cooking and liquefaction and more effective enzymatic breakdown of starch. In the USA where maize is the main feedstock for bioethanol production, milling of grains for bioethanol production may be carried out by either a "dry grind" or a "wet grind" process. Analogous processes can be considered for wheat, but the two species process quite differently. With a "dry grind", the whole grain is milled without any separation of grain components. This is the cheapest and most common process found in existing bioethanol production facilities, and is also common in potable alcohol distilleries. It is most likely that the planned bioethanol plants in the UK will use a simple dry grind process, starting with whole-wheat grain.

It should be noted that considerable process efficiencies might be achievable when designing new bioethanol plants, by employing additional dry processing technologies such as abrasive or roller milling to de-bran grain prior to the liquefaction and fermentation steps (Sosulski and Sosulski, 1994; Wang *et al.,* 1997). This would remove most of the fibre and protein from the grain (which do not contribute to fermentation), and would reduce the requirement for drying at the end of the process (when a significant input of energy is required).

In the case of maize, a "wet grind" process separates the grain into its constituent components, starch, fibre, protein and germ after a period of soaking (or steeping) in dilute sulphuric acid prior to milling. With wheat, the wet process is different because wheat contains a unique combination of proteins which form gluten. Existing wheat starch production plants in the UK use a wet process whereby wheat flour (either whole or white flour, depending on the factory and location) is wetted and kneaded to form a dough. The dough is then washed repeatedly to remove the starch granules from the insoluble gluten. Both gluten and starch are recovered as valuable products. Depending upon market conditions, the gluten can sometimes be the more valuable product (even though it is often considered as a co product).

Although energy intensive and more expensive, wet processes can theoretically increase the processing efficiency, as the concentration of starch entering the liquefaction and fermentation steps is greater, and less DDGS has to be dried. However, the overall economics of the process will rely on also being able to sell gluten as a high value co-product. To the authors' knowledge, none of the planned UK bioethanol plants intend to use this process.

2.2 Liquefaction/ Gelatinisation

A high temperature "cooking" step is commonly used to gelatinise the starch and make it more accessible to enzymes for degradation. The high temperatures also help to reduce microbial contamination. The milled grain is mixed with water to form a mash and heated to 120-150ºC. High temperature and high pressure cause mechanical shearing forces on the starch molecule. Release of pressure (blowdown) further disrupts the remaining endosperm structure.

The duration and the temperature of the cooking step must be carefully controlled; if the starch is cooked for too long or at too high temperature, browning (or Malliard) reactions may occur, resulting in reduced alcohol yields (Bringhurst *et al.,* 2003). Novel enzyme mixtures of α amylases and glucoamylases are now commercially available which are able to break down starch *in vitro* with no need for a high temperature liquefaction step (Genencor, 2005). Wilkin (1989) reviewed 'cold cooking' methods whereby ground grain is either not cooked before enzymatic saccharification or cooked at a reduced temperature (e.g. 80°C). These gave higher alcohol yields but the energy saved by cold cooking may be offset or even increased by the need to mill the grain more finely. Also, later steps may have higher microbial infection than when an initial cooking step is employed and problems with incomplete release and saccharification of starch could only be resolved by using exogenous enzymes.

2.3 Saccharification

In fuel alcohol production, after cooling to 90-100°C, a heat stable α-amylase is added to breakdown starch to smaller subunits. This step significantly reduces the viscosity of the mash and allows more efficient breakdown by further starch degrading enzymes. The mash is then cooled further to 80-90˚C and amyloglucosidase (also known as glucoamylase) is added. Amyloglucosidase removes successive glucose residues at the ends of the starch molecules.

Traditional distilling industries (e.g. Scotch whisky production) cannot use commercial enzyme preparations. However, germinating barley produces large amounts of enzymes well adapted to breaking down barley starch into sugars. These enzymes are produced in excess by germinating

barley grain and are therefore used to break down starch in unmalted wheat grains. The mashing step is carried out at 63-64ºC. Breakdown of starch leaves a sugar solution called 'wort'. The malt enzymes can only work efficiently on fully dispersed, gelatinized starch, so the cereals are first cooked under pressure and at high temperature (approximately 140°C).

2.4 Fermentation

Under anaerobic (oxygen limiting) environments, yeasts produce ethanol and carbon dioxide from sugars in a process called fermentation. In bioalcohol production, the mash from saccharification is cooled and yeast added. Fermentation typically occurs for 48-72 hours at approximately 30°C-35°C and results in wort with a typical final alcohol content of 8-12% depending upon the initial substrate level, amount of yeast added (pitching rate) and the degree of bacterial contamination. To maximise throughput and minimise costs, a maximal ratio of grain to water is desirable because water processing is both energy and cost intensive. Problems with viscosity may be encountered at high concentrations of dry matter and these are discussed below. Conditions for yeast growth are critical in maximising alcohol yields – where yeasts are stressed, 'sluggish' or 'stuck' fermentations may occur, significantly reducing yield (Ingledew, 2003).

2.5 Distillation and Dehydration

Distillation allows the concentration of alcohol to be increased by separating ethanol from water and other impurities in the mash. At sea level, ethanol vapourises at 78°C and water at 100°C, hence by heating the liquid, the ethanol and water can be separated to leave a 95% ethanol and 5% water azeotrope. Distillation for potable alcohol stops at this stage but for transport alcohol further dehydration is necessary. Molecular sieves are used to adsorb water, but not ethanol, so that pure, anhydrous ethanol is produced.

2.6 Stillage Separation

After fermentation and distillation, the residual mash, termed 'whole stillage' is separated by centrifugation or pressing and extrusion into wet grain (containing heavy particulate matter) and thin stillage (containing water and small particulate matter). The thin stillage fraction is dried to a syrup, then mixed with the wet grain fraction and dried further to form Dried Distillers Grains with Solubles (DDGS).

2.7 Co-Products

Storage carbohydrates (principally starch) and free sugars account for approximately 2/3^{rds} of the whole grain and are used in the fermentation process to produce alcohol and carbon dioxide. The

remaining 1/3rd of the grain consists of non-starch polysaccharides, non-degraded starch, proteins and lipids and if suitable markets can be found for these components, the revenue generated can contribute to the profitability of the process. Indeed, Wheals *et al.* (1999) estimated that in a maize alcohol facility, approximately 50% of the revenue is derived from co-products, and they suggested that there is still considerable scope to find uses for co-products other than in animal feeds, such as in pharmaceutical, nutraceutical and cosmetic products. Wheat has the potential to provide gluten (used in the baking industry and as an emulsifier or thickener; see earlier discussion on gluten co-processing), bran (used in cereal foods), germ (used in bakery products and for some high value cosmetic uses) and flour, in addition to DDGS, the standard co-product of bioethanol production (Tibelius and Trenholm, 1996). Generation of multiple co-products from a single feedstock does occur, but is rare at present owing to the costs involved. It is more common in wet grind facilities.

Where DDGS are the co-product of the alcohol production process approximately 305kg are produced per tonne of wheat. DDGS are used extensively in the UK as a feed for ruminants. Removal of starch concentrates the remaining components of the grain approximately three-fold, as shown in Table 2, so DDGS contains higher crude protein and fibre contents than grain, and similar levels of gross energy. However, utilisable energy, especially for non-ruminants, is much reduced in when compared to wheat grain. The composition of DDGS can be very variable depending on the source material, method of processing and processing efficiency. Feeding trials have shown that maximum inclusion levels of DDGS depend not only on the type of livestock but also the growth stage of the animal (Table 3). Because of their high fibre content, little DDGS are used in pig and poultry rations. For non-ruminants it is best suited to sows, but it is primarily thought of as a feed for ruminants. Some maize based DDGS is imported and produced in the UK, however the majority is wheat based (Bruce Cottrill, ADAS, *personal communication*).

Table 1 Nutritional composition of wheat grain and wheat DDGS (based on Nyachoti *et al*., 2005). Data is normalised to 100% dry matter and is based on values for Canadian wheats. Energy composition is given in terms of MJ kg^{-1} and chemical and amino acid composition is given in terms of g kg^{-1}. Figures do not include available carbohydrates since these are fermented in the bioethanol production process.

	Wheat	**Wheat DDGS**	**Concentration**
Dry matter	100.0	100.0	1.0
Nitrogen	2.3	6.8	2.9
Gross energy	1.8	2.1	1.2
Acid detergent fibre	5.2	13.7	2.6
Neutral detergent fibre	12.8	32.0	2.5
Ether extract	1.6	3.8	2.4
Ash	1.8	4.6	2.6
Total Phosphorous	0.4	0.9	2.2
Phytate P	0.3	0.2	0.6
Calcium	0.1	0.2	2.6
Essential amino acids			
Arginine	0.6	1.6	2.6
Histidine	0.3	0.8	2.5
Isoleucine	0.6	1.3	2.4
Leucine	1.0	2.9	2.8
Lysine	0.4	0.7	1.9
Phenylalanine	0.6	2.0	3.1
Threonine	0.5	1.4	2.9
Valine	0.7	1.8	2.7

Table 2 Maximum inclusion levels of DDGS as a percentage of total feeds for various livestock at differing growth stages (from Ewing, 1997)

Ruminants		**Pigs**		**Poultry**	
Calf	10%	Creep feed	0%	Chick	0%
Dairy	40%	Weaner	0%	Broiler	5%
Beef	40%	Grower	2.5%	Breeder	5%
Lamb	0%	Finisher	5%	Layer	5%
Ewes	0%	Sow	5%		

Studies with pigs have shown that the high fibre content of the DDGS promotes an increased flow of nitrogen and amino acids at the distal ileum. The digestibility coefficient for most nutrients, including the key amino acids lysine and threonine, is therefore lower than for the grain, resulting in reduced performance (Nyachoti *et al,*. 2005). Non-ruminants such as pigs and poultry lack the enzyme phytase that breaks down phytic acid to release phosphate (Jacques, 2003). Availability of phosphorus in DDGS is higher than in the grain, so DDGS may provide a cost-effective alternative source of available phosphorous in pig rations (Widyaratne and Zijlstra, 2004).

The main market for DDGS is currently in animal feed. If the maximum inclusion rate for DDGS in ruminants is assumed to be 40% and the annual market for ruminant feed is approximately 5 million tonnes this may provide a market for 2 million tonnes of DDGS. The exact market size is difficult to assess and it is possible that, if the price was right, farmers who mix their own feeds may provide an additional market of approximately 0.5 million tonnes (Bruce Cottrill, ADAS, *personal communication*). As production of alcohol increases, it is possible that changes to supply and demand in DDGS will affect its price. This raises questions and potential opportunities for the livestock industry and further research is needed to investigate more thoroughly the potential for DDGS incorporation into both ruminant and non-ruminant diets and other uses.

DDGS could also be burned to provide a source of combined heat and power (Morey *et al.,* 2005) for either the bioalcohol production plant or conventional power plants. The renewable fuels obligation requires power suppliers to source an increasing amount of their feedstocks from renewable sources; 10% by 2010 and 20% by 2020 (Department for Transport, 2005), and DDGS would be an eligible renewable source. Alternatively DDGS could be used as a feedstock for biogas (methane) production with the methane produced potentially burned in a boiler to heat and power the distilling process (Fleischer and Senn, 2005). Using the wet DDGS in anaerobic digestion would also remove the very significant energy costs associated with drying DDGS. The residues from biogas formation could then be used as a fertiliser. The fate of DDGS can have a very large impact on the energy and GHG balance of the biofuel, but at present prices their value as an animal feed is likely to be greater than as an energy source. This could change as markets develop and especially if sufficient economic value was derived from their use to meet the renewable fuels obligation and, potentially, improvement in GHG balance under the RTFO. Given the quantities of DDGS that are likely to be produced, and the contribution of co-products to the profitability of alcohol plants, further research is required on the possible uses for DDGS, both as an animal feed and more widely.

Approximately 280kg CO_2 is produced per tonne of grain (at 85% Dry Matter (DM) as a result of the fermentation processes. This can be captured and sold as an additional co-product. CO_2 is used in the carbonated drinks industry, to enhance agricultural productivity in greenhouses, in refrigeration and packaging industries, or in fire extinguishers (Senn and Pieper, 2000). However, a limited market currently exists for CO_2 and it is likely to be uneconomic to capture CO_2 once market capacity has been reached, unless values for carbon sequestration were sufficiently high.

2.8 Process Integration

Schultze *et al.* (2005) estimated that energy may account for between 10-16% of the total costs of an alcohol production facility, depending upon the location and the feedstock used. The energy costs associated with each stage of the process are outlined in Table 4 and are similar to figures suggested by Schultze *et al.* (2005).

Table 3 Approximate energy use in bioethanol sub-processes (adapted from Meredith, 2003).

Process	**Thermal energy use (% total)**	**Electrical energy use (% total)**
Grain recovery and milling	0	1
Cooking and liquefaction	4-6	0
Fermentation	1	0
Distillation and dehydration	43-48	0
Evaporation and drying of DDGS	31-36	3-4
Utilities	4-6	4-5
Building	1	0
Sub-totals	91	9

The largest energy costs are associated with steps that involve heating water, which is necessary at three stages: cooking, distillation and DDGS drying.

Good plant design can lead to substantial energy savings. In modern integrated plants, distillation can be integrated with other heat consuming systems such as dehydration or evaporation of the stillage to reduce the energy and costs (Schultze *et al.* 2005) and therefore save GHGs. Further environmental savings could be achieved if biomass were used as the energy source, especially if this was DDGS or straw. In the short term, economics dictate that fossil fuels will be the primary energy source. It is likely that most plants will utilise a combined heat and power (CHP) approach whereby natural gas is used to produce steam to produce electricity via powering turbines and for use in heating and distilling within the plant. Excess electricity could then be sold back to the national grid.

Two major feedstock factors affect energy usage during processing and hence operating costs and energy balance (A) the viscosity of the feedstock and (B) the amount of residual material after fermentation that needs to be processed. Viscosity is largely affected by the amount of non-starch polysaccharides (NSPs). Problems with viscosity can significantly affect the energy consumption of the plant. As discussed already, high viscosity slurries have a high heat coefficient. For example, rye processing requires more energy than other feedstocks due to high viscosity (Meredith, 2003). The viscosity problem can be reduced by using more water relative to dry matter but this merely increases the requirement for heating, cooling and evaporation. Energy costs associated with the evaporation

and heating of water are minimised by working with the highest concentration of dry matter. Maximum dry matters vary depending on the feedstock; Lurgi PSI of Tennessee recommend maximum solid levels of 34-35% for maize, 30% for wheat and 28% for barley (Pam Tetarenko, Lurgi PSI, *personal communication*). Viscosity problems can be reduced in fuel alcohol plants and in the neutral alcohol industry by using commercial enzyme mixes that digest the NSPs, however, this would not acceptable in the Scotch whisky distilleries. Feedstocks with low NSPs are therefore desirable, especially in the Scotch whisky industry.

2.9 Conclusion

The broad process for alcohol production is common to the potable and fuel alcohol industries. Starch is degraded to glucose, fermented to alcohol by yeast and alcohol is separated by distillation from the residual material, which is usually dried to produce the valuable co-product DDGS. The broad picture may be modified according to the differing needs of the target industry. The Scotch whisky industry is confined by the requirements of the Scotch Whisky Order (1990) and therefore can only use grains, water and yeast in production. The focus is on producing a high quality traditional product and therefore throughput is less important than for a fuel alcohol producer. Fuel alcohol producers are less constrained in the production processes employed, but working to tighter margins, process efficiency and throughput will be crucial. Enzymes and chemicals will be used where this results in reduced energy costs and increased processing efficiency.

Optimising processing parameters such as temperatures, pressures and flow rates for each stage of the process will differ between processing plants, and for the feedstocks used. However, in general terms, little is published in the public domain about the importance of the rate of processing on profitability relative to absolute alcohol yields and how this can be optimised by feedstock quality. The potential availability of thousands of tonnes of extra DDGS on the UK market raises questions of how this material could be best utilised for animal feed or for other uses.

3.0 Feedstock Quality

Feedstock represents between 55-70% of bioalcohol processing costs (Schultze *et al*., 2005). Feedstock quality can affect the total yield of alcohol, the ease of processing and the quantity and quality of the co-products. Grain giving a high alcohol yield per tonne not only provides more saleable product, but it reduces the amount of residual material, and associated water use giving considerable savings in energy costs through reduced heating, cooling and drying. The value of high quality grain in a large bioethanol plant could run into millions of pounds per year. Other effects on processing efficiency and rate can also have large impacts on operating and energy costs. Thus alcohol producers are closely concerned about all aspects of feedstock quality.

3.1 Alcohol Processing Yield

Alcohol processing yield depends on (A) the amount of starch present, (B) how much of this starch is converted to fermentable sugars, and (C) the efficiency with which these sugars are fermented into alcohol.

The *potential* alcohol yield from grain is set by the content of starch (plus other fermentable sugars). Starch hydrolysis requires one molecule of water per molecule of glucose, so that 1000kg of pure starch potentially yields 1111kg of glucose. Assuming perfect fermentation efficiency, this glucose would be converted to 568kg of ethanol, with a density of 0.789kg/l, so producing 720 l.

		Enzymes		**Yeast**			
	Starch	Water	Glucose		Ethanol	+	Carbon dioxide
Formula	**$(C_6H_{10}O_5)_n$**	→	**$C_6H_{12}O_6$**	→	**$2CH_2H_5OH$**	**+**	**$2CO_2$**
Molecular weight	$(162)_n$	18	180		92		88
Mass	1000 kg	111 kg	1111 kg		568 kg (720 l)		543 kg
With dry grain at 69% starch & 3% sugar	720 kg	77 kg	800kg		409 kg (518 l)		391 kg

Figure 2 Theoretical efficiency of starch conversion to glucose and glucose conversion to ethanol

So far, there is no standard approach to grain analysis for biofuel production in the UK, and so there is no standard grain specification. In Table 5 we therefore propose a 'benchmark' grain analysis to which all variation can be related. For benchmark wheat grain, containing 69% starch and 3% sugar on a dry matter basis, the stoichiometric relationship above indicates a potential yield of 518 litres of

alcohol per tonne of dry grain. Increasing starch concentration increases potential alcohol yield by 7.2 litres per 1% increase. Typical alcohol yields of UK wheat are in the region of 435 l/t dry grain (from data in Figure 2, adjusted to 11.5% protein), so it seems that apparent processing efficiency is currently around 84% of potential. However, yeast growth normally accounts for around 8% of the sugars available for fermentation, so processing efficiency is probably nearer to 92% of potential which still indicates appreciable scope for improvement.

Table 4 Benchmark composition of UK feed wheat (dry basis). A variety of sources were used to give values believed to represent wheat produced in the UK. Grain nitrogen (hence other constituents) was adjusted to the level achieved with optimal fertiliser use (as set out in RB209).

	Composition (%)	**Reference**
Starch	69.0	(By difference)
Sugar	3.0	Feed industry standard
Non-starch polysaccharides	11.0	Englyst *et al.,* (1999)
Crude protein (N x 5.7)	11.5	MAFF (2000)
Lipid	2.5	Feed industry standard
Ash	2.0	Feed industry standard
Lignin	1.0	Aman and Hesselman (1984)
Total	100.0	

There is considerable uncertainty however in how much starch is really present in typical UK wheat; starch is notoriously difficult to measure, different measurement techniques giving substantially different values (see Section 3.3). Alcohol yields, on the other hand, can be measured directly in the laboratory using a process mimicking commercial potable alcohol production (Brosnan *et al.,* 1998; see Section 3.3). Results for UK wheat vary between 410 and 480 l/tonne (Figure 3). Grain nitrogen can be measured most easily, accurately and precisely using either the Kjeldahl or the Dumas methods. Conversion of nitrogen to protein in cereal grains is conventionally (and reliably) based on a factor of 5.7 (Jones, 1931) (Note that the factor of 6.25 used for all feeds by the UK feed trade is less accurate for cereal grains, and can lead to inadvertent over-estimation of protein, hence under-estimation of starch.)

Due to the inverse relation between starch and protein, increases in alcohol yield are correlated with decreases in grain protein content (Figure 3). Results from The Scotch Whisky Research Institute (SWRI) in recent seasons show a relationship that approximates to direct replacement of starch by protein and 100% efficiency of starch conversion to alcohol (i.e. a *decrease* of 7.20 l alcohol per dry tonne for a 1% *increase* in protein). In this case, 1% protein corresponds to -7.36 l/t alcohol yield (r^2= 0.659). That the slope of this regression is so close to the theoretical 'replacement' relation between starch and alcohol is striking. Since efficiency of starch conversion is unlikely to approach 100%, variation in protein content almost certainly correlates positively with

variation in other unfermentable materials. Indeed, Coles *et al.* (1997) show that starch content of New Zealand wheat grain relates inversely to arabinoxylan content. The intercept on the y-axis of the 'replacement' line shown in Figure 3 indicates a total unfermentable fraction of 28%, equal to the sum of components that are not starch or sugar in the benchmark grain analysis (Table 5).

There is a need for much fuller investigation of the explanations for variation in alcohol processing yields and particularly how alcohol yield can be maximised. Current research is being undertaken in the GREEN grain project (HGCA Project 2979) and an associated HGCA & SWRI funded PhD Studentship with Heriot-Watt University.

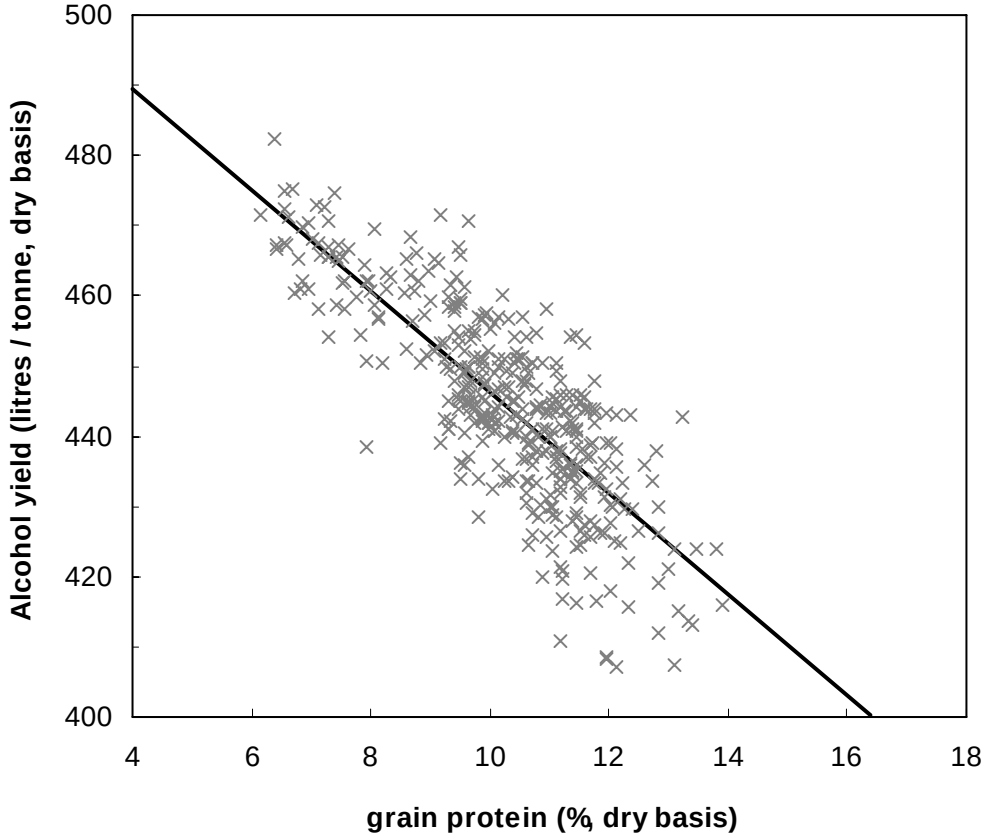

Figure 3 - Alcohol yields of grain samples from Recommended List (RL) variety trials against crude protein content. Data were measured by SWRI from many sites, harvests from 2003 to 2005, and from the GREEN grain project in 2005. The slope of the line represents direct replacement of starch by protein and complete conversion of starch to alcohol (see text for details).

3.2 Effect of Feedstock on Alcohol Yield

Figure 3 shows that differences in feedstock can give substantial differences in alcohol yield. A range of 2% protein (a range commonly seen at grain intake of UK wheats) gives a difference in alcohol yields of about 15 l/t. Assuming a value of ethanol of 40 pence per litre this difference would

be worth around £5/t grain to the bioethanol processor, plus potentially valuable savings in energy costs resulting from the reduced quantities of residue material. Set against this, production of DDGS would be lower.

The factors affecting alcohol yield are summarised in Figure 4 and each is discussed in successive subsections below.

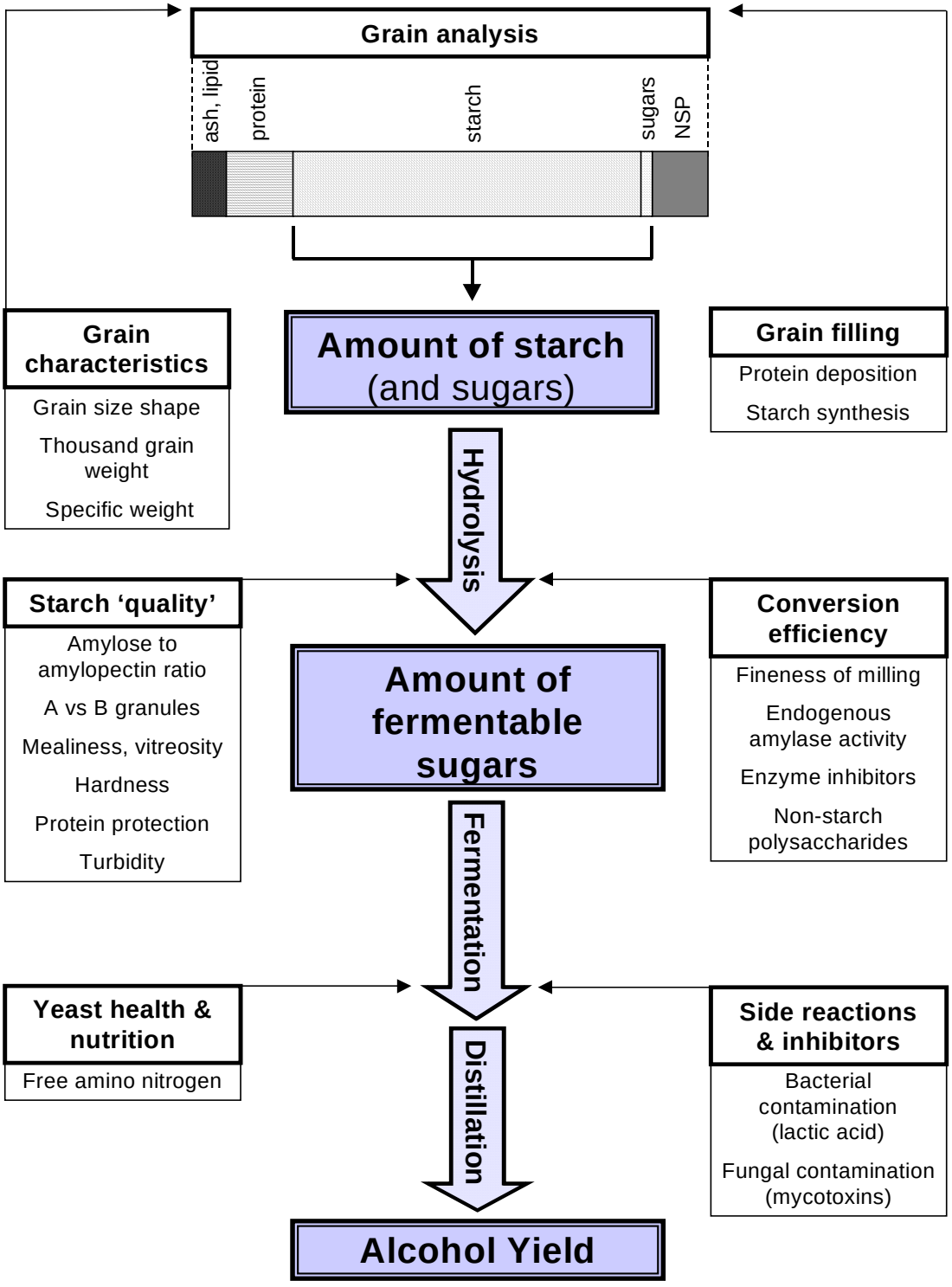

Figure 4 - Overview of the principle factors affecting alcohol processing yield and the main stages in processing at which these act.

3.2.1 Amount of Starch and Sugars

The most direct and obvious way of increasing alcohol yields is through increasing the amount of starch and sugar in the grain.

Starch is laid down during grain filling: starch deposition is more susceptible to poor conditions during grain filling than protein deposition. Factors that favour extended photosynthesis and grain filling will increase grain starch content, as will factors that reduce protein deposition. Large well-filled grains will contain more endosperm and therefore starch than poorly-filled shrivelled grains, as the endosperm will constitute a larger proportion of the grain in relation to the bran and germ. As such, factors such as grain weight and specific weight have an impact on alcohol yield. Grain width:length ratio can give an indication of the 'plumpness' of grains and this also has been associated positively with alcohol yield (Taylor and Roscrow, 1990; Swanston *et al.*, 2005a, Swanston *et al.*, 2006). However, these relationships tend to be fairly weak and do not apply across all varieties; the good distilling variety Glasgow has small grains but gives good alcohol yields, whilst Deben has large grains but gives low alcohol yields (Swanston *et al.*, 2005a). Good relationships between specific weight and alcohol yield have not been found (Taylor and Roscrow, 1990), although it is likely that samples with very low specific weights (<70kg/hl) will give poor alcohol yields.

The relationship between starch measured directly and alcohol yield is shown in Figure 5. Whilst the relationship is good (r^2= 0.78), the relationship with protein content is more precise (r^2= 0.85). A good relationship with protein is expected where differences in protein relate directly to differences in starch. This is most likely to be the case at low to medium protein where photosynthesis is ample for grain filling (sink-limited yield). Carbon from photosynthesis can either form protein or starch; if nitrogen is limiting then the starch content of the grain will be higher, whereas if nitrogen is abundant then the protein content will be greater and starch content lower. However, if photosynthesis becomes limited by drought or disease, protein contents will tend to be high and the relationship between protein and alcohol yield is expected to steeper. The combination of sink and source limited crops will tend to give curvilinear relationships between alcohol yield and protein content. Indeed, curvilinear relationships can be fitted to the responses in Figures 3 and 5, although without significant improvements in the amount of variation explained. Swanston *et al.* (2005a) found curvilinear responses of different varieties. Further work on the mechanics of grain filling is required particularly to elucidate how the partitioning of starch can be increased relative to

protein. Genetic and environmental effects on the protein and alcohol yield are explored further in Section 4.

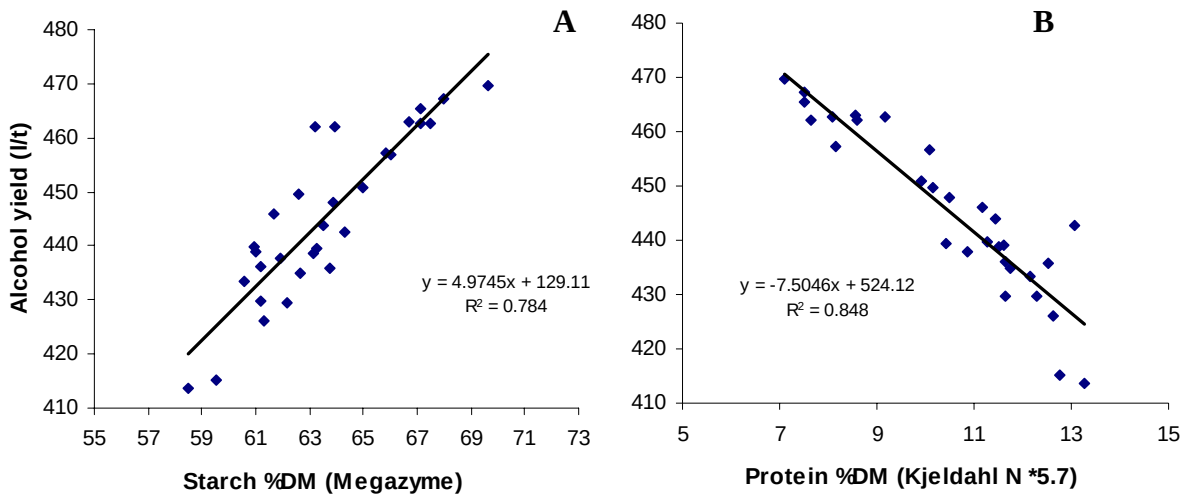

Figure 5 - Relationship of alcohol processing yield to starch (A) and protein (B) content of the grain. Starch was measured by the enzymatic method of McCleary *et al.* (1997). Data from a range of varieties, sites and agronomic treatments in the GREEN grain project (HGCA project 2979).

It is evident from Figures 5A and 5B that there is much variation in alcohol yield not explained by starch or protein alone. Whilst some of this is undoubtedly due to measurement difficulties, some at least must be due to differences in efficiency of starch conversion or fermentation.

3.2.2 Starch Conversion Efficiency

A 100% efficient conversion of starch to sugar by α amylase and amyloglucosidase will result in a mass of sugar that is 11.1% greater than the amount of starch processed. Modern fuel alcohol plants typically achieve a 10% increase of mass. Unconverted starch is carried through the process and ends up in DDGS. In practice, DDGS contain 1-2% starch, so the conversion efficiency of the starch breakdown is 98-99% (Kenneth Werling, Lantmannen Agroetanol, *personal communication*). Potable alcohol producers achieve a similar conversion rate.

Feedstock quality may affect the extent of starch conversion. Accessibility of the starch may be important, both in terms of physical and chemical structure of the starch itself, and in terms of protection by protein matrices etc. Also, other grain constituents may inhibit chemical conversion of starch to glucose. These issues will be explored in the following sections.

3.2.2.1 Starch Quality

Amylose and Amylopectin

Starch is composed of two polysaccharides; amylose, a linear chain of glucose residues, and amylopectin, a branched structure made up of a linear glucose backbone with occasional glucose side branches as shown in Figure 6. The ratio of amylose to amylopectin in starch contributes to its physical properties and its functionality and varies between species and varieties (Table 6).

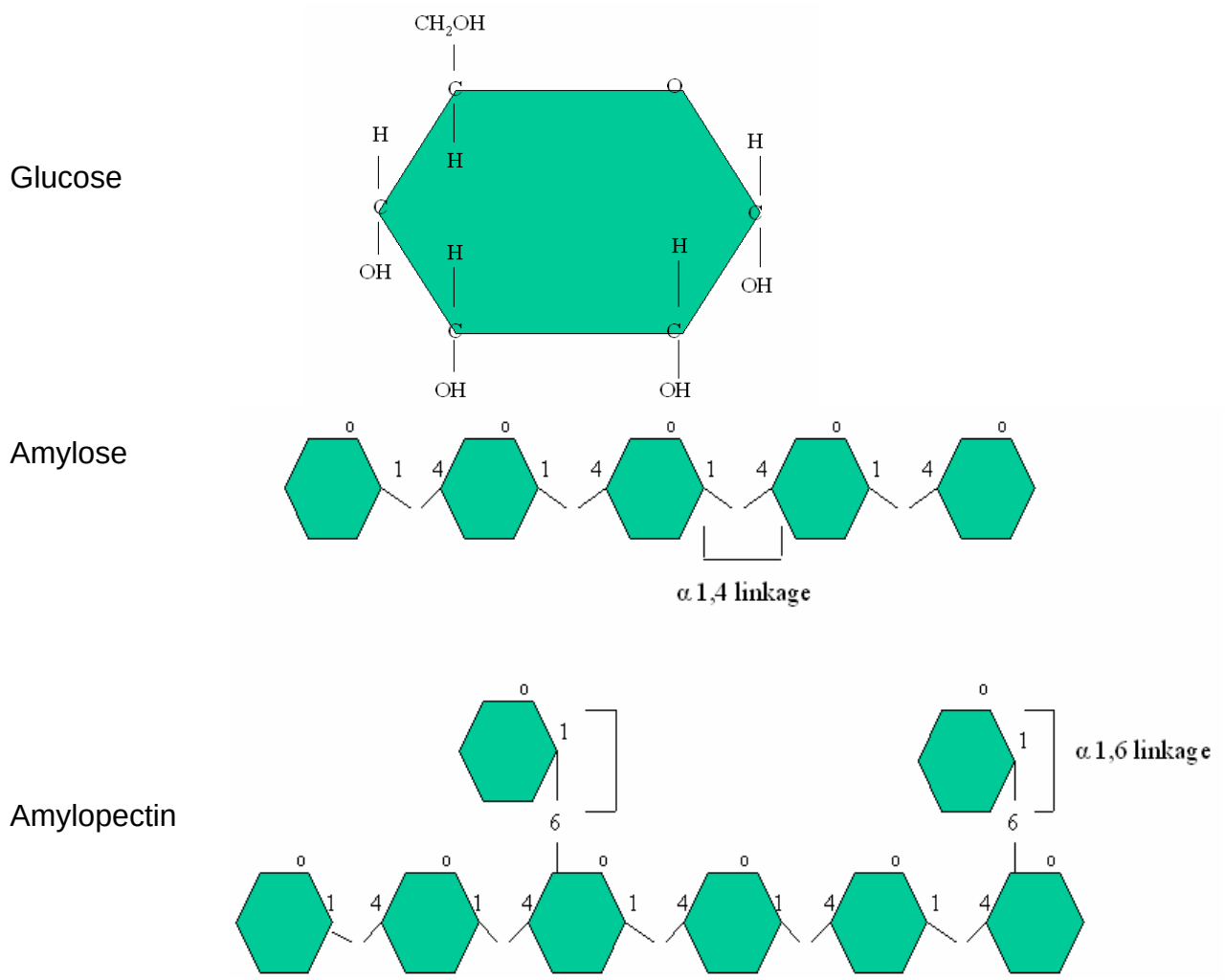

Figure 6 - Structure of glucose and amylose and amylopectin in starch. Glucose is a hexose sugar containing 6 carbon atoms [1]. Amylose is a linear chain of glucose residues linked by the carbons 1 and 4 of adjacent residues in an α 1-4 linkage while Amylopectin is a linear chain of glucose residues with occasional side branches linked by carbons 1 and 6 in an α 1-6 linkage. After Power (2003).

[1] The carbon atoms are numbered clockwise from the oxygen atom in the ring structure shown above

Table 5 - Percentage of amylose and amylopectin in starches from a variety of crops. Taken from Power (2003).

Starch Source	Amylose (%)	Amylopectin (%)
Wheat	25	75
Potato	20	80
Tapioca/cassava/manioc	17	83
Rice	20	80
Waxy rice	2	98
Maize	25	75
Waxy maize	1	99
High amylose maize	50-75	25-50
Sorghum	25	75
Waxy sorghum	<1	>99
Heterowaxy sorghum	<20	>80

Currently all UK wheats have a similar amylose content (*ca* 28%). High amylose starch has a low viscosity at a given temperature, because it requires much more energy to make it gelatinize and disperse into solution i.e. it will not gelatinize below 100°C, compared to gelatinization temperatures of *ca.* 60-70°C for standard starches. Although amylose may be more completely hydrolysed, high amylose starch is unlikely to be economic as a feedstock for alcohol production because of its high energy requirements for gelatinization. Conversely, starches with a high amylopectin content ('waxy starches') have a higher swelling power at a given temperature than standard wheat starch and disperse more readily into solution. Moreover, they do not 'set back' or retrograde to the same extent on cooling. Thus high amylopectin starch is generally advantageous to the alcohol processor. In the US, Japan and Australia, there are fully waxy (0% amylose) and partially waxy (*ca.* 21% amylose) wheats. Some breeders are reported to be developing waxy wheats for the UK. However, for the foreseeable future, it is unlikely that such varieties will be used for alcohol production, unless their grain yields become comparable to current feed wheats, and the benefits of a lower gelatinization temperature can be translated into a cost-advantage during processing.

Starch is packed into granules which, based on size, may be classified into large (A type) and small (B type) granule starches. The distribution of granule sizes in wheat affects its physical characteristics and has a small effect on wheat processing efficiency. In a study of 12 soft wheat cultivars in the United States, Raeker *et al.,* (1998) found significant cultivar-specific differences in the size distribution of starch grain sizes. These may also show some environmental variation. Large starch grains are more abundant in soft wheat than hard wheat varieties and they contain slightly more amylose (Raeker *et al.,* 1998, Capouchova and Maresova, 2003). Research on UK wheats indicates that total starch content (A+B granules) is more important for alcohol yield than the relative amounts of large and small granules (Brosnan *et al.,* 1998).

Starch Granules

Starch granules are embedded within a protein matrix within the endosperm, and grains can be classified as either mealy or steely according to their endosperm structure. A mealy grain contains starch granules loosely packed into a protein matrix providing air spaces within the endosperm, while a steely (or vitreous) endosperm contains a tightly packed matrix of starch, protein and cell wall material. Thus alcohol processing is favoured by mealy grains. Kolitsou and Palmer (2003) showed that barley varieties with a mealy endosperm released starch more readily and had a higher extract turbidity than steely endosperms. Swanston *et al.,* (2005b) investigated whether extract turbidity could be used as a predictor of spirit yield. The variety Consort combined high turbidity with high alcohol yield but turbidity did not accurately predict the alcohol yield of varieties such as Wizard. However, the results of the turbidity test are also affected by particle size of the flour after milling. Particle size after milling is influenced by grain hardness (i.e. sedimentation/turbidity tests can discriminate between hard and soft wheats), and hardness can therefore confound the interpretation of turbidity results. Genetically, mealiness is controlled independently of hardness (Weightman *et al.,* 2005). Further work is required to understand the effects of grain texture on alcohol yield.

3.2.2.2 Milling Effects

The fineness of milling can significantly affect alcohol yield; finely ground meal may yield 5-10% more ethanol than a coarser ground meal (Kelsall and Lyons, 2003). The fineness of milling is also known to affect starch digestibility in the context of poultry feeding. For example, in a study by Carré *et al.* (2005), starch digestibility was negatively correlated with hardness and particle size of flour prior to pelleting. However, as far as the authors are aware, there has been little study of the effects of fineness of milling on starch digestibility in the alcohol production process. It is generally assumed that because the flour undergoes a cooking and gelatinization step during processing, fineness of grinding will be less important than in poultry nutrition, where much of the starch remains ungelatinized.

3.2.2.3 Amylase Activity in the Grain

Starch reserves may be degraded *in vitro* by the action of endogenous α amylases. The extent of starch conversion can be assessed conventionally using the Hagberg (or Falling number) test, with which the grain trade industry is familiar. High endogenous α amylase activity (and low falling number) can be associated with pre-harvest sprouting and economic losses of grain dry matter, but may also result in starch conversion to sugars without any visible sprouting damage. It could be

argued that low Hagberg Falling Number (HFN) samples may give more efficient starch conversion because of higher levels of endogenous amylase, but conversely poor quality, e.g. sprouted samples may have already lost starch, and therefore alcohol yields might be reduced. Furthermore, a low HFN may cause browning reactions during the cooking step due an increase in free sugars. We have found no published data on the relationship between HFN and alcohol yield, but this may be worthy of further study in UK wheat. Indeed, a Defra-LINK study 'An integrated approach to stabilising HFN in wheat: screens, genes and understanding' is underway and may provide knowledge on whether HFN and alcohol yield are related. Exogenous starch degrading enzymes can be used in bioethanol production to overcome the differences in endogenous autoamylolytic activity and although costs are significant, they are not inhibitory. It is generally considered that endogenous enzymes are denatured at the cooking step and exogenous enzymes must be relied upon to ensure complete starch conversion.

3.2.2.4 Amylase Inhibitors in Wheat

The rate (or extent) of starch hydrolysis during processing may be affected by inhibition of amylase by other proteins naturally present in the wheat. Interestingly, such α amylase inhibitor levels were noticeably less in wheat (16 mU barley α amylase inhibited per gram of flour) than triticale (average 73) or rye (113-145) (Flintham *et al.,* 1993). It is not known to what extent amylase inhibitors in wheat affect the activity of commercial exogenous enzymes. Further work is required to assess their importance.

Due to the reliability, speed and effectiveness of commercial starch degrading enzymes it is unlikely that endogenous enzymes will be used for fuel alcohol production in the immediate future, especially since any endogenous enzymes would be inactivated by the high temperature initial cooking steps currently used to gelatinize the starch. This situation may change with the advent of novel enzymes that degrade starch without the need for a cooking step (i.e. Genencor, 2005). In the USA, Syngenta and Diversa have developed a transgenic maize line, AmylaseT™, which makes high levels of a thermotolerant α amylase endogenously and thus reduces or eliminates the need for added α amylases during processing. A similar approach may be feasible for wheats, although this would not be acceptable for use in the potable alcohol industry.

3.2.3 Fermentation Efficiency

Fermentation is the key step in alcohol production and yeast must be carefully treated to obtain the maximal conversion of fermentable sugars to alcohol. Management of fermentation is a

huge subject which has been well reviewed elsewhere (e.g. Kelsall and Lyons, 2003). Critically the temperatures, nutrients, sugar and alcohol concentrations should be at levels that do not starve or poison the yeast or encourage wasteful side reactions. The following sections just deal with the effects of feedstock on these influences.

3.2.3.1 Nutritional Factors

Yeast fermentation in ethanol production is often limited by a lack of free assimilable nitrogen. However, this is easily overcome by the addition of exogenous assimilable nitrogen such as urea or ammonium (Thomas and Ingledew, 1990). Exogenous proteases may also be used to break down wheat proteins to provide amino acids and can substitute for an exogenous nitrogen source (Jones and Ingledew, 1994, Genencor, 2006). Phytic acid makes up 60-80% of phosphorous in cereal grains and can form complexes with nutrients such as minerals and amino acids. This can limit their availability and have a significant anti-nutritive effect on the yeast (Kelsall and Lyons, 2003). In Scotch grain whisky production the addition of 10% barley malt for starch saccharification also provides sufficient free amino nitrogen to sustain fermentation.

3.2.3.2 Side Reactions and Inhibitors

Microbial contamination (particularly from *Lactobacillus* spp.) has been identified as a particular problem during the fermentation process in maize bioethanol plants - lactic acid and acetic acids can inhibit yeast growth and bacteria compete with yeast for sugar substrates (Skinner and Leathers, 2004; Narendranath *et al.,* 2000). In practice, bioethanol plants accept 5% bacterial infection. Bacterial contamination can have a significant negative effect on alcohol yield and it is desirable to reduce bacterial contamination as far as possible. Mycotoxins from fungal contamination of grain can certainly affect yeast growth – the mycotoxin Zearalenone can inhibit yeast growth at 50 ppm, Deoxynivalenol at 100ppm and Fumonisin at 10ppm, to the extent that they partly account for slow or stuck fermentations (Kelsall and Lyons, 2003). However, it seems unlikely that bacteria or mycotoxins in UK wheats would significantly affect alcohol processing yields.

3.2.4 Non-Starch Polysaccharides – Effects on Processing Efficiency

NSPs such as arabinoxylans (also called pentosans), β glucans and fructans can have significant effects on processing efficiency and yield. Quantitatively, the arabinoxylans are the most significant of the NSP in the whole wheat grain representing 8% of grain dry matter, out of a total

NSP concentration of 11% (Englyst *et al.,* 1992). They make up 70% of the endosperm cell walls (Sorensen *et al.*, 2006).

Non starch polysaccharides have a high water binding capacity and significantly increase the viscosity of the mash. Increased mash viscosities can have a number of deleterious effects on production, and consequently on alcohol yield and throughput. Viscous slurries are difficult to transport and mix, and may result in plant maintenance and hygiene problems. Viscous mashes also cause problems with uneven temperatures because they have high heat coefficients. This is particularly problematic in the processing of co-products such as DDGS after distillation. Viscosity tends to increase the energy needed to dry the DDGS.

In wheat, the main viscosity promoting polysaccharides are arabinoxylans. In a study of 22 French wheat varieties, Saulnier *et al.* (1995) found that different cultivars varied in the soluble arabinoxylan content. Cold water extract viscosity appeared to be largely determined genetically. Later, Martinant *et al.* (1998, 1999) identified quantitative trait loci (QTL) for water extractable arabinoxylans. Similarly, Weightman *et al.* (2001) showed that cold paste viscosity was influenced genetically, tending to be high in wheats containing the 1BL/1RS translocation.

Viscosity problems can be overcome by increasing the water content of the mash. However, as discussed in Section 2.8 producers aim to work with the minimum water content to maximise throughput and reduce the energy costs of drying. Enzymes that break down the non-starch polysaccharides, such as xylanases, are commercially available (Sorensen *et al.,* 2006) and have been included for many years in monogastric animal feed. Specific mixtures have been optimised for rye, wheat and barley based on the differing nature of their non-starch polysaccharides (Novozymes, 2006a) and so viscosity problems can be overcome at a cost. However, such enzymes cannot be used in potable alcohol production, except during co-product processing following distillation.

3.3 Assessing Feedstock Quality

Given the importance of feedstock quality to the alcohol processor, it is important that accurate and rapid methods are available for feedstock analysis. In general direct measurements are used to provide accurate estimates of quality attributes and indirect methods (calibrated from direct measurements) have been developed for rapid use at point of trade or in plant breeding.

3.3.1 Direct Alcohol Measurements

Alcohol yield is accurately determined by the method of Brosnan *et al.* (1998) and simulates the production process conditions in a potable alcohol grain distillery although in actual grain whisky production only malted barley would be used as the enzyme source. Wheat is milled to a fine grist and slurried with water. Termamyl™ α amylase is added, the mash is gradually heated to 85ºC before pressure cooking at 142ºC for 15 minutes in an autoclave. The cooked slurry is cooled to 85ºC and treated with Termamyl™ again to prevent starch retrogradation. Mashing occurs at 63ºC with high amylolytic enzyme malted barley for 1 hour. The mash is the cooled to room temperature and pitched with 0.4% (w/v) yeast and fermented at 68 hours at 30ºC. Distillation is then used to collect the alcohol. The alcohol yield is determined from the alcohol strength of the distillate measured by a Paar 5000 density meter. The results are quoted on a dry weight basis as litres of alcohol produced per tonne of cereal.

3.3.2 Direct Starch Measurements

The majority of quantitative starch analysis methods are based on a two-stage procedure with (i) the hydrolysis of starch into glucose by either acid or enzymatic means, followed by (ii) quantification of the glucose produced. These methods are summarised in Figure 7. Suitable analytical methods for determining glucose can include polarimetry, colorimetry, gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC). It is not the purpose of the present review to describe all analytical methods, but rather to comment on the main methods employed for starch analysis in commercial practice and to comment on their robustness and their relative costs.

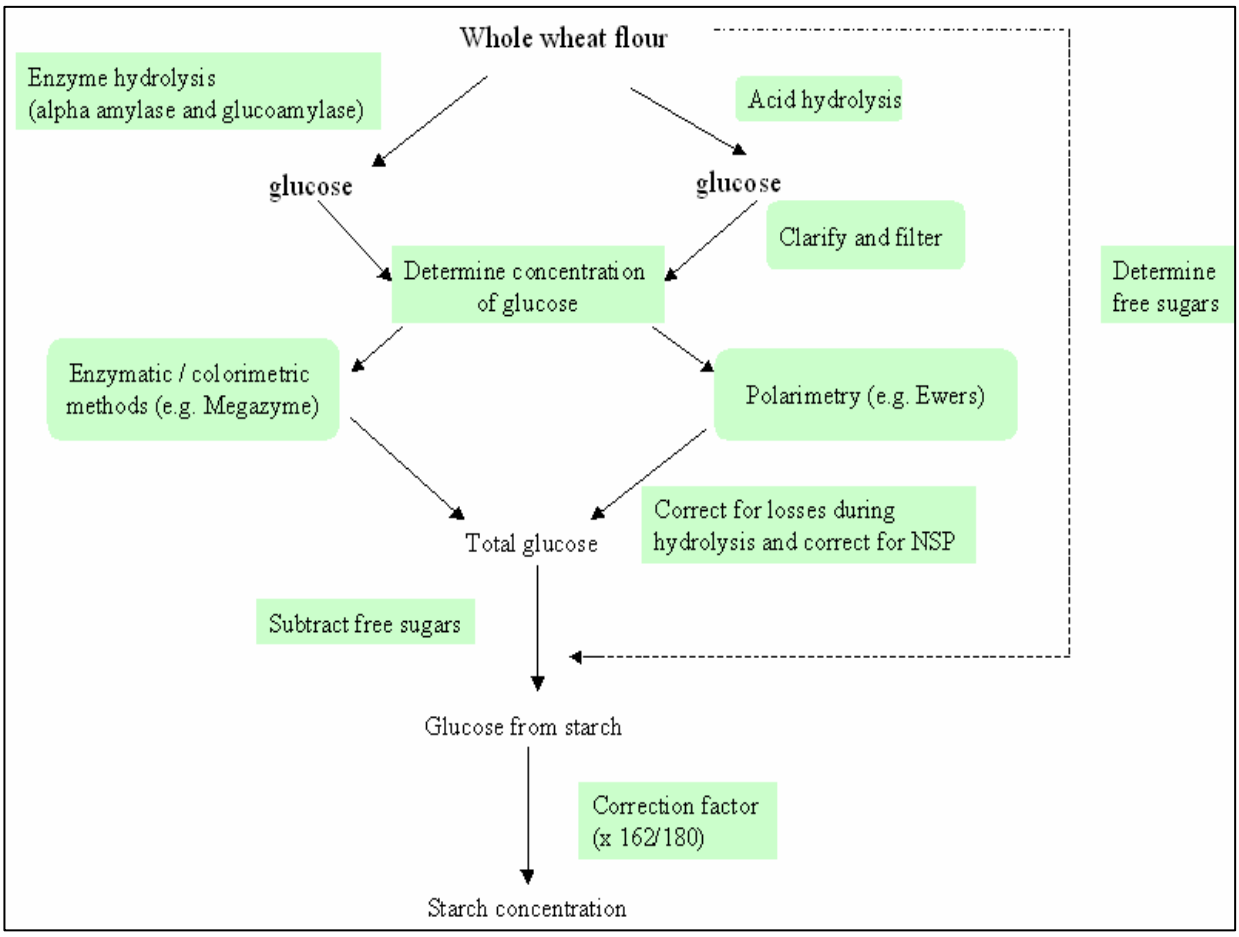

Figure 7 - Overview of the enzymatic and acid hydrolysis methods of quantifying starch.

3.3.2.1 Hydrolysis Methods Employed During Starch Analysis

Acid Hydrolysis

Dilute mineral acids can be used for starch solubilisation and hydrolysis. Such methods are relatively straightforward, assuming standardisation of temperature and time of hydrolysis. When analysing mixtures of glucose-containing polymers in plant samples, dilute sulphuric acid (e.g. 1M) is fairly selective for starch, leaving any cellulose intact (unless pre-treated with 12M sulphuric acid). Therefore it can be safely assumed that hydrolysis of cellulose will not interfere with starch determination at low acid concentrations (1-2N). However, there is always some loss of sugars during hydrolysis (*ca*. 10-20% dependent upon time, temperature and concentration of acid). Therefore, a correction factor (based on the loss during hydrolysis of a reference sample of starch) needs to be employed when estimating the actual starch concentration.

Enzyme Hydrolysis

Modern methods of analysis use enzymes rather than acid to break down the starch. The advantage is that there should be no loss of glucose through degradation (as in the case with acid hydrolysis). Three key enzymes are needed to break down the starch fully; α amylase (usually a thermostable amylase such as Termamyl™), pullulanase (to break down the 1-6 branch points of the amylopectin), and amyloglucosidase (to breakdown any residual glucans and maltose to glucose). α amylase can also break down 1-6 branch points of the amylopectin, albeit slowly. Because of this, and because it is very expensive, pullulanase is not used in industrial alcohol production.

The use of Termamyl™ (Novozymes, 2006b) and other heat stable α amylases (Richardson *et al.,* 2002) that work at 95°C is very important because the high temperature employed aids gelatinization and solubilisation of the starch. This is critical in ensuring complete hydrolysis. Some resistant starches (typically found in processed foods such as breakfast cereals and also in maize) require a pre-treatment step with an aggressive solvent such as dimethylsuphoxide at 100°C to fully disperse the starch prior to analysis (Englyst and Cummings, 1998). Insufficient gelatinization and dispersion is the most common source of inaccuracy in enzyme-based starch determinations, leading to underestimates of true starch content.

3.3.2.2 Polarimetric Measurement of Glucose for Starch Analysis.

In the polametric (or Ewers') method (USCL method 11-02), the starch is released from the milled wheat flour by boiling in dilute hydrochloric acid (HCl). This procedure effectively gelatinizes the starch granules and simultaneously hydrolyses the starch to glucose in a single step. The acid will also help break down the endosperm tissue, ensuring complete release of the starch granules from the protein matrix. Substances which may interfere with the measurement are removed by filtration/clarification and then glucose concentration is determined by measuring the angle of polarization or optical rotation (Senn and Pieper, 2000). Due to its simplicity this is a relatively inexpensive method. However, it has some potential sources of error that must be taken into account when using complex starting materials (like whole wheat) rather than pure starch. The free sugars which are present in the wheat grain (*ca*. 2-3% of dry matter; Lineback and Rasper, 1998) contribute to the estimate of glucose content. These free sugars are therefore measured separately by extracting a sample in ethanol solution, and subtracting the free sugars from the total glucose estimated by polarimetry. Errors can also arise using the Ewers' method because sugars can be generated by the acid hydrolysis of the NSP. As discussed earlier, NSP can account for *ca.* 10% of the dry weight of the grain. The monosaccharides released are mainly pentose sugars (xylose and arabinose) but these

can interfere with the quantification of glucose. While only a proportion of the total NSP will be hydrolysed by dilute HCl, it is likely that the contribution from breakdown of NSP could be of a similar order of magnitude to the free sugars. For this reason, the Ewers' method is stated not to be a suitable method for samples expected to have high levels of NSP or optically active substances which do not dissolve in 40% ethanol (Van Eys *et al.,* 2004). If the concentration of NSP is the same in every sample of wheat, then this can be discounted using a correction factor, but it is known that NSP levels in wheat are influenced by both genotype and environment. Results from the Ewers' method should therefore be treated with caution and will not be directly comparable to the results obtained by other methods.

3.3.2.3 Enzyme-Based, Colorimetric Measurement of Glucose for Starch Analysis.

Following enzymatic hydrolysis of the starch as described above, it is necessary to quantify accurately the glucose released. This is possible using GLC and HPLC, but the former requires a lengthy derivatization step making the technique relatively expensive, and the latter requires a specialised HPLC system. It is unlikely that these will be used in practice for routine starch determination. The availability of specific enzymes for the oxidation or phosphorylation of glucose means that the final step of starch measurement can be carried out accurately by enzymatic methods.

In the glucose oxidase method, the glucose is treated with glucose oxidase-/-peroxidase (GOPOD) reagent before quantification spectrophotometrically at 540nm. The amount of glucose in a sample is then determined from a standard curve. Typical of this approach, AACC method 76-11 employs the use of an autoclave to disperse the starch, and amyloglucosidase to hydrolyse the starch to glucose. Note however, that a single enzyme is employed with α amylase and pullulanase omitted, therefore this method can underestimate starch content in some processed cereal products and in high amylose starches.

An alternative enzymatic method of starch determination was described by McCleary *et al.,* (1997) and is derived from AACC method 76-11 using the GOPOD reagent, but utilising a thermostable α amylase to ensure full hydrolysis of the starch. This method can also be modified for samples with high levels of resistant starch through a pre-treatment step at 100°C in dimethylsuphoxide. This method has the advantage over method 76-11 in that it gives quantitative starch results even when the starch is resistant to degradation. Megazyme® supply all of the enzymes and standards in kit form and the method is fully certified by both AOAC and AACC (AOAC 996.11 and AACC 76-12).

3.3.2.4 Comparison of Starch Analysis Methods

Modern enzyme assay kits are of high purity and selectivity, but can be relatively expensive per sample compared to older (acid hydrolysis) methods. Enzyme–based methods can also be laborious, which contributes to their cost, and while in principle they are robust, they are more complex. With a large number of pipetting and transfer steps it is found in practice that they are only reliable in the hands of a skilled operator who uses them regularly. Therefore, when commissioning starch analysis using a contract laboratory it is advisable to ask about the performance in the laboratory e.g. repeatability of the method using a standard sample. In practice, although the Ewers' method is not selective for glucose and contains a number of correction factors, it has been found difficult to replace this by other methods (e.g. enzyme assays) in regulatory work (Jurgen Möller, FOSS, *personal communication*).

3.3.3 Protein Measurements

Protein measurement may be the most important determinant of wheat quality for alcohol production given that (A) the precision of protein methods is significantly better than starch methods, (B) there is a clear inverse relationship between protein and alcohol yield (as discussed above; see Section 3.1), (C) protein analysis is relatively inexpensive and is understood by growers, and (D) protein contents can be directly linked to agronomic practice, specifically N fertiliser inputs. Two main analytical methods for protein determination of wheat grain are used: the Kjeldahl method and Dumas methods. Both measure grain nitrogen content. Protein content is then estimated by multiplying nitrogen content by a factor of 5.7 (Jones, 1931; Draper and Stewart, 1979).

3.3.3.1 Kjeldahl Method

The Kjeldahl method is a wet chemistry method using digestion of organic matter with sulphuric acid and conversion to nitrogen to yield ammonium sulphate. In a separate step, the ammonium sulphate is refluxed with hydroxide and the ammonia is distilled and quantified by titration. The amount of nitrogen in the sample is proportional to the amount of acid needed to titrate the ammonium (Van Eys *et al*., 2004).

3.3.3.2 Dumas Method

In the Dumas method, the sample is burnt at very high temperature, and the nitrogen in the exhaust gases is measured directly. There are many variants of the Dumas procedure: typically in modern instruments (e.g. Leco Corporation, St. Joseph, Michigan, USA) the sample is burnt in a stream of high purity oxygen, the gases are scrubbed to remove CO_2 and water, and are passed over a heated copper catalyst to reduce the nitrous oxides to nitrogen gas. The concentration of N_2 is

quantified in the flow cell of an N-specific gas analyser. The Dumas method is faster than Kjeldehl, can be used on smaller samples and can be automated allowing high throughput. The Dumas method may give higher readings than the Kjeldahl method because it includes any nitrate, therefore the Kjeldahl method is the reference method for N and hence protein determination (Van Eys *et al.,* 2004).

3.3.4 Indirect Measurements

3.3.4.1 Near Infrared Reflectance (NIR)

NIR can measure rapidly a number of grain parameters such as protein, moisture and starch content. Two main factors influence the robustness of an NIR method, (A) the number and nature of the reference samples, and (B) the reference method by which they were analysed.

Samples are illuminated with near infrared light and the amount of light absorbed (i.e. not reflected or transmitted) is proportional to the amount of a particular component in the sample. Illumination at different wavelengths allows different components to be quantified. NIR is used on the whole grain so is non-destructive and because samples can be assessed in seconds rather than hours, is high throughput. NIR therefore has the potential to be useful for quality testing of grain at the factory gate or point of trade.

The relationship between the reflectance (or transmittance) of a sample and the amount of a particular substance is not predictable, and is therefore based on a calibration between the amount of substance determined by traditional laboratory based methods and the NIR reading. Reliable calibrations require a large number of samples representing the full range of varietal types and environments from which the NIR machine is likely to receive samples in practice. This limits precision. The sensitivity of NIR results also dependent on temperature, water content and reflectance from interfering compounds. These variables can be accommodated for by statistical corrections (Van Eys *et al.,* 2004).

Current NIR calibrations for starch appear to use Ewers' method as a reference. Despite its limitations and the fact that the starch values obtained for different species cannot be compared directly, Ewers' method is widely used in regulatory work. Given that Ewers' method can be affected by solubilisation and / or breakdown of NSP in a whole wheat sample, the main issue for the UK industry is whether (A) the reference set contains enough UK wheat varieties, and (B) whether it contains a wide enough range of starch contents, typical of UK wheats. In conclusion, NIR measurement of starch appears insufficiently precise or accurate to allow a reliable estimation of

alcohol yield. However, NIR calibrations for protein are more precise and robust and hence initially it is likely that protein content within grains will be used for predicting potential alcohol yields. Ultimately, the ideal measure would be NIR measures of expected alcohol yield, based on calibration measurements from samples directly measured for alcohol yield. Indeed, work by FOSS using samples generated by RL trials and the GREEN grain project, and analysed by SWRI, is aiming to produce an NIR measure of alcohol yield.

3.3.5 Tests for Processing Efficiency

There is currently no rapid test to determine whether a feedstock will lead to processing problems. A laboratory method, used by the potable alcohol industry to determine residue viscosity is the current standard. Residues from alcohol distillation (described in Section 3.3.1) are adjusted to a fixed volume (250ml) and centrifuged to remove the grain solids. The viscosity of the supernatant is measured at 20°C using an Ostwald viscometer in a similar fashion to that described by The Analysis Committee of the Institute of Brewing (IOB) (1997). No test currently exists for quickly determining NSP content which is a major contributor to viscosity problems.

3.4 Implications of Feedstock on Co-Product Quality

Grain quality (both proximate composition and microbiological quality) is important where the co-products from alcohol production are to enter the food chain. It is important that grain hygiene should be given a high priority by both growers and processors, so that microbial and chemical contamination during growing and storage are avoided wherever possible.

Mycotoxins are produced from fungi and moulds originating in the field (*Fusarium* species) or in storage (e.g. *Penicillium* species). Mycotoxins have detrimental effects on animal health and well-being and on human health. Legislation now limits the concentrations of mycotoxins allowed in foods and feeding stuffs and growers must not dispose of contaminated grain in the food chain. For instance it is not acceptable to mix contaminated grain with uncontaminated grain and the reader is directed to relevant guidance published by the HGCA (see Topic Sheet 91: Managing the *Fusarium* mycotoxin risk in wheat and Topic Sheet 78: Drying and cooling grain: an update). Mycotoxins are of particular concern for downstream products such as DDGS because mycotoxins are not destroyed by high temperature treatments. Moreover, because of the near complete fermentation of starch during bioethanol production, mycotoxins are concentrated approximately three-fold in the DDGS. Similarly, crops should not be grown where there is a chance of heavy metals accumulating in the grain. Grain health is therefore an important concern for processors and many ethanol producers have stringent grain quality guidelines, resulting in rejection of contaminated and damaged grains

(i.e. Lantmannen Agroetanol in Sweden). However, it should be noted that mycotoxin and heavy metal levels in UK wheats are rarely high enough to be problematic today.

The final nutritional profile of DDGS can affect livestock performance. Variations in the composition of nutrients other than starch are magnified approximately three-fold in DDGS. The essential amino acid lysine for example, is a key limiting amino acid for animal growth and if varieties that had increased lysine content were used in processing this would make the DDGS more nutritionally valuable. Processing may also affect DDGS nutrient composition. Uneven drying of DDGS because of high viscosity (see earlier discussion on the effects of NSP in wheat) causes localised overheating and results in sugar binding to amino acids through the Maillard reaction. This causes the grains to become dark (hence the alternative name Distillers Dark Grains with Solubles) and makes amino acids unavailable for digestion, thus reducing the nutritional benefits of DDGS. This is less of a problem in modern alcohol plants where sugar fermentation is more complete and better heat distribution systems and lower drying temperatures can avoid burning effects. The likely nutritional value and potential variation in DDGS from UK fuel alcohol plants compared to that from traditional distilleries has not yet been explored but will be of interest to the livestock industry.

3.5 Current Wheat Grain Feedstock Specifications

3.5.1 Potable Alcohol

The potable alcohol industry uses a relatively simple quality specification for wheat. Aside from standard parameters such as moisture, the specification is for soft wheat with a specific weight over 72 Kg/l and as low nitrogen as possible. Hard wheats have proven to process poorly in potable alcohol distilleries and are therefore unsuitable for distilling. Although single wheat varieties are not specified, distillers often have a preferred list of varieties that they accept which is based on varieties rated as acceptable for distilling on the HGCA and SAC Recommended Lists. Varietal effects, including hardness and the 1B/1R translocation, are discussed in the next chapter.

3.5.2 Fuel Alcohol

The specifications of wheat used for fuel alcohol production may vary between different producers depending upon the production process employed and the co-products produced. The basic requirement for European producers Abengoa and Sudzucker (utilising wheat as a feedstock and producing only ethanol and DDGS) is for standard feed grade. Feed wheat rather than milling wheat is desirable because of its low protein content (hence high starch content) and low price. In contrast, the Canadian producer Permolex rejects feed wheat in favour of specified varieties typically used in bread making (Permolex, 2006). This is because Permolex utilise a “wet grind” procedure where high

protein is desirable for production of gluten. A high starch content is not so critical in this process since ethanol is only one of a number of valuable products, and Canadian feed wheat yields are closer to those of milling wheats.

Sudzucker and Lantmannen Agroetanol (who use a dry grind) specify the requirements for high quality standard feed wheat with low mycotoxin levels, freedom from ergot and no heavy metal contamination (Marie Afors, Lantmannen Agroetanol, *personal communication*; Bernhardt Dahmen, Sudzucker, *personal communication*). Most processors also reject grain with over 15% moisture content (Permolex, Lantmannen Agroetanol) due to the potential for mould contamination of grain in storage.

A premium would provide an incentive for growers to select varieties and grow crops that would maximise desirable grain characteristics. Lantmannen Agroetanol in Sweden for example, offer a supplement to growers for grains that have a starch content greater than 71% (Marie Afors, Lantmannen Agroetanol, *personal communication*) whilst Sudzucker are considering introducing a premium for low protein contents due to the difficulties in accurately assessing starch content (Bernhardt Dahmen, Sudzucker, *personal communication*).

3.6 Conclusion

Current specifications for alcohol production in the UK are based on feed wheat. However, as shown in this chapter, the suitability of feed wheats for alcohol production can show significant variation between varieties, sites and growing systems.

The quality factor of primary importance to the alcohol producer will be the amount of starch present. However, this may not be directly measurable and producers may be better off using protein content as a more precise guide to predicting alcohol yield. In the future, premiums for specific varieties or starch contents may be introduced to provide growers with an incentive to grow the varieties best suited to the processors requirements. In both potable and fuel alcohol industries, a high starch grain content is desirable because it maximises alcohol yield per tonne of grain processed. High starch contents also improve processing efficiency as more of the feedstock is converted to alcohol and consequently less material is left over as a residue, giving considerable savings in energy costs associated with heating, cooling and drying. Centaur Grain in the UK (Centaur Grain, 2006) and Lantmannen Agroetanol in Sweden have offered premiums for growers based on starch content, yet in our opinion, current methods for starch measurement are inaccurate. We suggest that processors should consider introducing a low protein premium for grains because grain N is inversely

related to starch content and can be accurately and easily measured using high throughput devices such as NIR. Any premium based scheme will have costs to the processor, through increased feedstock costs and also analysis and logistical costs, and potentially reduced marketing flexibility. There is a commercial need on the part of the biofuel industry to explore in more detail the economic consequences that differences in feedstock quality have on profitability of the alcohol plant, and the potential costs and benefits of operating a premium based system.

As discussed in Section 2, the technology employed in each of the alcohol industries varies and this may have a significant effect on the feedstock required. Viscosity is potentially a major processing problem in all alcohol production industries. In the fuel alcohol industry viscosity can be reduced by using enzyme mixes. In the Scotch whisky industry the use of such enzymes is not allowed. Therefore, while both industries can benefit from growers supplying grains of varieties that are known not to have viscosity problems, it is more important for suppliers of the Scotch whisky industry. If the viscosity issue is a major concern to the processor, the most appropriate course of action may be to avoid problematic varieties given the lack of appropriate tests for NSP and the general lack of understanding in this area. It is likely that fuel alcohol processors will use a wider range of varieties than traditionally used by distillers and therefore there may be a requirement to test the alcohol production traits of a wider range of varieties using a method which more closely relates to the fuel alcohol process.

4.0 Agronomic Effects on Variation in Feedstock Quality

The significant differences between wheat feedstocks in processing efficiency and alcohol yield can be associated with species, varieties, environment or management. This Chapter describes variation caused by these factors in more detail. However, it must be recognised that, unless premiums are offered for feedstock quality, or unless carbon accreditation schemes are introduced at the farm level, the primary considerations for growers of wheat for alcohol production will be to maximise grain yield and minimise costs of production. Since the fuel alcohol market is global and highly competitive, success for the fledgling fuel alcohol industry in the UK will depend very much on minimising costs of feedstock production as well as minimising processing costs. The principles of low cost grain production are well rehearsed in the farming industry, so these need not be repeated here. Rather, our emphasis will be on accounting for variation in feedstock quality, whilst noting any interactions that important factors may have with crop productivity or growing costs.

4.1 Genetic Differences in Feedstock Quality

Differences in feedstock quality between species are usually larger than between varieties, so initially we consider how wheat compares to maize, rye, barley, oats and triticale. Despite a few studies on genetic effects from Germany (Fleischer and Senn, 2005, Rosenberger, 2005; Aufhammer *et al.,* 1993; Aufhammer *et al.,* 1994; Aufhammer, 1996) there is currently a dearth of reliable information in the literature.

4.1.1 Cereal Species

Table 7 shows data from the literature on alcohol yields for different cereal species. Results tend to be confounded by environmental effects, because grain samples have been collected *ad hoc* and so also represent a range of growing conditions. In a comparison of rye, triticale and wheat in Germany, Rosenberger (2005) found that wheat showed the greatest range in protein concentration, starch content and consequently alcohol yield. Alcohol yields were found to increase with starch content (measured by Ewers' technique) to a similar extent in both wheat and rye (4.7 l / tonne / % starch), but triticale gave less alcohol per unit of starch and was less responsive to increases in starch content. This contrasts however with other work where triticale has been found to give higher alcohol yields at a given protein content than wheat (Fleischer and Senn, 2005; Aufhammer *et al.,* 1994; Aufhammer, 1996).

Table 6 - Starch content and extrapolated alcohol levels of production for several cereal species grown in the UK.

Source	Literature		On-farm statistics		Trial statistics		Reference
Species	**Starch content (%) DM**	**Alcohol yield (l/t)**	**Yield (t/ha) #**	**Alcohol yield (l/ha)***	**Yield (t/ha) #**	**Alcohol yield (l/ha)***	
Wheat	69	444	7.73	2914	10.0	3770	Rosenberger, 2005
Triticale	69	441	4.37	1636	7.0	2621	Rosenberger, 2005
	63	403	4.37	1496	7.0	2396	Sosulski *et al.*, 1997
Rye	65	438	5.78	2152	7.6	2829	Rosenberger, 2005
	64	397	5.78	1949	7.6	2563	Ingledew *et al.*, 1999
	64	409	5.78	2009	7.6	2642	Sosulski *et al.*, 1997
Barley	59	367	5.71	1781	8.2	2558	Sosulski *et al.*, 1997
Oats (hulled)	51	318	5.88	1587	8.1	2187	Thomas and Ingledew, 1995
Oats (naked)	60	353	5.88	1765	8.1	2432	Thomas and Ingledew, 1995
Maize	72	439			7.1	3117	Putnam *et al.*, 1991

Data from Rosenberger (2005) are based on German varieties of wheat (81 observations), triticale (18 observations) and rye (31 observations) grown in Germany in 2003 and 2004. Starch contents were measured by Ewers' technique and are the means of observations in 2003/2004.
Data from Sosulski (1997) for barley, triticale and rye are based on one Canadian variety per species. The starch measurement method was not reported
Data from Ingledew (1999) are means of 7 rye varieties. Starch measured using an enzymatic method similar to that of Stitt *et al.* (1989).

#On farm grain yield in tonnes per ha at 15% moisture averaged for UK 2001-2005 (Defra 2006). Trial grain yields are derived from fungicide treated plots as presented in the HGCA RL 2006/2007 (HGCA 2006) or from NIAB trials in 2003/2004 (Maize Growers Association, 2005).

*Alcohol production (l/ha) is estimated from the alcohol processing yield (in l/ t) multiplied by the grain yield (t/ha) corrected to 100% DM.

It must be recognised that production estimates will be inaccurate because they are products of data from different sources.

In order to indicate likely alcohol production by each of these species, Table 7 also shows their alcohol processing yields multiplied by grain yields from trials (HGCA RL trials) and by national average farm yields (Defra, 2006). This comparison shows wheat to have much the greatest potential alcohol production of all cereal species. However, there is a need to evaluate more thoroughly how maize, wheat, barley, oats, rye and triticale compare as potential feedstocks for alcohol production under UK conditions. Triticale also holds promise, not least due to its perceived low input requirements and lack of (reported) viscosity problems in modern varieties. Rye is not considered suitable for potable alcohol production in the UK due to its high levels of pentosans which causes problems with viscosity. Viscosity could be overcome in fuel alcohol production facilities using enzymes and is not seen as an unsurmountable problem in Germany where rye is used routinely. However, rye co-products are worthless.

4.1.2 Modern Wheat Varieties

Wheat varieties vary greatly in their suitability for specific end-uses. As well as breeding for grain yield, wheat has traditionally been bred for the milling and baking industry, where high protein contents and specific protein qualities are required. Although wheat breeders have aimed to maintain specific weight, which may benefit alcohol processing, there has been little emphasis thus far on breeding directly for alcohol yield.

Information on quality and agronomic characters of current UK wheat varieties are contained in the HGCA RL (for example, HGCA 2006b). Whilst quantitative data on distilling value are not currently given, a classification on whether a variety is suitable for distilling is provided, based on information funded by the potable alcohol industry through the Scotch Whisky Research Institute (see below). In addition, information on protein content, endosperm texture and specific weight are given and may provide some guide to value for alcohol production as shown in Table 8.

Table 7 -Variety information from the HGCA RL 2006-2007

Variety	Yield (% control, 10.0 t/ha)	NABIM group*	Endosperm texture	Suitability for distilling	Protein content	Specific weight
Robigus	104	3	Soft	Y	11.6	76.4
Deben	104	3	Soft	(Y)	11.2	76.0
Nijinsky	102	3	Soft	Y	11.8	74.8
Wizard	101	3	Soft	Y	11.8	76.5
Claire	100	3	Soft	Y	11.7	76.1
Consort	99	3	Soft	Y	11.7	76.9
Riband	98	3	Soft	Y	11.7	74.8
Ambrosia	106	4	Soft	-	11.6	77.0
Glasgow	107	4	Soft	Y	11.1	75.9
Alchemy	107	4	Soft	Y	11.6	77.3
Istabraq	106	4	Soft	Y	11.1	78.0
Brompton	106	4	Soft	-	11.6	73.6
Gladiator	105	4	Hard	-	11.8	77.1
Gatsby	104	4	Hard	-	11.8	76.8
Hyperion	104	4	Soft	-	12.1	77.9
Napier	104	4	Soft	-	11.5	75.4
Access	103	4	Hard	-	11.1	75.2
Richmond	103	4	Hard	-	11.9	78.3
Welford	103	4	Hard	-	11.6	74.4
Savannah	102	4	Hard	-	11.3	76.6

* NABIM (National Association of British and Irish Millers)

Experience in the potable alcohol industry has shown that wheat varieties differ both in alcohol processing yields, and in the ease with which they can be processed. Over the past 15 years the variety Riband has become very well-liked by distillers, and has continued to be grown in Scotland despite being out-yielded by newer varieties. The soft-milling candidate varieties from the 1st and 2nd year of National List trials (NL1 and NL2) and RL trials are tested for alcohol yield and distilling properties each year by SWRI, from about six (predominantly Scottish) sites. This information, together with experience from the industry, is used to state whether or not varieties are deemed suitable for distilling.

Up until the present time, the work on distilling value of new varieties has principally been funded by the potable alcohol industry. Whilst varieties that are good for traditional distilling are expected to be good for fuel alcohol production also, there may be a case for a more systematic analysis of the potential alcohol yield of varieties that would not be considered by the traditional distilling industry, for example, hard varieties and those containing the 1B/1R

translocation (see below). Additionally, if differences in the value of varieties for fuel alcohol production are different to that for the traditional distilling industry, there would be a strong case for testing the full range of varieties using fuel alcohol methodology.

Alcohol yield measurements by SWRI from HGCA NL and RL trials in the harvest years 2003-2005, together with protein content and residue viscosity in order to ascertain the varieties best suited to alcohol production. In each year at least six trial sites were assessed, at least four Scottish sites and two English sites. At each site, at least eleven soft varieties were tested. Additional varieties have been tested in 2005 as part of the GREEN grain project. Not all varieties have been tested at all sites or in all years, so Restricted Maximum Likelihood analysis was used to account for site and year effects and to give a fair basis for comparison of varieties. Two sites from Aberdeenshire in 2003 were excluded from the analysis due to high incidence of shrivelled grain in some of the varieties (this unfairly skewed the averages of certain varieties). Varieties were included where they were represented by more than four analyses from more than one site. The number of sites for each variety for each year is shown in Table 9 below. Associated grain yields (t/ha) were taken from *RLplus* for each individual trial and combined with alcohol processing yield (l per dry tonne) to give alcohol production (l per ha) for each of the varieties.

Table 8 - Number of sites tested for alcohol yield by SWRI in each year for each variety in the period 2002-2005. This data is made up of varieties in NL and RL trials as well as some data from the GREEN grain project.

	Glasgow	**Zebedee**	**Istabraq**	**Alchemy**	**Riband**	**Consort**	**Ambrosia**	**Atlanta**	**Robigus**
2002	1	*	*	*	*	1	*	*	*
2003	*	1	8	1	6	9	*	*	8
2004	6	1	6	1	4	7	6	6	6
2005	7	7	7	6	5	6	1	*	7
	Nijinsky	**Dickson**	**Piranha**	**Claire**	**Wizard**	**Deben**	**Kipling**	**Hyperion**	
2002	*	*	*	1	*	*	*	*	
2003	8	8	*	9	8	8	1	1	
2004	6	6	*	7	*	6	1	1	
2005	6	7	7	7	1	1	6	7	

It is likely that alcohol production will have been overestimated on a UK basis by this approach because Scotland is over-represented, and gave protein contents lower than would be expected in England, and the English trial sites were specifically chosen to give reasonably low protein contents. It is evident from Figure 8 that protein % for the varieties measured in these trials is often lower than the averages reported in the RL

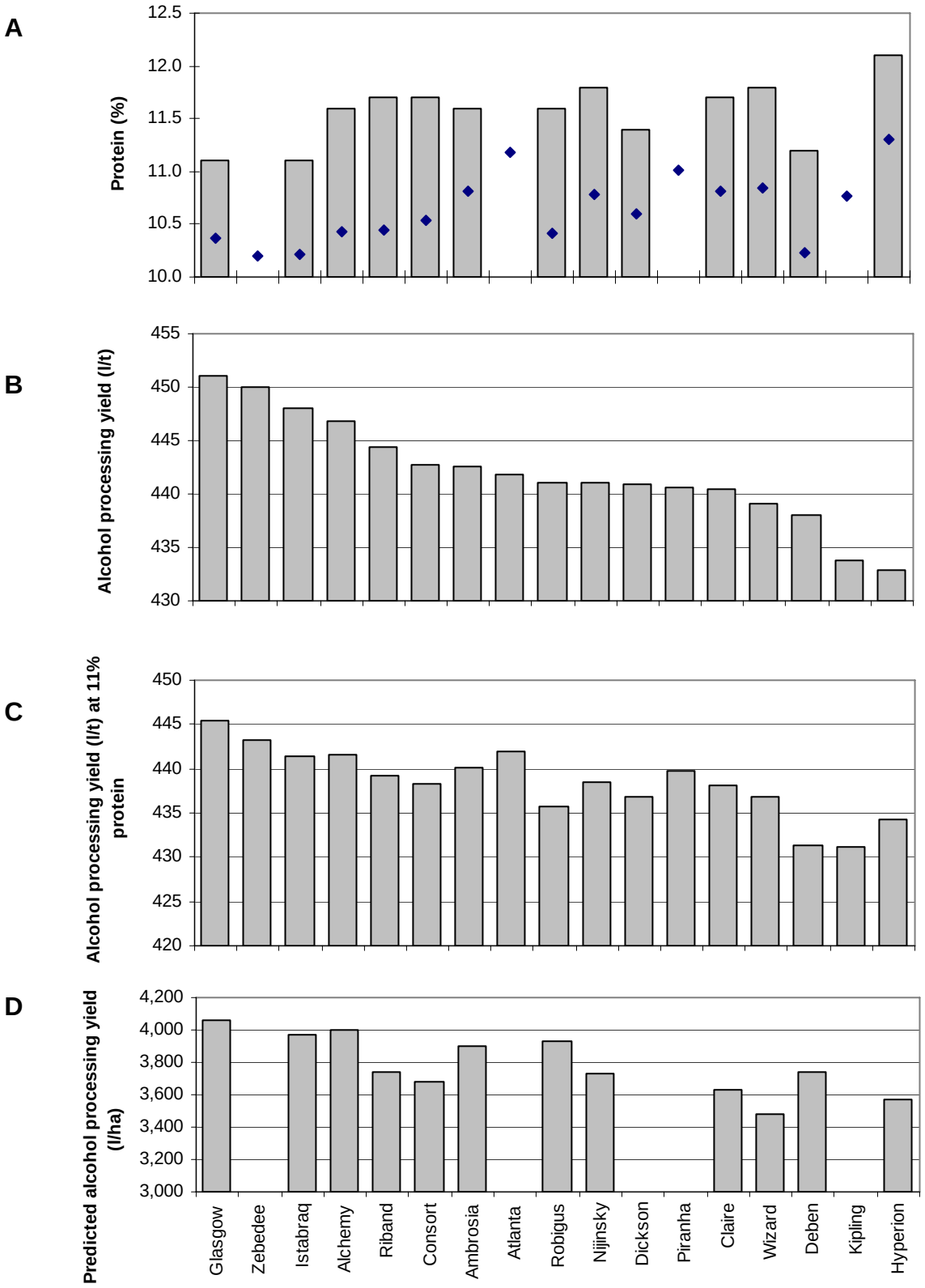

Figure 8 – Protein and alcohol yield in varieties tested in NL and RL trials in 2002-2005 and as part of the GREEN grain project in 2005. A) Protein content in the RL list (bars) against actual measured protein (dots) B) Alcohol yield in l/t C) Alcohol yield (l/t) normalised to 11% protein D) Predicted alcohol yield in l/ha based on alcohol yield in l/t multiplied by grain yield per ha for each variety

Figure 8B shows a wide range in alcohol processing yields of current and candidate wheat varieties, with Kipling and Hyperion averaging around 430 l / dry tonne and Glasgow and Zebedee producing around 450 l / dry tonne. To an extent this reflects the difference in protein contents of these varieties. It seems reasonable to take the view that protein differences in a variety trial commonly relate inversely to grain yield, whereas on-farm fertiliser use tends to be adjusted by yield level and so protein levels are more consistent. Thus, using the replacement slope of 7 litres of alcohol per tonne per 1% decrease in protein, we compare the expected alcohol yields of different varieties at a constant protein concentration (Figure 8C). Although this shows less variation between varieties, Glasgow and Zebedee still come out high, whilst Hyperion, Deben and Kipling come out poorly. This suggests that it is more than just differences in protein that distinguish these varieties.

In a perfect market (whether economic or environmental), the objective of the UK agriculture and alcohol industries should be to optimise production of alcohol per ha. Figure 8D shows potential alcohol yield per ha from the varieties analysed on the RL. Glasgow, Alchemy and Istabraq combine high grain yields and high alcohol processing yields to give an alcohol production of around 4,000 l alcohol per ha. Indeed, the well recognised inverse relationship between grain yield and grain protein in different varieties (Simmonds, 1995), together with the inverse relationship between alcohol yield and grain protein, suggests that high yielding varieties will tend to give high alcohol yields. The residue viscosities shown in Figure 9 give an indication of the likely processing problems that would be associated with these varieties. Very generally, varieties that give high alcohol yields tend to give low residue viscosities and varieties giving low alcohol yields tend to give higher residue viscosities. Kipling stands out as a variety that gives low alcohol yields and very high residue viscosities. This may be indicative of differences in NSPs, with varieties such as Glasgow having more starch and less NSPs at a given protein content.

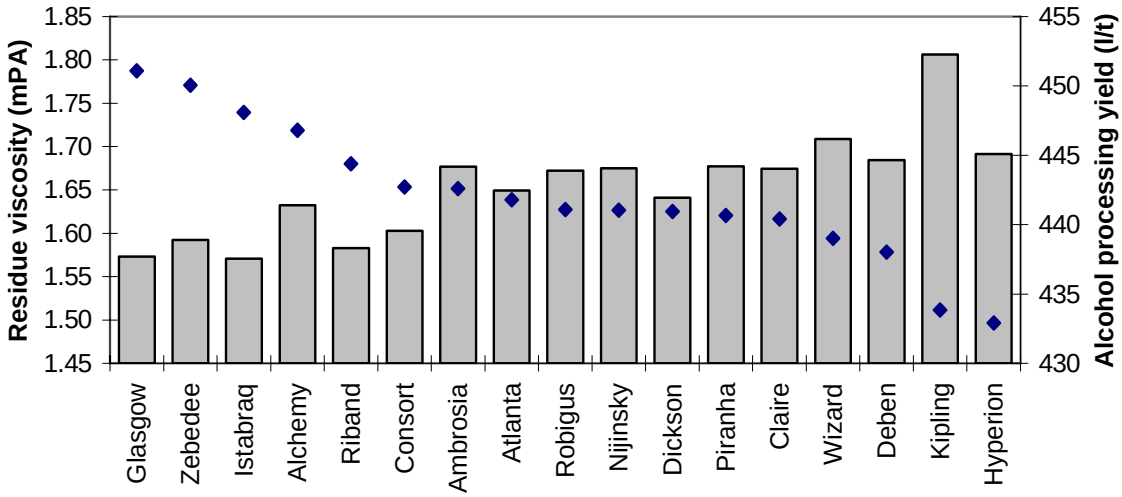

Figure 9 - Residue viscosity (mPa.s) (bars) and alcohol processing yield (l/t) (dots) measured by SWRI on varieties tested in NL and RL trials in 2003-2005 and as part of the GREEN grain project in 2005.

To test the importance of protein concentration in explaining differences between varieties (and sites and seasons) a regression analysis was carried out on a subset of varieties that were measured in all years and at all sites (the full set of data are shown in Figure 3). The general form of the relationship in Figure 3 clearly complies with the notion of replacement of starch by protein. There is some justification for considering the relationship to be curvilinear – the fall-off in alcohol processing yield increasing at high protein concentrations. However, the curvilinear effect was generally not significant here, the changes in alcohol processing yield and protein content that will now be discussed are treated as linear for individual varieties and seasons.

The regressions suggest that all varieties follow the same response to protein. Analysing the data on a year by year basis allows a wide range of varieties to be assessed (Figure 10). Despite the number of replicates (=sites) of each variety being limited, there is significant justification for fitting differing intercepts to the varieties in 2003 and 2005 (in 2004 the lack of significant differences maybe due to reduced replication – 6 sites rather than 8), but there is no justification for fitting different slopes. Slopes in 2003, 2004 and 2005 were –9.6, -6.0 and –7.3 l per tonne per % protein. The differences in intercept indicate that Deben (also Kipling and Hyperion) consistently gave less alcohol per tonne at a given protein concentration than Glasgow, Istabraq and Zebedee. A majority of varieties shared an 'average' intercept, including Claire, Consort, Robigus and Nijinsky. In 2005, Glasgow gave considerably higher alcohol yields than other varieties at a given protein concentration.

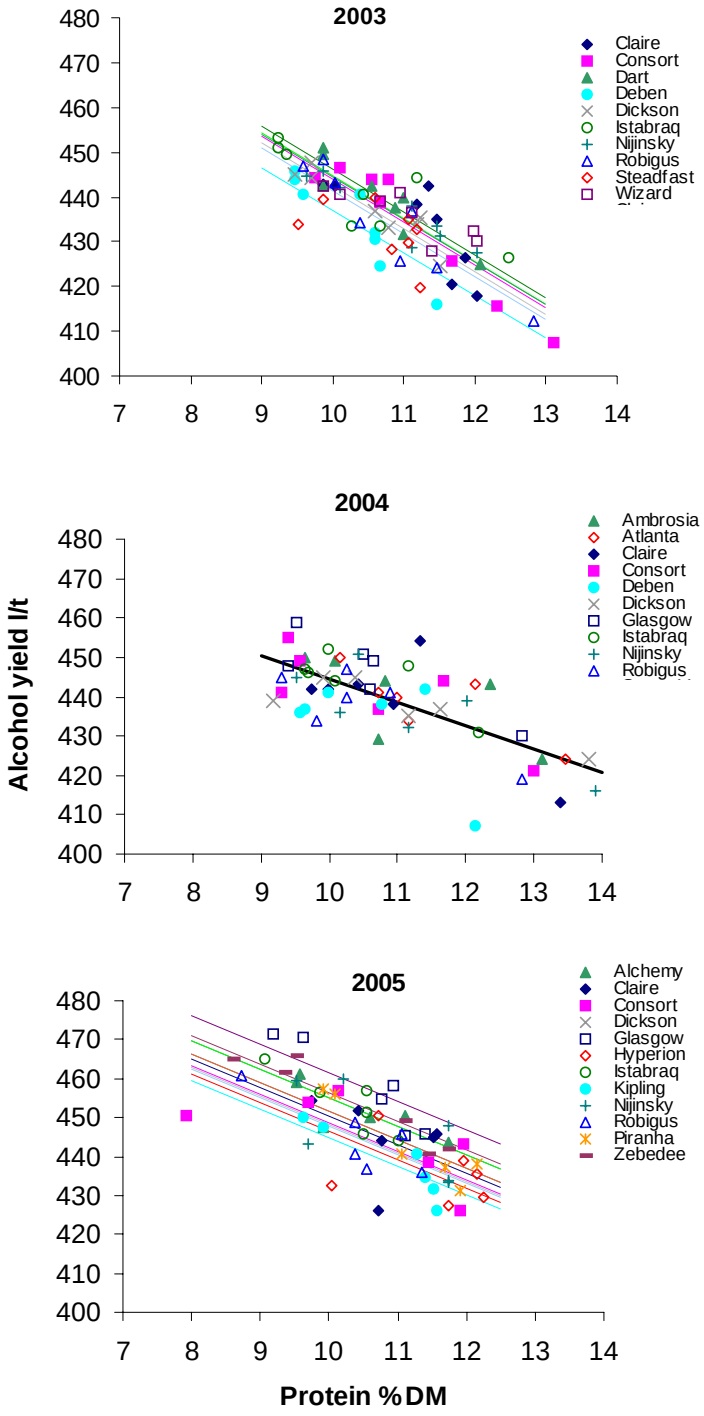

Figure 10 - Response of alcohol processing yield of different varieties to protein concentration across sites within a year. In each year all varieties are represented at all sites. Regression analysis shows justification for parallel lines in 2003 and 2005 and a common line in 2004.

4.1.3 Hard vs Soft Wheats

Wheat can be classified as either hard or soft. The primary importance of soft wheats is that they are easier to mill. Puroindolines are present in soft wheats where they interfere with the adhesion of the starch granule to the protein matrix. Absence of PIN isoforms (PIN-a and PIN-b), or mutations of PIN-b in the presence of PIN-a can lead to hardness. Traditionally, the alcohol industry has preferred soft wheats, although this does not preclude use of hard wheats for bioethanol production. Whilst hard wheats take marginally more energy to mill, effects on overall production costs are probably negligible. Hard wheats are generally associated with greater protein contents, and this may be the main reason for distillers' dislike of them. Hardness has also been associated with processing problems, the starch perhaps being less accessible and handling problems occurring (Taylor *et al.*, 1993). However, little work has been done to show whether hard wheats do actually have lower distilling value than soft wheats. Taylor *et al.* (1993) found no effect of grain hardness on alcohol processing yields.

4.1.4 The 1BL/1RS Translocation

The 1BL/1RS chromosome translocation from rye to wheat occurred with certain varieties (e.g. Slejpner, Hornet) in the late 1980s and provided new disease resistance traits. It was then found to give yield benefits, so the translocation has been retained in a subset of modern wheats, even though the disease benefits no longer apply. However, the 1BL/1RS translocation has been associated with negative qualities for animal feed and it is also associated with increased residue viscosity. Information about varieties containing the 1BL/1RS translocation is not readily available to growers and processors. However, as with hard wheats, distillers have identified varieties with 1BL/1RS that are undesirable. Experience in the feed industry suggests that not all 1BL/1RS varieties have a problem with viscosity, and this is likely to be borne out with alcohol yield. For example, Ambrosia has the 1BL/1RS translocation but has not shown very high residue viscosities whereas Kipling (also 1BL/1RS) consistently gives high viscosity and poor alcohol yields.

4.1.5 Future Breeding and Genetic Improvement

Wheat breeders are now breeding for alcohol as a specific market. Indeed, a Defra and Scottish Executive Environmental and Rural Affairs Department (SEERAD) sponsored LINK

project, in which HGCA is a partner, aims to facilitate the breeding of high energy varieties for feed and alcohol production (GREEN grain; HGCA Project 2979; www.greengrain.org). In addition to traits associated with grain processing yield and efficiency, breeding of varieties for alcohol production is likely to require somewhat different prioritisation of agronomic traits. For instance, lodging resistance may have increased importance to allow early nitrogen applications, and earliness may have increased importance to minimise grain-drying costs. Current analogous research in America by Monsanto and Pioneer is attempting to identify hybrids which maximise alcohol production in maize (Bryan, 2002). Swanston *et al.* 2005c (and others) have suggested that variety mixtures may be suitable for alcohol production, giving greater consistency of yield and potentially offering sustainability benefits. However, as with cropping for other markets, grain yield and quality from mixtures tend to under-perform the better variety in a mixture.

4.1.6 Conclusions on Varietal Effects

Current varieties (Groups 3 & 4 in the HGCA RL) show variation in grain yield from 99% (Riband) to 107% (Glasgow & Alchemy) of control and variation in alcohol processing yield from 432 (Hyperion) to 452 (Glasgow) litres per dry tonne (4.5% difference; Figure 10). Grain yield is therefore responsible for somewhat more of the variation in alcohol production (l/ha) than alcohol processing yield. However, this may change in future as breeders turn their attention to improvement of processing performance and as interactions with growing conditions (especially N status) are exploited. At present, there is a very useful positive association between both components of alcohol production, exemplified by Glasgow. However, it remains to be seen whether this will apply generally in future genetic improvements. In the case of Glasgow, suitability to alcohol production is not perfect since this variety has relatively poor resistance to lodging, which may preclude use of early N applications to maximise grain quality.

4.2 Environment and Management Effects

Grain yields from wheat in the UK are amongst the highest in the world (Sylvester-Bradley *et al.*, 2005). The combination of high yielding varieties giving high alcohol yields may well provide the UK industry with a competitive advantage over other countries. However, costs of production are also high in the UK and the prospect of a significant alcohol market only serves to emphasise the importance of maximising grain productivity (per unit of cost). The following section examines the potential to locate and manage wheat crops with high value for the biofuel market, whilst also maximising their productivity.

4.2.1 Year and Site Variation

Figure 11 represents the data in Figure 10, but showing the different sites. Alcohol processing yields were generally higher in 2005 than in 2003 or 2004, and protein contents generally less. The range in alcohol yields across sites and varieties is sizeable at 40-50 litres per dry tonne and fairly consistent across years.

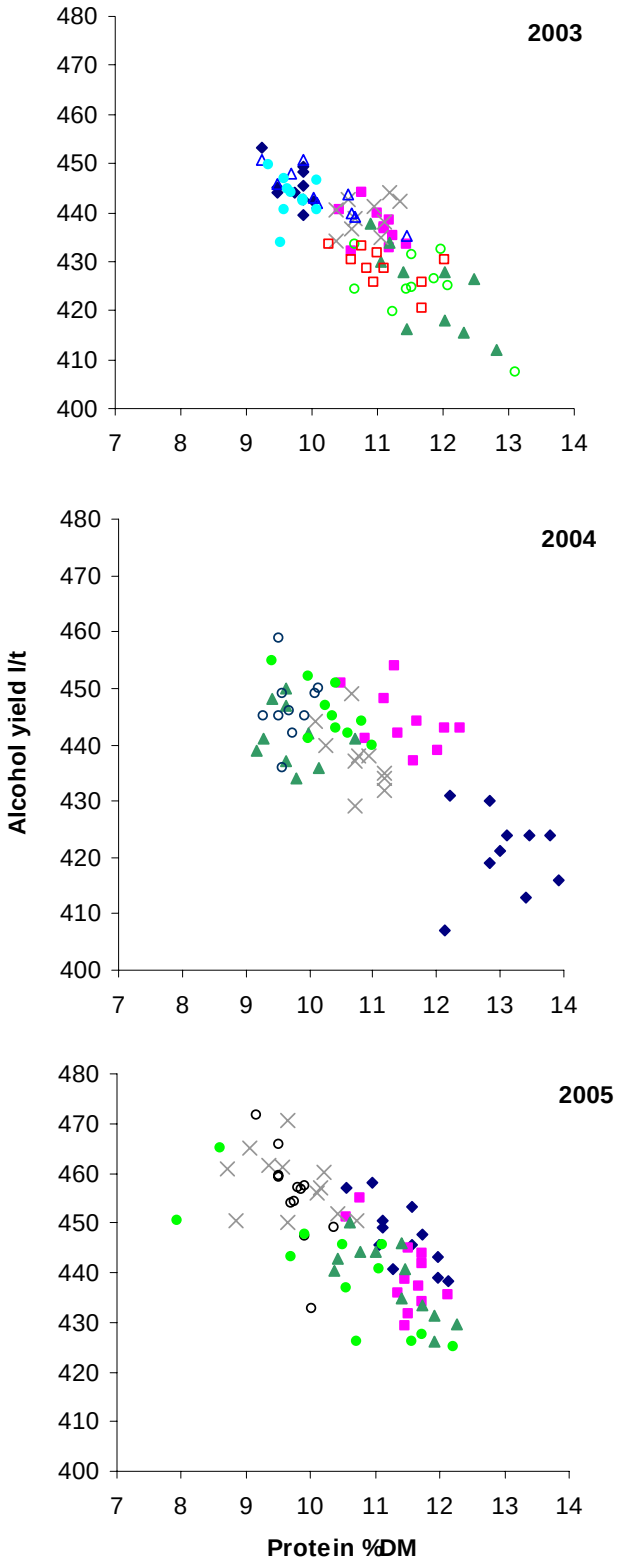

Figure 11 - Response of alcohol yield to protein content across varieties at different sites within a year. In each year all varieties are represented at all sites. Regression analysis justifies parallel lines in each of the years, but with no difference in slope between sites and is not shown for clarity.

Variation between sites is generally greater than variation between varieties – the sites tend to cluster markedly, often with a fairly limited range in alcohol yields and protein contents within each site. Across varieties within a site the protein-alcohol yield relationship does not generally hold up. Causes of inter-site variation may be numerous, including location, soil types. management, fertility, yield potential and weather. These will now be discussed.

4.2.2 Location (North vs South)

Comparing alcohol yields from Scotland to those from England (Figure 12) indicates that trials in Scotland generally give lower grain proteins and higher alcohol yields. This probably results from a combination of climate and soil characteristics that has been exploited traditionally by the Scottish distilling industry. Scottish soils generally have greater organic matter contents, but the organic matter has a high C:N ratio hence releases less N through mineralisation. The cooler climate also provides less evapo-transpiration and more leaching of nitrogen, and it gives a longer grain-filling period which increases starch deposition relative to the amount of nitrogen taken up.

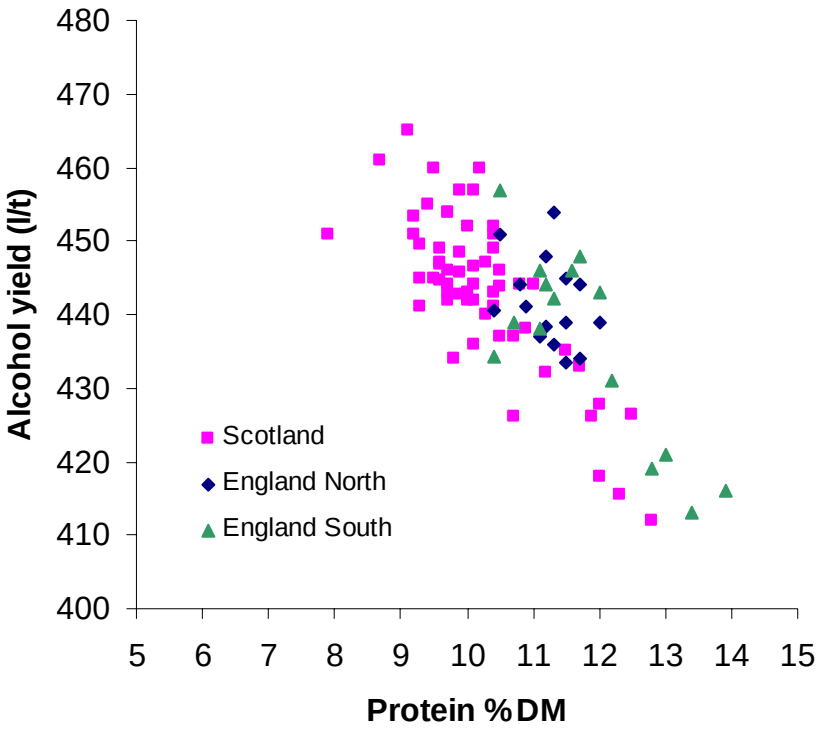

Figure 12 - Response of alcohol processing yield to protein content, grouped by growing region.

4.2.3 Soil Type

Other than the comparison between Scotland and England above, there is little empirical evidence on which to base deductions about soil type. Suffice it to say that soils capable of high yields and least at risk of drought are likely to give lowest protein contents and therefore highest alcohol yields. Organic or peaty soils will tend to give high protein levels and low alcohol yields.

4.2.4 Management

The management of crops for biofuels ideally needs to meet a range of objectives; (A) maximise financial profitability, (B) optimise grain quality for alcohol production (C) minimise GHG emissions per unit of bioethanol produced and (D) maximise energy balance of the system and minimise other adverse environmental effects of growing the crop (Loyce *et al.*, 2002). Generally, these objectives will be achieved by optimising grain yields with judicious use of mechanical power and agricultural inputs, especially N fertilisers (Rosenberger *et al.,* 2001, Rosenberger, 2001).

Costs of growing wheat are shown in Figure 13 in both economic and energy terms. It is clear that if cost considerations change from a solely economic basis to include some measure of GHG emissions there must be a shift in optimum production practices, particularly with respect to use of nitrogen fertilisers, but also to minimise drying costs, perhaps by adoption of earlier-maturing varieties. Such changes are likely to bring other environmental benefits.

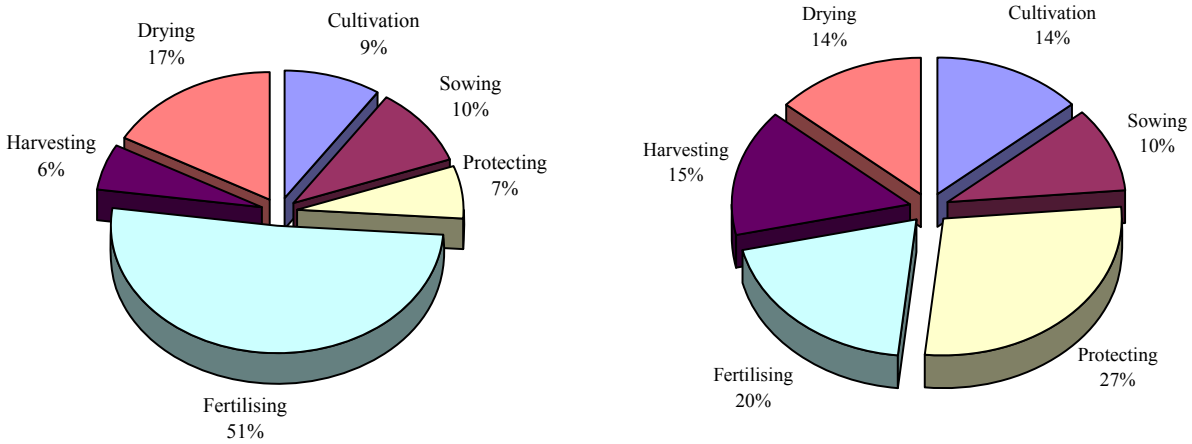

Figure 13 - Relative costs of growing a crop of wheat yielding 8 t/ha grain in terms of energy (left) **and money** (right). Costs of each operation include materials, labour and machinery (Source: ADAS)

If the farming system includes a sequence of cereal crops, it is likely that those grown for alcohol should be grown first, to capitalise on the high yields and lower grain protein of the 'first wheat' niche (Vaidyanathan, 1987). (Conversely, wheat crops grown for breadmaking will tend to benefit in terms of grain protein content from being grown following another cereal.) Given the expected expansion of the oilseed rape area for biodiesel it seems likely that suitable opportunities for biofuel wheats will increase within UK arable rotations. However, there are implications for crop nutrition and protection of these expected changes, and further research will be needed to verify the best ways of maximising profit when both biodiesel and bioethanol markets are operational.

To maximise GHG benefits through in-season crop management, in broad terms, growers should seek to minimise the number of passes and minimise agricultural inputs. It is likely that this approach will only show real benefits when a carbon accreditation scheme emerges. For example, in circumstances where penalties on weed control are minor, the use of minimum tillage may reduce the farm diesel input per ha. On the other hand, reducing inputs such as fertilisers and pesticides to below the economic optimum for yield will result in significant yield losses and reduced profitability.

Optimum input levels for maximising profitability of inputs such as fertilisers will change if sufficient economic incentives are introduced. In the case of producing grain for alcohol, processors may pay premiums for grain of suitable quality, as is done in the milling industry. Grain that gives 480 l/alcohol per dry tonne is obviously worth more to processors than grain that only yields 410 l/alcohol per dry tonne, and it makes sense for the processor to pass some of this added value back to the grower to incentivise the market.

In the case of GHG emissions, it is likely that there will be some form of 'carbon assurance' for biofuels that are used to meet the RTFO requirement of 5% by 2010. Whilst in the first instance this is likely to be a relatively straightforward reporting procedure, it is conceivable that in future growers could be rewarded financially for producing grain for alcohol with reduced associated GHG emissions. Work surrounding this issue is currently being undertaken through the HGCA Research Project "Facilitating Carbon Accreditation Schemes for Biofuels: Feedstock Production".

Management strategies for wheat for alcohol will be explored in the following sections. Recognising that the market is in its early development, we shall assume no financial or regulatory incentives for quality or GHG reductions, but will show how growers should be seeking to optimise alcohol yields per ha.

4.2.5 Crop Establishment

In terms of profitability and quality for alcohol production the most important decision at drilling is variety choice (see Section 4.1). This must represent a compromise between grain yield, grain quality and agronomic characteristics.

Establishment can be very important for yield (Blake *et al.,* 2003). In general, cultivations, seed rates and sowing dates for biofuel wheat should be no different than for conventional markets. However, grain quality considerations may dictate choice of a variety with high lodging risk (e.g. Glasgow). Also the rotation and fertiliser strategies to enhance alcohol yield may increase lodging risk. Thus later sowings and/or lower seed rates than normal may be preferable.

4.2.6 Crop Nutrition

Requirements for potassium, phosphate and minor nutrients will be the same with wheat for alcohol as for conventional markets, and nutrients should be applied as appropriate for the rotation (see RB209, (MAFF, 2000)). N fertiliser is the single most important management factor to be considered when growing wheat for bioethanol, due to its large effects on grain yield, alcohol yield as well as GHG emissions and energy balance.

Figure 14 shows a response curve to N fertiliser developed over many sites and seasons from experiments on feed wheats on clay soils. Initial increases in N fertiliser give substantial increases in yield, but as the quantity of N applied increases the increments in yield reduce, until a plateau is reached. Larger N applications may result in reduced yields. Individual N response curves vary due to available soil N, soil type and achievable yield, but the shape of the response is generally consistent and can definitely be used to explore effects of N fertiliser on alcohol production.

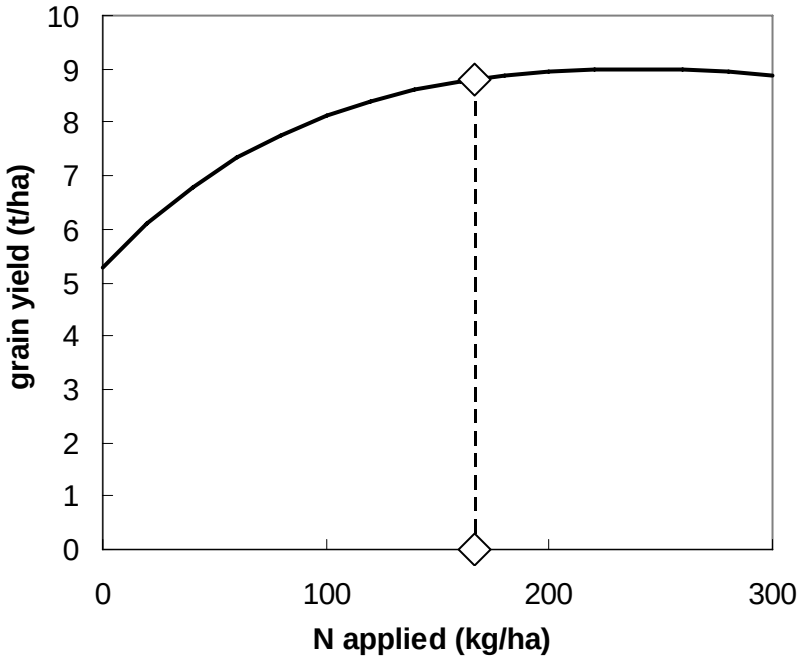

Figure 14 - Standard yield response of wheat grain to N fertiliser. The vertical line shows the economic optimum assuming a grain price of £75/t and a fertiliser price of £150/t ammonium nitrate (34.5% N).

When growing feed wheat, growers should apply the quantity of nitrogen fertiliser that provides the largest financial return per ha, i.e. the economic optimum. This optimum is dependent on the price of wheat relative to nitrogen (the break-even ratio). Using the response curve in Figure 14 and current prices of £75 per t for grain and £150 per t ammonium nitrate (breakeven ratio of about 6:1) the optimum N rate is about 165 kg/ha. Historically, higher grain prices and lower fertiliser prices have given a break-even ratio of about 3:1, and optimum N rates of about 190 kg/ha. Modern volatile markets mean that N rate decisions should be linked to pricing strategies on the farm.

When growing for a milling market, growers need to meet minimum specifications for protein content (usually 13%) and therefore generally apply more N than the optimum for yield. In the case of alcohol, the value of the grain to the processor, in terms of alcohol yield, decreases as protein concentration increases. Figure 15 shows the typical response of grain protein to N fertiliser, taken from the same experiments as in Figure 14, and the effect of N on alcohol processing yield can be inferred using the replacement relationship of 7.2 l alcohol per tonne per % decrease in protein (see Figure 3). Increasing fertiliser N from zero to 300 kg/ha increases protein content from 8.5% to 12.5% and is expected to reduce alcohol processing yield from 455 l/t to less than 430 l/t. At the optimum N application for grain yield (165 kg/ha) the expected alcohol processing yield is 439 l/t, considerably below its maximum of 457 l/t.

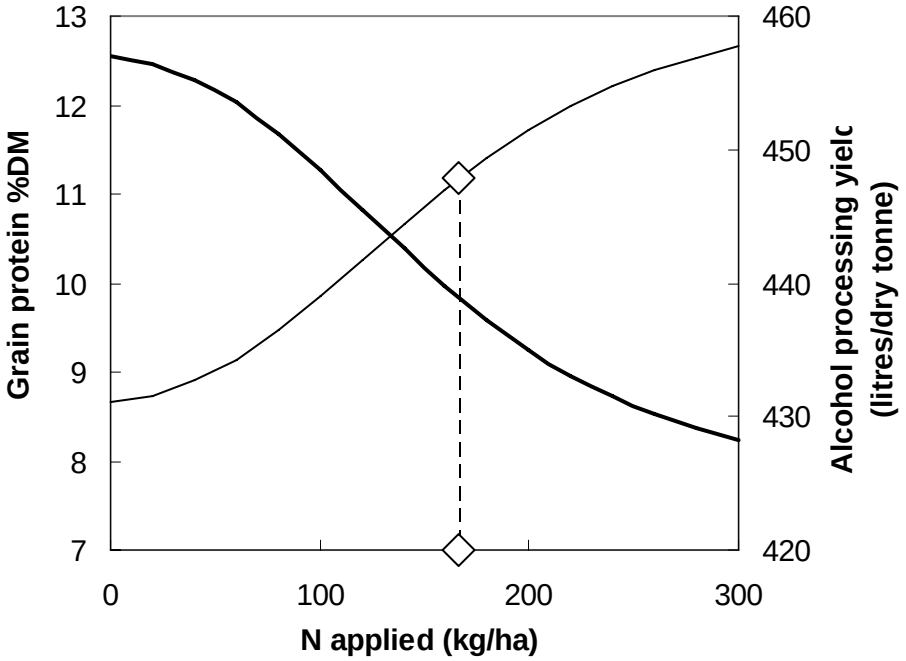

Figure 15 - Effect of N fertiliser on grain protein and alcohol processing yield.

Whilst the grower will be interested in optimising grain yields, and processors in optimising processing yields, the ultimate objective of the industry as a whole should be to optimise alcohol yields per ha. The effect of N fertiliser on alcohol production per ha can be seen by multiplying grain yield per ha by alcohol yield per tonne (Figure 16). This suggests that *maximum* alcohol yields per ha are achieved at 200 kg/ha N, a greater N rate than the current optimum for grain yield. Thus the effect of N on grain yield is more important in determining alcohol production per ha than the effect on alcohol processing yield.

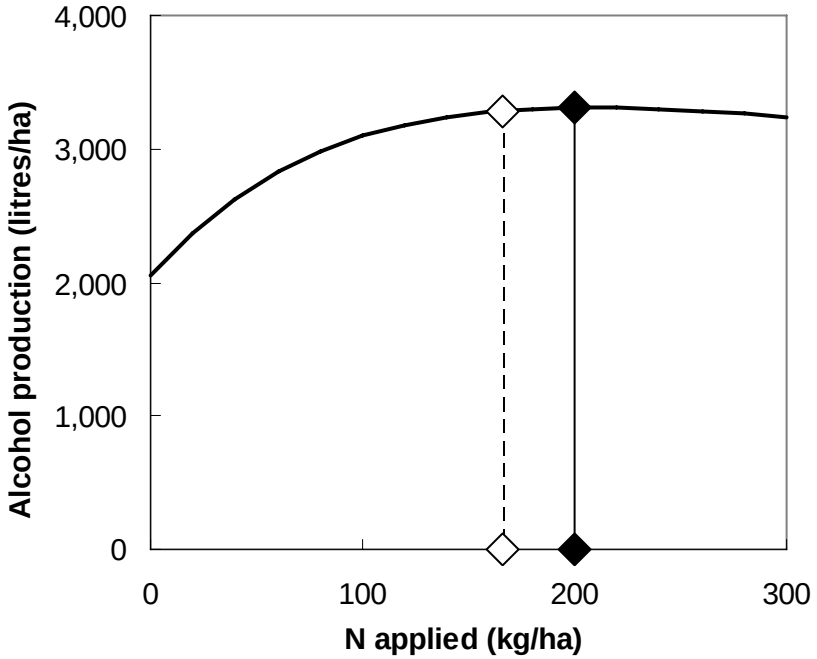

Figure 16 The effect of N fertiliser on alcohol production per ha. The economic *optimum* for grain yield (open diamonds) is 35kg/ha less than the N rate giving *maximum* alcohol production (closed diamonds).

Given the effect of N application on alcohol processing yield, and the potential value of increased alcohol processing yield to the processor, it may prove worthwhile for processors to offer premiums for high alcohol yields (as discussed in Section 3.5). This would have the effect of reducing optimum N rates, which would have additional benefits of reducing GHG emissions. If premiums are not offered for alcohol yield, then the economic optimum N rate for a farmer growing wheat for bioethanol will be exactly the same as for growing feed wheat; the N rate which gives the optimal *grain* yield/ha. If, for arguments sake, the bioethanol producer was growing the wheat crop, the economic optimum N rate would be that which gives the optimal *alcohol* yield/ha; this would depend on the relationships between grain yield, protein content, alcohol processing yield and N fertiliser previously described, and the price of alcohol relative to nitrogen fertiliser. Further analysis is required, exploring various economic and accreditation scenarios, so as to maximise the economic benefits to all in the supply chain, and hence optimise nitrogen nutrition for bioethanol production.

The responses of alcohol production to N fertiliser (in Figures 15 and 16) have yet to be validated in full, but they are supported by alcohol yield measurements on samples from one site-

season within project "Genetic Reduction of Energy Use and Emissions of Nitrogen through Cereal Production" (GREEN grain) (HGCA Project 2979) where grain from N response experiments using the varieties Riband and Option had been tested with N rates from zero to 240 kg/ha N (Verhoeven *et al.*, *in preparation*).

In the context of alcohol production, there may be further considerations affecting processing efficiency; N application has been shown to decrease the auto-amylolytic rate significantly (Aufhammer *et al.* 1996), and this is consistent with effects found on amylase activity or Hagberg falling number in the context of breadmaking (MacDonald and Vaidyanathan 1987; Kindred *et al.*, 2005). This effect is best explained through the differences in grain ripeness at harvest caused by N. It is unlikely to be sufficient to influence N applications, but it may indicate potential to manipulate harvesting strategies to optimise alcohol processing.

4.2.7 N Timing

Timing of N application is known to affect grain protein content of wheat (Dampney, 1987). The effect of applications of nitrogen during stem extension and grain filling on the quality of wheat grain used for breadmaking (Bhandari *et al.,* 2006) and of barley (Lord and Vaughan, 1987). Taylor and Roscrow (1990) have shown that N timings can also affect alcohol yields. Whilst late applications of N improve grain protein for milling markets (Dampney *et al.*, 2006), applying N fertiliser early is likely to result in lower grain proteins, hence benefiting alcohol processing yield. However, there may be subsidiary effects of earlier N application on early growth, tillering and lodging risk which are detrimental, particularly for relatively fast developing, lodging prone varieties such as Glasgow. There is the possibility also that earlier N applications are less efficiently taken up by the crop and may result in greater N losses due to leaching or denitrification to nitrous oxide. Recently funded work is looking at the effects of N timing on potential alcohol yield through field experiments (Extension to HGCA Project 3084; Optimising fertiliser nitrogen levels for modern cereal crops).

4.2.8 Crop Protection

Maximising grain yield and grain filling by ensuring the crop is weed, disease, pest and lodging free is likely to increase grain yields as well as starch contents and alcohol processing yields. In addition to choosing resistant varieties, fungicides can be especially important in protecting grain filling; samples from HGCA RL untreated plots analysed as part of the GREEN grain project had shrivelled grain with high protein contents and very low alcohol yields.

Fusarium ear blight may result in high levels of *Fusarium* mycotoxins, which should be avoided because these can enter the food chain through the DDGS. However, given normal *Fusarium* mycotoxin levels in the UK it is unlikely that wheat crops for biofuel would warrant a specific fungicide ear spray. Latest advice on this is given in Topic Sheet 91 "Managing the *Fusarium* mycotoxin risk in wheat".

4.2.9 Harvesting and Drying

Whilst growers should seek to harvest grain in a timely fashion to minimise drying costs and risks of sprouting in the ear, perhaps by preferring early-maturing varieties, harvesting early to avoid low Hagberg Falling Numbers will be less critical than for milling wheats. Indeed, low HFN may even be beneficial if endogenous enzymes are relied upon to breakdown starch (see Section 3.2.2.3), and delays in harvest have been shown to increase endogenous starch degrading activity in some varieties (Aufhammer, 1996). However, advanced sprouting will be associated with losses of starch and sugars by respiration and germination, and this is likely to reduce alcohol processing yields if the process advances too far.

Whilst it is important that grain is adequately dry to avoid fungal and mould contamination, and to avoid storage problems, it is possible that grain with moisture contents greater than 15% may be accepted by fuel alcohol producers at lower penalties than in other markets. As moisture contents increase the proportion of the traded grain which is starch will obviously decrease, so some discount for high moisture grain is inevitable. Very high moisture grain can cause problems with processing, and is unsuitable for long term storage, but, depending on the set-up of the plant, moisture contents of up to, say, 16-17% are unlikely to cause the processor significant extra costs provided intake is immediate.

Drying wet grain presents a substantial economic cost to growers, and can also make up a very large proportion of the total energy balance and GHG emissions from bioethanol production (Table 4). From these perspectives it may prove advantageous to avoid drying grain for bioethanol as far as is possible. Because of the storage problems associated with high moisture content, the scope for accepting high moisture content grain would be most around harvest, though it should be possible to store grain of 16% moisture for periods of up to a month (see HGCA Safe Storage Time Calculator; www.hgca.com).

4.2.10 Conclusions on Effects of Environment and Management

At this stage, grain yield will remain the dominant driver of crop management for alcohol production. Only small changes to management for the feed market are envisaged. The most important consideration at present is variety choice. However, with the introduction of premiums for grain quality and of accreditation schemes for GHG emissions changes to crop manangement may be larger. Given the large contribution that N fertiliser can make to the GHG balance, and its importance in determining grain yield and alcohol yields, it is envisaged that significant adjustments to N management may be required. Eventually, carbon accreditation may also encourage changes in cultivations, harvesting, drying and storage strategies to reduce GHG emissions on-farm. The major implications of all agronomic practices on GHG emissions are being investigated more thoroughly in HGCA project MD-0607-0003 (Facilitating carbon accreditation schemes for biofuels - feedstock production).

Much of the variation in alcohol processing yield is associated with variation in grain protein content. There is little evidence that the response of alcohol yield to protein is much affected by agronomic factors. Thus, of all management factors, fertiliser N has the greatest effect on alcohol yields. However, alcohol yield *at a given protein concentration* can also be much affected by year, site, management and variety, with somewhat greater variation between sites and years than between varieties. It will therefore be important to explore effects on components of the grain other than protein and starch; principally non-starch polysaccharides.

Current knowledge suggests that the key points to consider when growing wheat for biofuel or alcohol production may be summarised as:

1. Grow a soft-milling, high yielding variety (see HGCA RL)
2. Select a high yielding situation
3. Avoid over application of fertiliser N
4. Avoid late application of fertiliser N
5. Manage grain production and drying to avoid mycotoxin development.

5.0 Final Perspective

It seems inappropriate at this early stage in development of the UK bioethanol industry to draw final conclusions. Rather, we offer here our perspective on the important considerations, going forward.

The principal stakeholders in alcohol production from wheat are the growers and processors, who seek financial returns, and the consumers who seek GHG savings. We believe that full stakeholder satisfaction is feasible, but that it will arise most quickly through some concerted industry integration.

The processors primarily look to their feedstock for a good alcohol yield. Good feedstocks give increased saleable product, and also reduced energy costs in heating and drying residual materials. The primary driver for increasing alcohol yields is the amount of starch. While saccharification and fermentation efficiencies are undoubtedly of concern, they are less important than starch content *per se*. It is evident that starch content is increased with reduced protein. Whilst the potential for reducing protein contents genetically, hence increasing alcohol yields, is being investigated by the GREEN grain project (HGCA 2979), there is also a need to investigate how alcohol yields can be increased through crop management, particularly N fertiliser strategies.

Differences in protein content do not fully explain differences in starch content or alcohol yield. Aside from protein and starch the only other significant constituent of the grain is non-starch polysaccharide (NSP). It seems highly probable that much of the variation in alcohol yield not accounted for by protein content must be due to differences in NSPs. We deduce from comparisons of varieties reported here that genetic variation in NSPs can be large and, since ash and oil contents are small, this variation must cause differences in starch at a given protein content, as well as influencing residue viscosity. There is therefore a major need to address NSPs, when researching how grain constituents affect alcohol yield. Given that 80% of NSPs are found in the bran, another promising approach should be to consider grain components histologically (i.e. germ, bran and endosperm), to explain effects of grain size and shape. HGCA, with SWRI, are funding a studentship at Heriot-Watt University on this. An inverse relationship between starch and NSP may explain why viscosity is generally inversely related to alcohol yield. Certainly, the variation in NSPs amongst current varieties, and variation in NSPs caused by environment and management, are worthy of new research.

In terms of measuring feedstock quality at intake, the ideal test would be a rapid prediction of potential alcohol yield from an individual grain sample. This may indeed be possible if work by FOSS UK Ltd., SWRI and others (in the GREEN grain project) fulfils its promise of an accurate and reliable NIR calibration for alcohol yield. NIR has the significant advantage that it is familiar to the grain and alcohol industries. However, direct prediction of alcohol processing yield may still be some years from commercialisation. In its absence, assessment of other grain constituents may be the best alternative. Since starch is so difficult to measure, protein provides the best alternative. (NSPs and viscosity cannot currently be measured on a sufficiently rapid or cost effective basis to be used in a grain intake test.) Protein has a number of advantages as an intake test; it is linked to alcohol yield by a well recognised relationship, its measurement is understood and accepted by the grain trade, it is reasonably well defined for different varieties, it is directly linked to N fertiliser strategies, and it is understood by growers as being so.

To help account for scatter in the protein-alcohol relationship, it may be best to include variety differences in alcohol yield in prediction approaches. Within a variety, the protein: alcohol yield relationship seems to be reasonably consistent across sites and N nutrition levels, though this is less true across varieties. It is possible that these reasonably consistent variety effects may be due to differences in NSP contents. We suggest that the response of alcohol yield to protein of -7.2 l/t per % protein could be used with specific base values for different varieties, to give a workable method of predicting alcohol yield. The analyses of distilling value of certain RL varieties by SWRI allow this to easily be achieved for varieties of interest to the potable distilling industry (i.e. by extrapolation from Figure 8C). However, because fuel alcohol producers are likely to use a wider range of varieties than have previously been considered by the distilling industry, there is a need to test the distilling value of all varieties on the HGCA Recommended List. The deviation in alcohol yield from the –7.2 l/t per % protein response could be quantified for all varieties likely to be used for fuel or potable alcohol production, allowing processors to predict the likely alcohol yield of the feedstock at grain intake with reasonable accuracy.

In practice, growers are unlikely to grow and produce grain of perfect distilling value without an economic incentive. Premiums based on both protein and variety would help to incentivise the market, maximise financial returns to the processor and the grower and provide appropriate price signals to breeders and others in the agricultural supply industry. Because of the strong associations between grain protein and alcohol yield, and because of the large energy and

GHG costs associated with N fertiliser use, the issues surrounding N fertilisers are potentially of great importance to the bioethanol industry. Economic instruments to reward reductions in GHG emissions could have large impacts on how N fertilisers are used. Without a market mechanism to reward lower proteins it is unlikely that the market will work as effectively as it could: in striving for high grain yields growers may use unsuitable varieties and over-generous N rates that compromise alcohol processing yields and processing efficiency, giving reduced economic returns and increased energy costs to the processor. In particular, GHG emissions could be higher than necessary. Further work is required to explore the size of premiums and potential market mechanisms required to optimise the supply chain for each of its objectives: maximising financial return to growers and processors and minimising GHG emissions.

Other parameters used in standard feed contracts (e.g. specific weight, moisture content, freedom from ergot, mycotoxins, heavy metals etc.) are probably suitable for alcohol distilling. Whilst it may be possible for bioethanol producers to accept lower quality grain than traditional markets this would have to be at a price discount. If DDGS are to be sold for animal feed then grain 'health' is of importance, but if DDGS are used for non-food uses then contracts may be less restrictive.

In conclusion, the generally high yields and starch contents of UK wheats mean that UK growers are well placed to produce suitable feedstocks for bioalcohol. If the progress with varietal improvement and crop management for bread-making markets over the past thirty years is anything to go by, and adequate investment is made, then it should be possible to maximise alcohol production, maximise financial returns to growers and processors and minimise GHG emissions.

Future Research Needs

The most urgent areas for future research are as follows:

- Optimising crop management, especially with respect to rotations and N fertilisers
- Wider testing of varietal effects on alcohol yield
- Development of a rapid and accurate test for assessing alcohol yield in the laboratory, using fuel alcohol methodology
- Investigation into new and novel uses of co-products from alcohol production

- Investigation into novel processes which can minimise energy usage and maximise rate and efficiency of processing in fuel alcohol processing.
- Investigation into the fermentable constituents of the wheat grain and their interactions with non-fermentable constituents.
- Economic consideration of how the supply chain could best be optimised to deliver maximal alcohol production, maximal financial returns for growers and processors, and maximal GHG emission savings.

6.0 Glossary

Biodiesel - A renewable fuel produced from oil rich crops such as rape or waste fats such as tallow and used cooking oil. Biodiesel can be added to conventional diesel in a 5% biodiesel, 95% diesel blend without major engine modifications and can be used in some engines as straight biodiesel.

Bioethanol - An alcohol produced from starch rich crops such as wheat and maize and sugar rich crops such as sugar beet and sugar cane which can be used in industrial, fuel and potable industries. Fermentation of the sugars produces ethanol that is distilled to 95% volume for the potable alcohol industry and dehydrated to over 95% volume for the fuel industry.

Biofuel - A fuel made from recently living biomass (compare with fossil fuel). Biofuels are renewable as they are derived from recently living plant material. Biodiesel and Bioethanol are examples of biofuels.

Carbon Dioxide (CO_2) - An atmospheric gas that is produced by combustion of carbon rich materials and through respiration and used in photosynthesis by plants to form sugars, storage compounds such as starch and biomass. Carbon dioxide is a yeast fermentation product and can be captured as a co-product of the fermentation process and sold for carbonation of drinks or as a gas for industry.

Cold Cooking - Milled grain is either enzymatically saccharified without a cooking step or cooked at a low temperature (80°C) prior to enzymatic saccharification.

Distillation - Process where different components of a liquid are separated on the basis of their boiling point by heat.

Dried Distillers Grains with Solubles (DDGS) - A co-product from the production of bioethanol from starch rich crops that is used as a protein and fibre rich food for livestock markets.

Dry Grind - Process in which the whole grain is milled and processed for bioalcohol production.

Fermentation - The production of ethanol from sugars by yeast species in anaerobic conditions with the production of CO_2.

Fossil Fuels - Fuels formed from the remains of ancient animals and plants. Rich in carbon and have taken millions of years to form under extreme pressure and heat. Coal, gas, peat and oil are fossil fuels, petroleum and diesel are derived from oil. Fossil fuels are non-renewable and are limited in supply, once they are used up they are gone forever.

Flexible Fuel Vehicle - A vehicle which can run on any blend of ethanol and petroleum up to 85% ethanol 15% petrol. Sensors in the engine automatically detect the ratio of ethanol to petrol and adjust the fuel injection to suit the fuel.

Greenhouse Gas - A gas such as carbon dioxide, carbon monoxide, water and methane which absorbs some of the longer wavelength infrared radiation (heat) that the Earth radiates back and therefore traps heat in the atmosphere.

HCGA Recommended List (RL) - Booklet containing information on the suitability of UK cereals and oilseed varieties which for a variety of end uses, farming systems and location.

Lignocellulosics - Materials containing a high proportion of cellulose, hemicellulose and lignin cell wall components. The composition each component varies significantly depending upon the feedstock; and determines its processing efficiency. Typical feedstocks are wood materials, straw, stover and paper.

Malliard Reaction - Where sugars and amino acids form a complex under heat treatment caused by the reactive carbonyl group of the sugar interacting with the nucleophilic amino group of the amino acid. Can also be known as a "browning reaction"

Malt Whisky - Whisky produced entirely from barley malt as the cereal source.

Mycotoxin - A class of toxins such as aflatoxin, aflatoxins, ochratoxin A, fumonisins, zearalenone, trichothecenes and deoxynivalenol (DON) which are toxic to man and animals and produced by moulds such as *Fusarium* species in the field or *Penicillum* species in storage.

Near Infrared Spectroscopy (NIR) - A technique for rapidly assessing the amount of a specific component of a biological material. Near infrared light is passed through a sample. Illumination results in changes in the sample so that the amount of light absorbed is proportional to the amount

of a particular component in the sample. Illumination at different wavelengths allows different components to be quantified.

Neutral Alcohol - Ethanol containing 95.5% alcohol and 4.5% water which has no colour, taste or aroma. Neutral alcohol is used as a basis for the production of vodka, gin and liqueurs.

Potable Alcohol - An alcohol which is fit for human consumption (includes neutral alcohol and Scotch whisky). Also called 'beverage alcohol'.

HCGA Recommended List (RL) - Booklet published by the HGCA containing information on the suitability of UK cereals and oilseed varieties for a variety of end uses, farming systems and location.

Retrogradation ('set back') - The change from a dispersed amorphous state to an insoluble crystalline state that occurs when gelatinised starch begins to re-associate upon cooling. Starch in this state is resistant to degradation and causes processing problems due to high viscosity and results in low alcohol yield.

Renewable Transport Fuels Obligation (RTFO) - UK government target for the amount of biofuel to be included in conventional fuel supplies from 2008. A way to ensure long term confidence in the biofuels market in the UK and reduce emissions of greenhouse gases. Targets set is a 2.5% biofuel component by 2008, 3.75% biofuel component by 2009 and 5% biofuel component by 2010/11.

Saccharification - The conversion of starch and dextrins to fermentable sugars such as glucose and maltose by enzymatic hydrolysis.

Scotch Grain Whisky - Whisky made from approximately 90% unmalted cereals (typically wheat) and 10% high enzyme malted barley for saccharification, whose products conforms to the Scotch Whisky Order (1990). Grain whisky is mixed with malt whisky to produce blended whisky which accounts for 90% of Scotch whisky sales.

Stillage - Whole stillage is made up of dissolved solids, dead yeast, proteins and water after the fermentation step. This is separated into the liquid components "thin stillage" and solid components "whole stillage". The whole stillage may be dried to form Dried Distillers Grains.

The water component of thin stillage is evaporated to leave a syrup which can be added to the whole stillage component and dried further to form Distillers Dried Grains with Solubles (DDGS).

Viscosity - The thickness or the resistance to flow or stirring by a liquid.

Wet Grind - Grain is soaked (or steeped) in sulphuric acid for 24 hours and grain components such as germ, gluten and bran removed before the starch component is milled and used in alcohol production. Separation of grain components at wet grain facilities allows the production of a number of economically valuable co-products as well as ethanol.

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