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Feasibility of co-producing arabinoxylans and ethanol in a wheat biorefinery: Fractionation studies on UK wheats

by

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Contents

Abbreviations used

AY	Alcohol yield
AX	Arabinoxylan
DDGS	Distillers Dried Grains and Solubles
DM	Dry matter
EtOH	Ethanol
ha	Hectare
L	Litre
N	Nitrogen
NSP	Non starch polysaccharides
RL	Recommended List
SKCS	Single Kernel Characterisation System
SWRI	Scotch Whisky Research Institute
t	Metric Tonne
TGW	Thousand grain weight

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1 Abstract

Arabinoxylans (AX) are a promising candidate for co-production alongside bioethanol in an integrated wheat biorefinery, having both food and pharmaceutical uses. However, the economics of a biorefinery depend strongly on wheat composition.

Ten wheat varieties, representative of the range of compositional variation in UK wheats, were analysed for grain size and shape, protein, starch and arabinoxylan contents, in order to assess effects on biorefinery economics. The wheats were also fractionated by pearling and milling to produce a total of 100 different milling fractions, and the arabinoxylan, protein and ash contents quantified in order to identify suitable fractions for AX extraction. Following debranning, the 4% pearlings fraction (dominated by the outer bran layers) was found to have higher arabinoxylan contents than other fractions. In contrast, protein, which acts as a contaminant in AX production, was concentrated in the inner bran layers.

Economic simulations based on arabinoxylan extraction from the 4% pearlings showed the effects of starch and arabinoxylan contents on ethanol and AX costs, respectively, and identified the most promising wheats for processing into bioethanol and AX. Some wheats were suitable for both AX and bioethanol production, whereas some were suitable for neither. Actual measurements of alcohol yield of selected grain and flour samples indicated that bioethanol yield could not be predicted from starch content alone, as the two good distilling wheats had higher alcohol yields than would be predicted by their starch contents alone.

2 Project Summary

2.2 Objectives of the study

The objectives of the study were to: study debranning technology for production of starch rich endosperm and clean bran; determine how grain size and shape affect debranning and fractionation; quantify the starch and protein contents of individual cereal fractions; quantify the arabinoxylan content of different cereal fractions, and; identify the opportunities for the biorefinery processing industry to extract added value from grain.

2.2 Background

The UK government has highlighted the importance of biorefineries as a means of producing chemicals and other valuable renewable materials from crop by-products and residues. At its simplest, a biorefinery can be defined as the processing of biomass in a sustainable manner into many marketable products and energy in a manner analogous to an oil refinery.

In order to make bioethanol production from cereals economically competitive and commercially feasible, the ethanol must be produced as one of several co-products within an integrated biorefinery. After starch and protein, the nonstarch polysaccharide (NSP) fraction is quantitatively the major component of whole wheat grains (*ca*. 11%). At present the nonstarch polysaccharides pass through the distillery and are recovered in the fibre fraction of the distillers dried grains and solubles (DDGS) for sale into the animal feed market. Adding value to the NSP fraction is highly desirable, in order to enhance the economics of wheat-based biorefineries.

In wheat approximately 50% of the NSP are formed of a class of complex carbohydrates called arabinoxylans (sometimes called pentosans). The arabinoxylans are found mainly in the bran layers and are a key component of the dietary fibre fraction of cereals, which have commonly accepted health benefits in the human diet. They also have pharmaceutical uses, such as their inclusion in hydropolymer wound dressings.

Previous HGCA-funded work (Project Report 425) concluded that the cost of arabinoxylan (AX) production in a biorefinery also producing bioethanol could be sufficiently low to make feasible the creation of a commercial market for AX as a food ingredient such as a food thickener. It was felt that this conclusion justified further research into the extraction, functionality and end-use of wheat-derived AX. The conclusion was based, however, on an assumed representative wheat composition. The economics of a wheat biorefinery co-producing bioethanol and AX would depend strongly on the composition of the wheat used, particularly its arabinoxylan content. However, the range of arabinoxylan contents occurring in UK wheats has not previously been established. Similarly, the milling fractions from which an AX product might be most economically extracted have not been identified.

The aim of the current work was to measure the range of compositional variation occurring in representative UK wheats and their milled fractions, to identify differing fractionation patterns among different wheats, and to interpret these ranges in terms of their effects on biorefinery economics utilising the model developed previously (Project Report No. 425). The objective was to study a range of wheats representing the full range of variation seen in grain size, shape, hardness, protein and starch content for standard UK wheats.

2.3 Materials and methods

92 individual wheat variety samples from a single National List trial site were assessed for grain size and shape, and 30 samples exhibiting the widest range selected. The starch and protein contents of these 30 samples were then determined, and 10 wheat variety samples identified which represented the full range of variation in the original 92 for starch, protein and grain size. The 10 wheats were then subjected to a detailed milling and fractionation procedure, which yielded 10 different fractions of grain, bran or flour per wheat sample (100 grain fractions in total) for further analysis. The ash, arabinoxylan and protein contents of each of the 100 samples were determined. The data were used to estimate the price of ethanol and arabinoxylans produced using the 4% pearling fraction for arabinoxylan extraction. Finally, the starch and bioethanol yields of both pearled flour and the 4% pearling fraction were determined, for 5 of the wheat samples showing the biggest variation in protein content.

2.4 Results and Discussion

2.4.1 Differences in grain composition between wheat samples

The samples studied were chosen to be broadly representative of UK wheats grown with standard agronomy at a single site, yet showing appreciable variation in grain size, shape and protein content. Prior to this study there were no published data on the arabinoxylan content within UK wheats, an essential pre-requisite to optimising feedstocks for co-production of arabinoxylans and ethanol. In the 10 wheats analysed, arabinoxylan contents ranged from 4.5-9.0% dry matter in the whole wheat, comparable and slightly higher than the 4.8-6.9% range reported for French wheats.

Of particular interest was the fact that the good distilling wheats Zebedee and Glasgow had the lowest arabinoxylan contents. Anecdotal evidence would have predicted that these wheats had the lowest arabinoxylan contents because they are preferred by the distilling industry partly due to their very low residue viscosities when processed, and wheat viscosity is to a large extent governed by the levels of soluble arabinoxylans.

2.4.2 Distribution of arabinoxylan and protein in milling fractions

Milling the whole wheats gave Coarse Bran with arabinoxylan contents in the range 11.5-22.5%, Fine Bran with arabinoxylan contents of 6.4-11.4%, and Flour with much lower arabinoxylan contents of around 1.6%. The 4% pearlings had substantially higher arabinoxylan contents than any other fraction (21.4-34.5%), supporting the earlier suggestion that this fraction may be advantageous as the feedstock for arabinoxylan extraction.

With increasing length of time, debranning broke deeper into the grain, and the arabinoxylan content tended to reduce, giving marked differences in concentrations of arabinoxylans between pearling fractions for the hard wheats. However, for the soft wheats (Zebedee and Glasgow) the difference in arabinoxylan content between the pearling fractions was much less distinct. This suggested either that the arabinoxylan was distributed differently in the soft wheats (i.e. that it was less concentrated in the outer bran layers in these wheats) or that debranning the soft wheats broke through more deeply into the inner bran layers and endosperm, diluting the arabinoxylan in the resultant pearling fractions with starchy endosperm. On this basis, it would appear

that the soft wheats are less useful for arabinoxylan extraction, even though they tend to be favoured at present by distillers for alcohol production.

Protein content varied differently between milling fractions, being concentrated in the inner bran layers, principally the aleurone cells. Since protein tends to co-extract with the arabinoxylan under alkaline conditions, it causes problems during manufacture of AX, as the protein is a contaminant of the finished product. This suggests that the outer bran layers (in the 4% pearling fraction) which would be lowest in protein, would again be most appropriate tissues for extraction. The results also imply significant differences in the compositional and structural arrangements of the kernels from different wheat varieties, and hence differences in their responses to processing.

2.4.3 Economics of co-production of arabinoxylans with ethanol

In the current work, the effects of compositional variation represented by the ten wheats were illustrated by calculating, for each wheat, the cost of producing ethanol and an extracted AX product, based on the starch content of each wheat and the arabinoxylan content of the 4% pearlings.

The cost of AX (£/kg) produced is inversely proportional to the absolute amount of arabinoxylan extracted, so the greater yield from pearlings could offset the higher costs calculated in the previous HGCA–funded project (Project Report No. 425). From the current work, it is now evident that the 4% pearlings are consistently more enriched in arabinoxylan, so it was appropriate to allow for this in the simulations. The arabinoxylan content in the 4% pearlings is on average over 60% greater than in the Coarse Bran obtained from milling whole wheat. If this is translated into 60% more AX product extracted from the same quantity of bran (i.e. if the AX is equally extractable from both sources), then this lowers the cost per kg by 37% and would make AX product extracted from pearlings around 25% cheaper than AX extracted from Coarse Bran. However, it should be emphasised that the functionality of the AX product from these different bran sources would need to be evaluated.

In the current work, economic analyses were performed based on extraction of arabinoxylans from the 4% pearlings, assuming 45% yield of the arabinoxylan contained in the bran. The cost of the AX product depends on the yield of arabinoxylan, but also on the yield (and hence cost) of ethanol, which in turn depends on the starch content of the whole wheat. In the analysis, the cost of ethanol was

calculated as a function of starch content for each wheat, rather than being based on a measured alcohol yield. The prices of ethanol and AX for each wheat were calculated, from which the most appropriate wheats for co-production of these two products were identified. Some wheats exhibit a positive combination of high starch content and high arabinoxylan content in the pearlings, leading to a combination of low ethanol and AX product prices; such wheats would be ideal for a biorefinery co-producing ethanol and AX. Wheats Hereward and NSA02-1422 in the present study were in this category. Other wheats were better suited for either ethanol or AX production, but not both, while others such as Zebedee and Glasgow were suitable for neither. However, an actual assessment of bioethanol yield for these latter two wheats in the laboratory indicated that ethanol yield was higher than would be expected from starch content alone, indicating that further information on predicted alcohol yield is needed for simulation models.

2.5 Key conclusions

The work described here is the first to simulate the economics of co-production of AX with ethanol, based on measured starch and arabinoxylan contents of UK wheats, and builds on the earlier simulation studies. The following conclusions are drawn from the work:

1. The present study has confirmed that the AX content of pearlings is higher than that of coarse bran, and that using pearlings can reduce the price of AX produced, implying that capital investment in debranning equipment may be a cost effective approach to integrating AX and bioethanol production.

2. Further practical work to extract and purify AX from milling fractions at pilot scale and to test functionality of the isolated AX in food products is required to fully test the concept.

3. The removal of AX from the co-product stream will result in differences in composition of the DDGS and at present this cannot be predicted accurately, because there are so little data on the variation in composition of wheat grain in terms of the non-protein components (starch, NSP, lignin, lipid, ash).

4. Future work will be needed to take into account environmental as well as genetic variation in AX and starch content in wheat grain.

5. Simulation models will need better predictors of alcohol yield for different wheat varieties at a given starch content, otherwise false conclusions may be drawn,.

6. Wheats for distilling may well have higher starch contents, and hence ethanol yields, than those studied here, therefore low protein grain should also be studied in the context of integrating AX and bioethanol production.

7. The implications of the distributions of grain components, particularly protein between bran/pearlings and endosperm/flour, need to be considered further in terms of breeding approaches to low protein grain, and in particular whether screens could be developed which specifically focus on reducing protein storage in the bran layer.

8. This study supports the earlier work (Project Report No. 425), that AX can be produced economically at prices which are competitive in the marketplace, and has extended the earlier work to include actual concentrations of AX in the pearling fractions of UK wheats.

9. The study indicates that there is scope for optimising the choice of wheat variety which can lead to reductions in the prices of both ethanol and AX produced in an integrated biorefinery.

3 Introduction

The UK government has highlighted the importance of biorefineries as a means to producing chemicals and other valuable renewable materials from crop by-products and residues (Anon, 2006). At its simplest, a biorefinery can be defined as the processing of biomass in a sustainable manner into many marketable products and energy in a manner analogous to an oil refinery.

Cereal biorefineries will necessarily be part of the mix of sustainable chemical and energy providers in the 21st century. Conceptually, the process of manufacturing food products such as starch and gluten from wheat can already be considered as biorefining: Starch extraction plants for instance separate and purify the major components and sell these on, or convert them into other related products such as sugar syrups or chemically-modified starches. However, biorefining on a larger scale, in terms of producing bulk chemicals from a single factory which can compete with volumes traded in the chemical industry (e.g. >20,000 t/annum) have been slow to develop in the UK. This is despite rapid developments in biorefining elsewhere such as the US and continental Europe where plants have been designed to hydrolyse and ferment corn starch into building blocks such as lactate, for production of plastics such as polylactic acid. Nevertheless with the anticipated production of bioethanol in 2009/10 by at least two commercial operations in the UK, each designed to process one million tonnes of wheat per annum, it is timely to study the opportunities for advanced cereal biorefineries in the UK.

In order to make bioethanol production from cereals economically competitive and commercially feasible, the ethanol must be produced as one of several co-products within an integrated biorefinery. After starch and protein, the non-starch polysaccharide (NSP) fraction is quantitatively the major component of whole wheat grains (*ca*. 11%, Smith *et al*., 2006). At present the NSP pass through the distillery and are recovered in the fibre fraction of the distillers dried grains and solubles (DDGS) for sale into the animal feed market. Adding value to the NSP fraction is highly desirable, in order to enhance the economics of wheat-based biorefineries (Campbell *et al*., 2006).

3.1 Why arabinoxylans ?

In wheat approximately 50% of the NSP are formed of a class of complex carbohydrates called arabinoxylans (AX). They have also traditionally been called 'pentosans' because they are principally made up of the two pentose sugars, arabinose and xylose. The AX are structural polysaccharides forming part of the cell wall matrix associated with cellulose and pectins. In wheat the AX are quantitatively found mainly in the bran layers (including aleurone cell walls), and the thin cell walls within the endosperm. AX are a key component of the dietary fibre fraction of cereals, which have commonly accepted health benefits in the human diet (Alldrick, 1991).

AX have several interesting functional properties relevant to food or pharmaceutical use. However in comparison with the research which has been carried out on proteins and starch, much less has been carried out on AX, and the fine structure and understanding of the functional properties were not elucidated until the early 1980s (Table 1).

Table 1. Key papers tracing the increasing knowledge of arabinoxylans and their development in food and pharmaceutical applications.

Authors	Area of research / discovery
Durham (1925)	Oxidative gelation of wheat flour suspensions
Norris & Preece (1930)	The hemicelluloses of wheat bran
Montgomery & Smith (1955)	Water soluble hemicelluloses from endosperm of wheat
Timell (1967)	Chemistry of wood hemicelluloses
Nieduszynski & Marchesault (1972)	Structure of xylan hydrate
Kunz (1974)	Chemically modified polysaccharides from cereal brans
Hoseney (1984)	Functional properties in baked goods
Izydorczyk *et al*. (1990)	Oxidative gelation of water soluble pentosans from wheat
Greenshields & Rees (1993)	Gel production from plant matter (AX from maize bran)
Doner & Hicks (1997)	Alkaline hydrogen peroxide extraction of hemicelluloses
Hollmann & Lindauer (2005)	Pilot scale extraction of arabinoxylans
Chen & Englemann (2007)	Pentosan polysulphate in pharmaceutical applications

Hoseney (1984) described the role of AX role in water binding in wheat flour doughs, and discussed their influence on dough properties such as increasing loaf volume through supporting gas retention in bubbles. Hoseney also discussed their putative role in oxidative gelation through cross-linking reactions with other AX molecules, or with gluten proteins. This latter phenomenon, the ability to carry out oxidative gelation and form a covalently linked network, was originally reported early in the 20th Century (Durham, 1925) but commercially, specific high value applications for arabinoxylans making use of this property were not reported until much later: Greenshields and Rees (1993) described the extraction of AX from cereal brans in order to produce a purified AX material which could be cross-linked to form a hydropolymer for use in medical devices such as wound dressings. More recently Chen and Engelmann (2007) reported the use of 'pentosan polysulphate' as a pharmaceutical molecule.

It is also important to characterise the A/X ratio of the AX in different fractions because it signifies the degree of arabinose substitution on the xylan backbone; a high A/X ratio indicates a more branched structure which is likely to affect the functional properties of the AX, most notably tending to render it more water-soluble (Courtin and Delcour, 2002). A functional food ingredient such as a water-soluble polysaccharide may have a value up to five times that of flour, and pharmaceutical molecules up to ten times that of flour. They thus provide considerable opportunity to add value to wheat grain.

3.2 Previous research into arabinoxylan extraction and isolation

Quantitatively, the principal location of AX is in the bran layer, and is in a form which is insoluble in water. The AX typically have to be solubilised by alkaline extraction, to break down hydrogen bonds and protein-polysaccharide linkages, with a hot potassium hydroxide (KOH) solution as the solvent of choice. Variants use different concentrations of alkali (typically 0.05-1M KOH) and also alkaline peroxide extraction (e.g. Doner and Hicks, 1997).

Following solubilisation, neutralization and filtering of the extract, the polysaccharides then have to be precipitated from solution in order to recover them in solid form. This is achieved by adding alcohol (ethanol, iso-propanol, industrial methylated spirits) until the alcohol concentration is *ca*. 85%. The polysaccharides precipitate out of

solution and can be dried by solvent exchange and/or vacuum drying. The liquid waste stream, an aqueous ethanolic mixture (containing degraded proteins and sugars), has to be redistilled to recover the alcohol for subsequent processing, or else must be disposed of in an environmentally sound way. Both of these options (distillation or disposal) bear significant costs. Bearing in mind the fact that the alcohol must be purchased in the first place, this has always meant that arabinoxylan extraction has been commercially non-viable at least from the point of view of producing AX products which can compete with existing hydrocolloids in the food market. However, the option of being able to carry out AX extraction in the context of a cereal biorefinery manufacturing bioethanol means that two limiting factors have potentially been removed: a) the plant can manufacture its own ethanol, so this does not need to be bought-in, and b) the alcoholic waste can be recycled and the ethanol recovered by distillation and re-used.

3.3 Integration of bioethanol and arabinoxylan production

As a first step towards co-product processing and the highly integrated biorefinery concept, AX therefore appear a promising candidate for extraction, and the co-production of ethanol and AX was modelled in a previous HGCA-funded project (Mustafa *et al*., 2007; Sadhukhan *et al*., 2008; Du *et al*., 2009; Misailidis *et al*., 2009). The extraction of AX was facilitated using ethanol, and employed the process described by Hollmann and Lindhauer (2005).

Economic analysis of co-production of bioethanol and AX from wheat in an integrated biorefinery, based on the UK context, concluded that the cost of AX production could be sufficiently low to make feasible the creation of a commercial market for AX as a food ingredient (Mustafa *et al*., 2007; Misailidis *et al*., 2009). It was felt that this conclusion justified further research into the extraction, functionality and end-use of wheat-derived AX. The conclusion was based, however, on an assumed representative wheat composition. The economics of a wheat biorefinery co-producing bioethanol and AX would depend strongly on the composition of the wheat used, particularly its AX content. However, the range of AX contents occurring in UK wheats has not previously been established. Similarly, the milling fractions from which AX might be most economically extracted have not been identified.

Previous work reported by Mustafa *et al*. (2007) and Du *et al*. (2008), and supported by Barron *et al*. (2007) and Saulnier *et al*. (2007) had suggested that AX is more concentrated in the outer layers and hence in the 4% pearlings fraction. The current work sought to confirm this for a wider range of wheats.

3.4 Aim of the project

The aim of the current work was to measure the range of compositional variation occurring in representative UK wheats and their milled fractions, to identify differing fractionation patterns among different wheats, and to interpret these ranges in terms of their effects on biorefinery economics utilising the model developed previously by Mustafa *et al*. (2007) and refined by Misailidis *et al*. (2009), based on the extraction procedure of Hollmann and Lindhauer (2005). The objective was to study a range of wheats representing the full range of variation seen in grain size, shape, hardness, protein and starch content for standard UK wheats.

4 Materials and Methods

4.1 Samples

An initial selection of 92 samples from a National List (NL) trial at Malden in Essex harvested in 2006 were provided by Nickerson-Advanta Ltd. Some were named varieties and are familiar from the HGCA Recommended List (RL), while others were coded as NL samples. Each sample was available in 10 kg quantity which gave sufficient material to enable pearling and milling studies to be carried out. A two stage screening procedure then took place in order to reduce the number of samples to manageable quantities, while preserving the range of grain size, shape and composition.

Stage 1

The full set of samples (92) were screened initially for grain size and length:width ratio: Grain dimensions (mean length, mean width) were determined using a Marvin digital seed analyser (GTA Sensorik GmbH), and grain length:width (L:W) ratio calculated from the primary data as described by Kindred *et al*. (2008). Thousand grain weight (TGW) was estimated concurrently using the digital seed analyser, and a sub set of 30 samples was identified showing the maximum range of each character.

Stage 2

The 30 sample subset was analysed for starch and protein (see methods below) and a final subset of 10 samples identified which encompassed the range of starch and protein and grain size and shape seen in UK wheats. After starch and protein content, kernel size and shape were used as the principal criteria for sample selection, on the reasoning that kernels varying substantially in size and shape should therefore vary in bran content, and that this should be reflected in compositional variation. These were then subjected to a detailed milling protocol which yielded a range of milling fractions on which further chemical analysis was carried out. The procedures are explained in more detail below.

Note: As the grain samples received were unreplicated within the site, it was not possible to make statistical comparisons between varieties.

4.2 Milling protocol

The 10 wheats were each processed into ten fractions (100 sample x fraction combinations), as illustrated in Figure 1. Fraction 1 was the whole wheat. 2 kg of the whole wheat samples were conditioned to 16% moisture, then milled using a laboratory-scale Buhler mill to yield three different fractions: Coarse Bran (Fraction 2), Fine Bran (Fraction 3) and Flour (Fraction 4). Another 2 kg of the conditioned wheats were subjected to a pearling process using the Satake TM05 debranner (Satake Corporation, Japan). The 4%, 4-8% and 8-12% pearlings were removed by successive pearling and were labelled as Fractions 5, 6 and 7, respectively. The pearled kernels were then milled using the Buhler mill as before to yield fractions of Coarse Bran, Fine Bran and Flour, labelled as Fractions 8, 9 and 10, respectively (see summary in Table 2). Additionally at each stage of pearling, the grains were passed through a Single Kernel Characterisation System (SKCS; Perten Instruments) in order to measure grain weight, diameter and hardness.

Table 2. Sample codes for milling and pearling fractions.

Sample code	Description
1	Whole wheat flour (starting material)
2	Unpearled bran
3	Unpearled fine bran
4	Unpearled flour
5	4% pearlings
6	8% pearlings
7	12% pearlings
8	Pearled bran
9	Pearled fine bran
10	Pearled flour

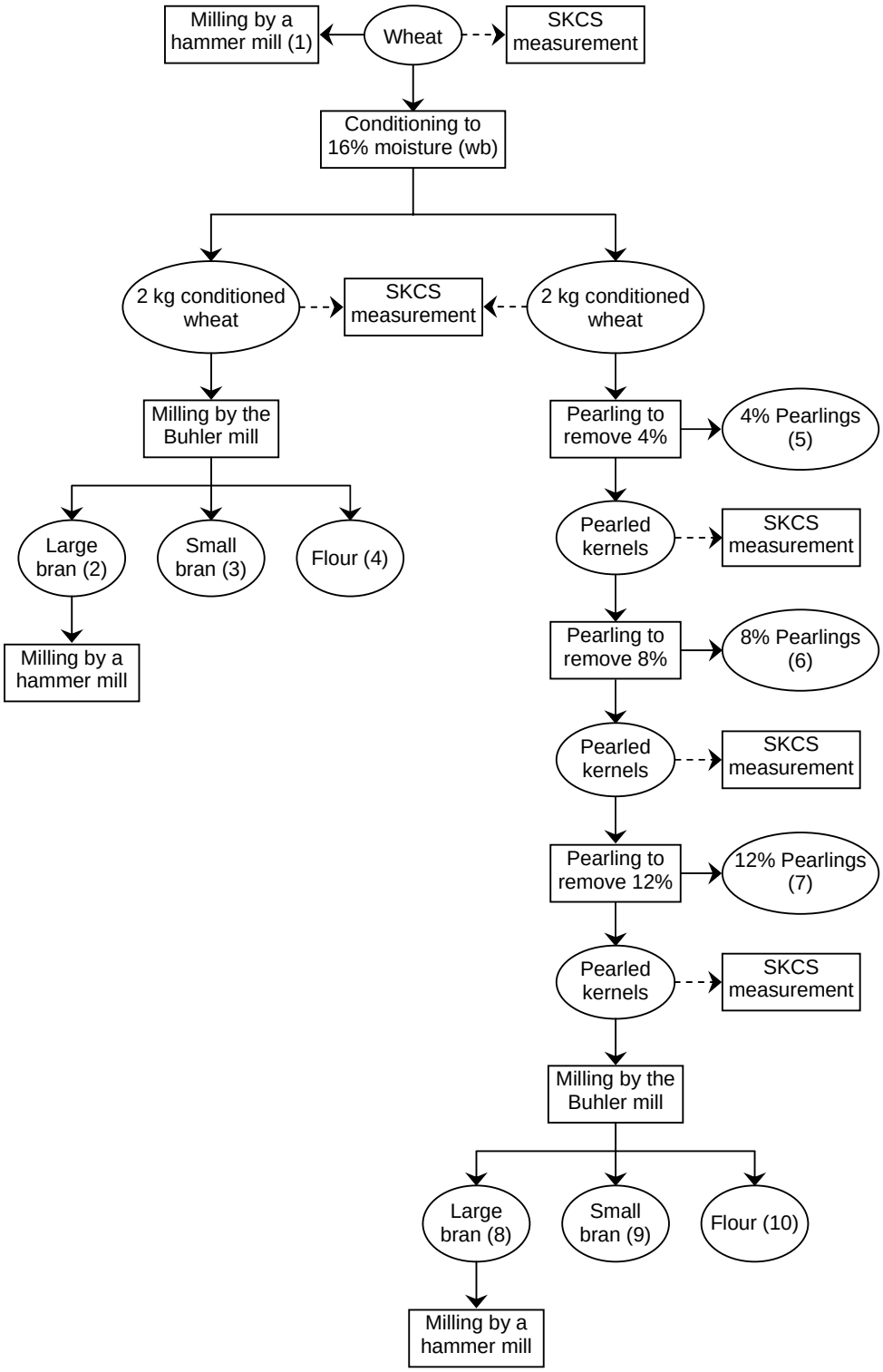


Figure 1. Processing diagram to produce wheat fractions. Numbers in brackets indicate sample codes.

4.3 Analytical methods

Grain size and texture

Mean grain weight (mg), width (mm) and hardness index were measured using the Single Kernel Characterisation System (SKCS). Thousand grain weight was then estimated as 1000 x mean grain weight.

Proximate analysis

Protein was determined for each of the 100 milling fractions x wheat samples. Protein was estimated as Nx5.7, following determination of grain N content by Dumas combustion. Starch was determined on the starting material only (i.e. Fraction 1) using the Ewers polarimetric method as described by Kindred *et al*. (2007b).

Ash analysis

Ash content is generally taken to be broadly indicative of bran content of wheat milling fractions. However, the ash content of the various bran layers and of aleurone varies, such that bran fractions obtained by pearling are likely to vary in their ash contents for different wheats. Ash content of the ten fractions was therefore measured in the current work by placing 1 g of material in a furnace at 560°C overnight.

Arabinoxylan concentration

AX concentration was measured as follows: Whole wheat (Fraction 1) was ground using a Glen Creston mill fitted with a 2 mm screen, while the other pre-prepared samples (Fractions 2-10) were used as received following the processing regime described above. The moisture content of each fraction was determined by drying overnight at 100^{o}C until reaching constant weight. Individual neutral sugars were determined by hydrolysing each fraction in 2N sulphuric acid (2 h, 100^{o}C) following a pre-treatment with 72% sulphuric acid (1 h, 30^{o}C) according to Saeman *et al*. (1954). Inositol was added as an internal standard (IS) prior to hydrolysis. The individual sugars were reduced, acetylated and analysed as their alditol acetates by gas chromatography (Englyst & Cummings, 1984) using an Agilent DB-225 column, and detected using a mass spectrometer operating in selective ion mode (Agilent 6890 GC with 5975 Mass Selective Detector; Agilent Technologies, Cheshire, UK). Response factors for individual sugars were determined using a standard solution of monosaccharides plus IS and hydrolysed using 2N sulphuric acid in the same conditions as above. Concentrations of monosaccharides were converted to their

anhydro-polymer equivalents by multiplying by the following factors: 0.896 (rhamnose), 0.88 (arabinose (A), xylose (X)) or 0.9 (mannose, galactose, glucose) before correcting concentrations to a 100% DM basis. Samples were analysed in duplicate, and the standard deviations estimated for each sugar. The coefficients of variation (CV) for measurements of the analytes (and their derivatives) reported here were: arabinose 6.1%; xylose, 5.5%, A+X, 6.1%; A/X ratio, 3.0%; protein, 5.2%; starch, 0.7%. These analytical errors were relatively small, and for this reason error bars are omitted from the figures (other potentially larger sources of error, for instance those resulting from environmental variation were not considered in this study).

Alcohol yield determination

Alcohol yield and viscosity were determined in duplicate on a subset of five flour samples using an ADAS method adapted from that of the Scotch Whisky Research Institute (SWRI; Agu *et al*., 2006). For the original grain (Fraction 1) the wheat grain was milled using a Glen Creston hammer mill fitted with a 2 mm screen. For Fraction 10 (pearled flour) no further preparation was required as this had been Buhler-milled as described above. In both cases the moisture content of the flour was determined on a subsample by drying overnight at 100°C. Flour (15 g of whole flour for Fraction 1; 12.75 g of white flour for Fraction 10 both on a fresh weight basis) was placed in a stainless steel beaker with 40.5 mL of water to which 53 µL of a thermostable alpha-amylase (Spezyme Xtra, Genencor, Lieden, Netherlands), 75 µL of a protease (Fermgen, Genencor) and 6.8 µL of a beta-glucanase (Optimash BG, Genencor) were added (in excess) to rapidly break down starch to oligosaccharides. The slurry was then heated in a waterbath set at 60°C for 35 minutes with frequent stirring, before the temperature of the waterbath was increased to 74°C and the sample was stirred for a further 60 mins. The sample was then autoclaved at 126 °C for 11 min before being returned to the waterbath set at 88°C and a second dose (53 µL) of the alpha-amylase added to minimise retrogradation. This cooked slurry was then mashed for a further 60 mins at 88°C before being removed from the waterbath and allowed to cool to approximately 30°C. The slurry was then pitched with distillers yeast (0.4% w/w) as well as further enzyme additions; 75 µL of the protease and 13 µL of a saccharifying enzyme (Fermenzyme L-400, Genencor) before being fermented at 30°C for 68 hours after which the slurry was distilled and the distillate measured for alcohol content using an Anton Paar density meter.

4.4 Economic evaluation

As reported later, the current work confirmed for a range of wheats that the 4% pearling fraction is high in AX, such that it may be the most suitable fraction for AX extraction within a wheat biorefinery; economic analyses were therefore based on this fraction. Using the starch content of the whole wheat and the AX content of the 4% pearlings, economic analyses were performed based on the biorefinery simulator presented by Misailidis *et al*. (2009), to calculate the costs of ethanol and AX production for the ten wheats. In accordance with common practice, costs are given in US$, although are selected to be relevant to the UK context. A ratio of US$2 = £1, which was appropriate at the time the work was performed, was used in all cases.

5 Results

5.1 Characteristics of wheats studied

The range of grain sizes and dimensions of the initial 92 wheats are shown in Table 3. At each stage, the subsets chosen managed to retain the wide range of variation seen in the original samples. A more detailed analysis of the final 10 wheats chosen for analysis are shown in Table 4. The selection included both hard milling wheats (e.g. Hereward) as well as good distilling wheats (Zebedee and Glasgow).

5.2 Milling and fractionation studies

Grain hardness is shown in Table 5 for the starting grain and the material at each stage following pearling. Glasgow (sample 39) was the softest wheat with a hardness index of 42 for the original grain and NSL04-5070 (sample 66) was the hardest grain with a hardness index of 78. The hardness affected the rate of debranning (or pearling) as shown in Figure 2: The soft wheat Zebedee (sample 37) lost material during debranning at a faster rate than the eight hard wheats. Based on these 'pearling curves' the time could be estimated at which a particular wheat sample needed to be debranned in order to remove a fixed amount of material. The debranned material (or pearlings) were then used for further chemical analysis, knowing that each represented a similar proportion of the initial grain across the different samples.

As the wheats had successively more of the outer layers removed by debranning, their reported SKCS hardness values decreased (Table 5). The average reduction was 7.8 hardness units between the unpearled conditioned grain and removal of 12% of the mass as pearlings. The overall reduction (i.e. following removal of the 12% pearlings) represented an average loss of 13.8% of the initial hardness. However, the effect of debranning on grain texture was greater for the soft wheats, with Zebedee (sample 37) and Glasgow (sample 39) losing 9 and 13 hardness units (21 and 46% of their initial hardness) respectively, whereas the hard wheats NSL04-5070 (sample 66) and Hereward (sample 160) lost *ca*. 9 hardness units (~12.5% of their initial hardness). It should be noted that the hardness index reported by the SKCS is in arbitrary units,

and assumes intact wheat kernels; the reduction is hardness is indicative of how the SKCS algorithm interprets the crush profiles of pearled kernels.

Figure 3 shows the yields of each fraction obtained for the ten wheats (note that Figure 3 and similar figures employ line graphs when bar graphs might be considered strictly more appropriate; however, line graphs have been used because they communicate the patterns considerably more clearly than would bar graphs). Buhler-milling of whole wheat resulted, on average, in production of around 18% Coarse Bran, 10% Fine Bran and 72% Flour. Pearling removed on average 12% material (three lots of 4%), after which Buhler milling of the pearled kernels yielded on average 9-10% of both Coarse and Fine Bran and 68.5% Flour. Thus pearling reduced Coarse Bran yield (as expected), with little effect on Fine Bran yield, and slightly reduced Flour yield.

Table 3. Grain size and shape as determined by the Marvin digital analyzer, and the starch and protein contents of NL wheat samples, going through the selection process from an original 92 samples down to a final subset of 10 for fractionation studies.

	Thousand grain weight (g)	Width (mm)	Length (mm)	L:W ratio	Starch (%)	Protein (%)
Original 92 samples						
Min	38.5	2.30	5.10	1.79	-	-
Max	55.3	3.10	6.20	2.36	-	-
Mean	47.2	2.73	5.71	2.10	-	-
Initial 30 sample subset						
Min	38.5	2.30	5.10	1.79	67.0	11.1
Max	55.3	3.10	6.20	2.36	71.0	13.6
Mean	47.2	2.71	5.66	2.09	69.0	12.5
Final 10 sample subset						
Min	38.5	2.30	5.10	1.79	67.4	11.1
Max	55.3	3.10	6.20	2.36	71.0	13.6
Mean	47.2	2.72	5.63	2.08	69.2	12.6

Table 4. Grain weight and diameter measured by SKCS, length:width ratio measured by Marvin digital analyzer, and protein content for ten samples of wheat selected for fractionation studies.

ID	Variety	TGW (g)	Diam. (mm)	L/W Ratio	Protein (%)	Rationale for inclusion
37	Zebedee	49.8	2.86	2.14	13.1	Distilling var., high TGW
39	Glasgow	42.0	2.62	2.12	12.1	Distilling var., low TGW
66	NSL04-5070	54.8	3.25	1.90	11.4	Highest width
104	NSL05-3341	46.3	3.03	2.36	12.3	Highest L:W ratio
146	NSA02-1422	55.3	2.89	2.04	13.5	Highest TGW
148	Ochre	46.5	3.03	1.79	12.2	Smallest L:W ratio
157	Gulliver	51.5	3.16	2.14	13.6	Highest protein
160	Hereward	45.4	3.04	2.04	13.2	High starch & high protein
168	NSLWW81	38.5	3.03	2.26	13.5	Low TGW & low diam.
268	NAWW5	46.9	3.07	2.00	11.1	Lowest protein & low starch

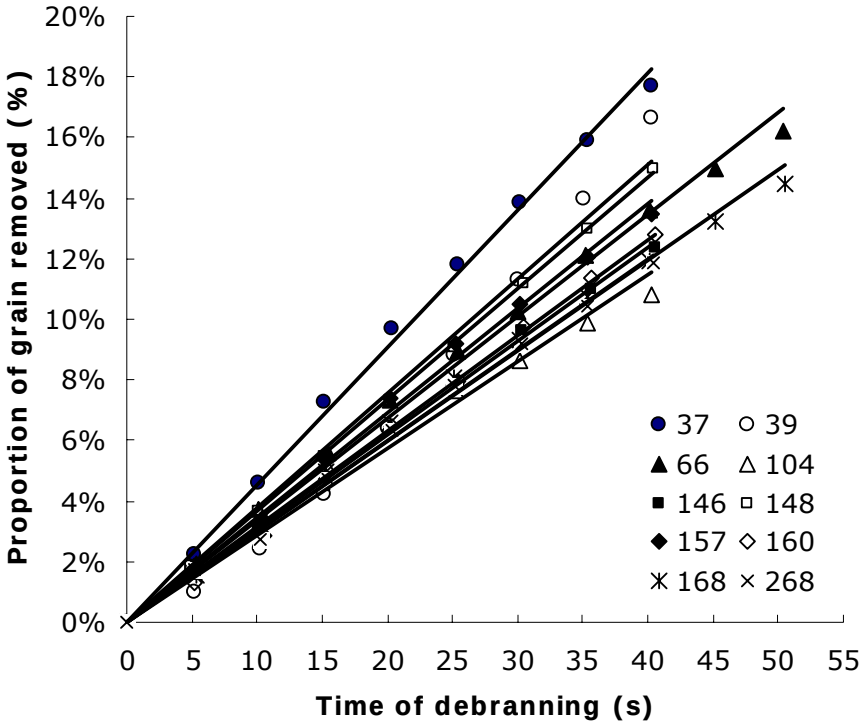


Figure 2. Pearling curves for ten individual wheat samples, showing proportion of grain removed by debranning with time.

Table 5. Grain weight, size and hardness during pearling, measured using the Single Kernel Characterisation System.

Wheat sample	Description	Weight (mg)	Diameter (mm)	Hardness index
37	Original wheat 37	47.0	2.86	44.1
	After conditioning	48.8	2.96	41.9
	After pearling for 4% removal	44.1	2.54	38.2
	After pearling for 8% removal	39.6	2.20	34.6
	After pearling for 12% removal	36.3	2.01	32.9
39	Original wheat 39	42.1	2.62	28.2
	After conditioning	43.5	2.72	28.6
	After pearling for 4% removal	40.5	2.43	22.5
	After pearling for 8% removal	38.3	2.23	20.3
	After pearling for 12% removal	36.3	2.12	15.6
66	Original wheat 66	52.6	3.25	78.1
	After conditioning	56.8	3.32	76.4
	After pearling for 4% removal	50.6	2.87	73.3
	After pearling for 8% removal	42.0	2.46	69.3
	After pearling for 12% removal	38.4	2.30	67.2
104	Original wheat 104	48.6	3.03	86.6
	After conditioning	48.7	2.98	84.5
	After pearling for 4% removal	45.6	2.64	82.7
	After pearling for 8% removal	42.7	2.54	81.0
	After pearling for 12% removal	39.3	2.50	80.0
146	Original wheat 146	47.0	2.89	79.2
	After conditioning	48.1	2.98	78.2
	After pearling for 4% removal	44.1	2.62	74.7
	After pearling for 8% removal	40.1	2.48	71.8
	After pearling for 12% removal	38.6	2.36	70.8
148	Original wheat 148	47.6	3.03	81.7
	After conditioning	47.2	3.04	78.2
	After pearling for 4% removal	43.1	2.67	74.6
	After pearling for 8% removal	40.4	2.55	72.0
	After pearling for 12% removal	36.6	2.45	70.0
157	Original wheat 157	50.1	3.16	80.2
	After conditioning	52.9	3.29	76.5
	After pearling for 4% removal	49.5	2.91	73.1
	After pearling for 8% removal	45.2	2.66	71.5
	After pearling for 12% removal	39.6	2.43	69.3
160	Original wheat 160	47.3	3.04	74.6
	After conditioning	47.0	3.09	74.2
	After pearling for 4% removal	44.1	2.79	69.3
	After pearling for 8% removal	40.8	2.60	67.3
	After pearling for 12% removal	37.5	2.43	64.8
168	Original wheat 168	42.9	3.03	88.3
	After conditioning	41.9	2.94	85.2
	After pearling for 4% removal	40.0	2.66	82.8
	After pearling for 8% removal	37.3	2.40	80.9
	After pearling for 12% removal	36.3	2.28	80.1
268	Original wheat 268	48.5	3.07	81.4
	After conditioning	51.5	3.10	75.2
	After pearling for 4% removal	45.1	2.67	73.4
	After pearling for 8% removal	41.6	2.47	71.9
	After pearling for 12% removal	37.5	2.32	70.4

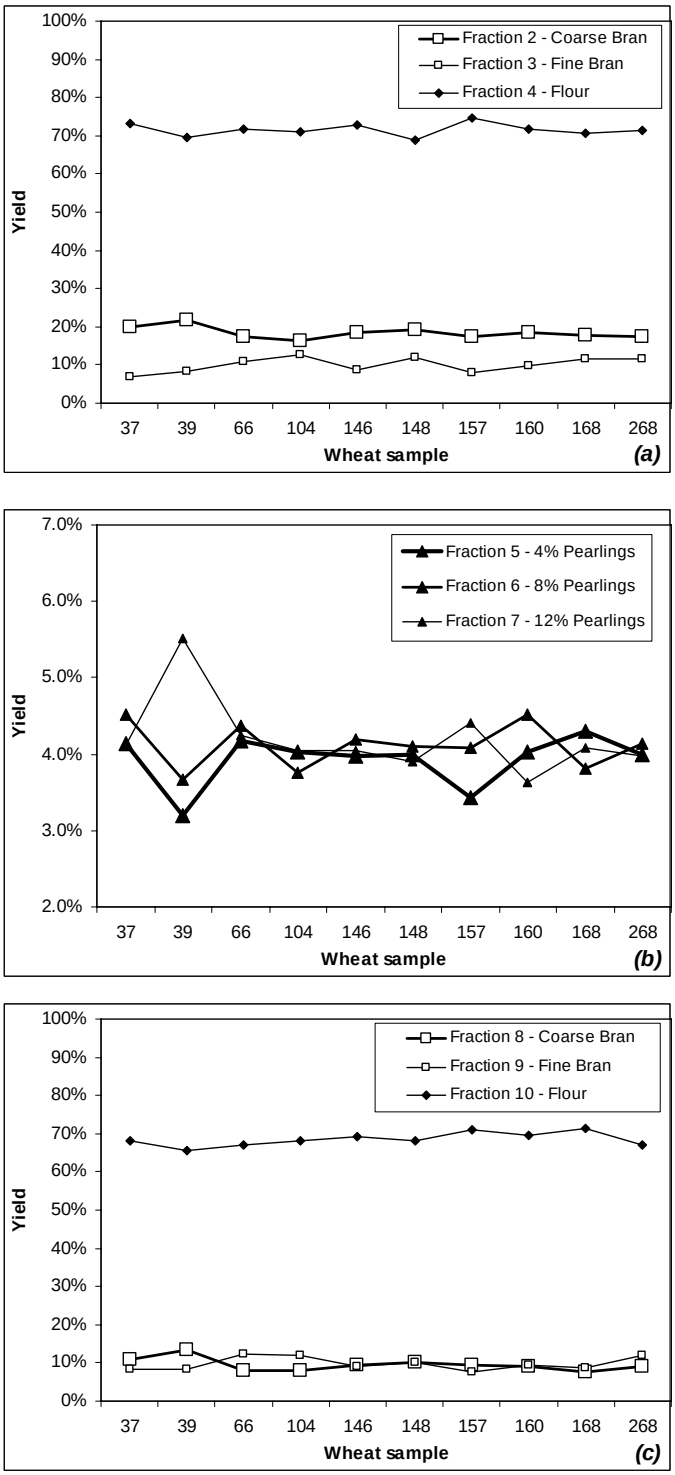


Figure 3. Yields of fractions following milling of the ten wheats: (a) yields of Coarse Bran (Fraction 2), Fine Bran (Fraction 3) and Flour produced from Buhler milling of the Whole Wheat; (b) 4% (Fraction 5), 4-8% (Fraction 6) and 8-12% (Fraction 7) Pearlings; (c) Coarse Bran (Fraction 8), Fine Bran (Fraction 9) and Flour (Fraction 10) produced from Buhler milling of the Pearled Wheat.

5.3 Arabinoxylan distributions in wheat milling fractions

Figure 4 shows the AX contents and the A/X ratios of the ten fractions, for each of the ten wheats. AX contents ranged from 4.5% to 9.0% dry matter in the whole wheat (Figure 4a). Milling the whole wheats gave Coarse Bran with AX contents in the range 11.5-22.5%, Fine Bran with AX contents of 6.4-11.4%, and Flour with much lower AX contents of around 1.6%. A/X ratios varied in the opposite manner to the AX content (Figure 4d), with Flour having higher A/X ratio than Fine Bran, which was higher than Coarse Bran. Figure 4b shows the AX contents of the three pearling fractions, demonstrating that the 4% pearlings had substantially higher AX contents than any other fraction, supporting the earlier suggestion that this fraction may be advantageous as the feedstock for AX extraction. In contrast to the milling results, for these pearling fractions, A/X ratio varied directly with AX content, being highest for the 4% fraction. This indicates that the nature of AX varies in different parts of the wheat kernel, highlighting the importance of knowing both the relative AX contents of the different kernel components as well as the characteristics/functionality of the AX from those different components.

Figure 4c shows the AX contents of the Coarse and Fine Brans and the Flour produced from pearled kernels; these follow the same trends as for the corresponding fractions from whole wheat, but with the Coarse Bran in this case having a lower AX content, owing to the removal of high AX material in the pearlings. Again, the A/X ratios are highest in Flour, lowest in Coarse Bran.

5.4 Protein distributions in wheat milling fractions

Protein was distributed differently to AX, being richer or more concentrated in the inner bran layers (Figure 5; Annex Table A1) meaning that for unpearled wheat fractions (i.e. following a conventional milling procedure) the protein was most concentrated in the Coarse Bran fraction (F2). However, for pearled grains, the protein content increased in the order of pearling fractions 5-7, being lowest in the 4% pearling fraction (F5) and highest in pearled bran (F8). The protein content was always lowest in the flours (F4 and F10).

There was a weak but positive relationship between the concentration of protein in the various bran fractions, and the total grain protein when data for all ten wheats were pooled (Figure 5a). However, the relationships were much improved when the two

soft wheats (Nos 37 and 39) were removed from the analysis and the relationship plotted for hard wheats alone (Figure 5b).

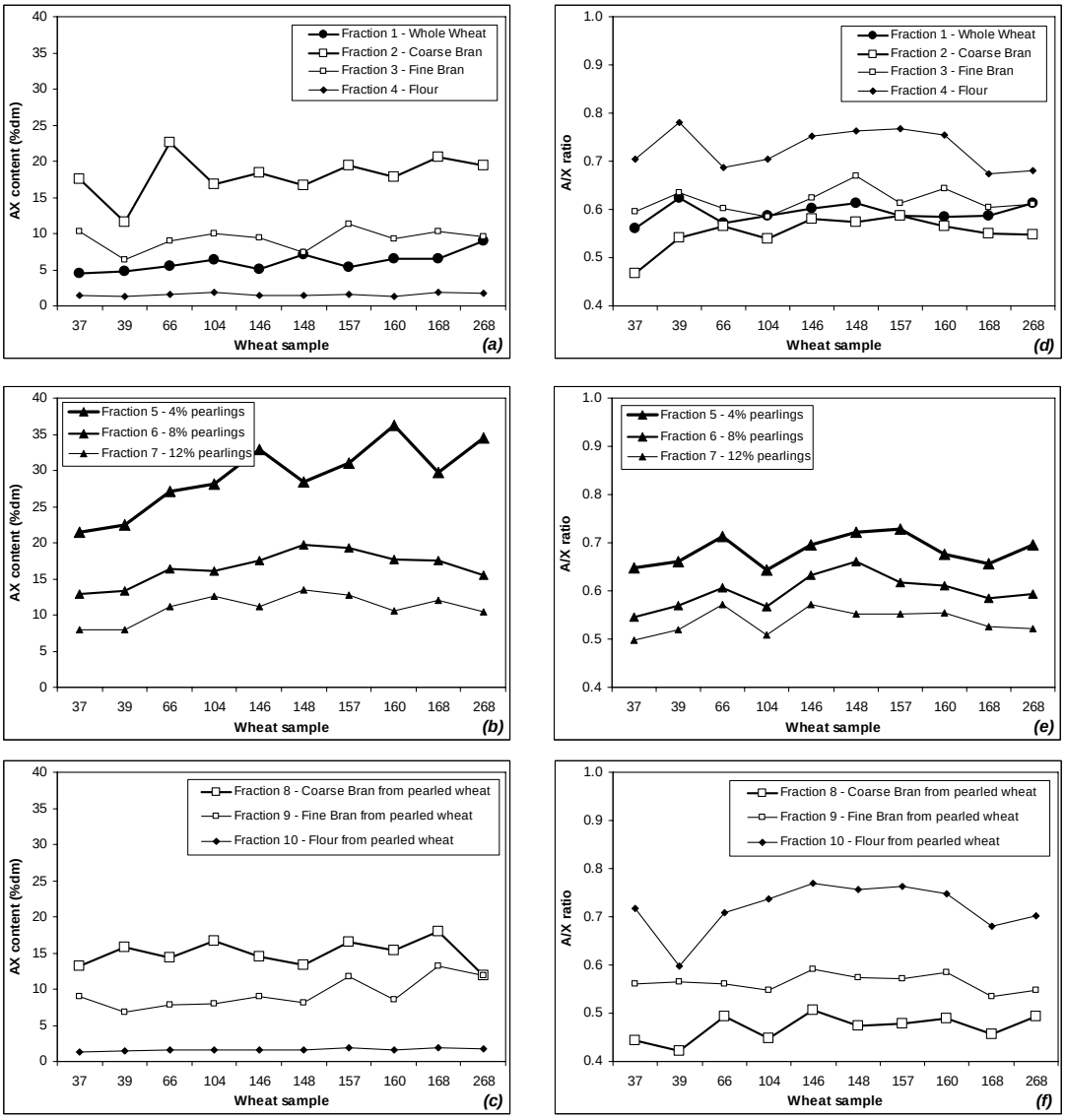
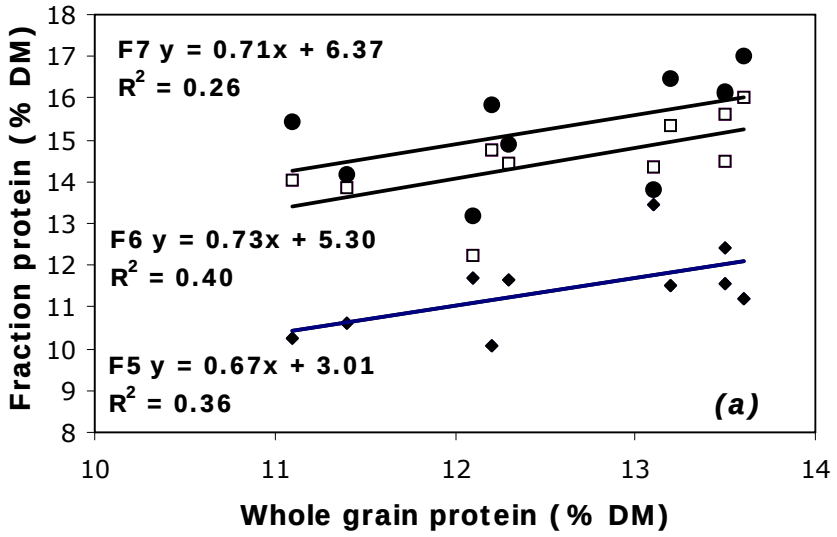


Figure 4. AX contents (left) and A/X ratios (right) for ten wheats and their milling and pearling fractions: AX contents of (a) Whole Wheat (Fraction 1), Coarse Bran (Fraction 2), Fine Bran (Fraction 3) and Flour produced from Buhler milling of the Whole Wheat; (b) 4% (Fraction 5), 4-8% (Fraction 6) and 8-12% (Fraction 7) Pearlings; (c) Coarse Bran (Fraction 8), Fine Bran (Fraction 9) and Flour (Fraction 10) produced from Buhler milling of the Pearled Wheat; (d), (e) and (f), A/X ratios of the fractions.



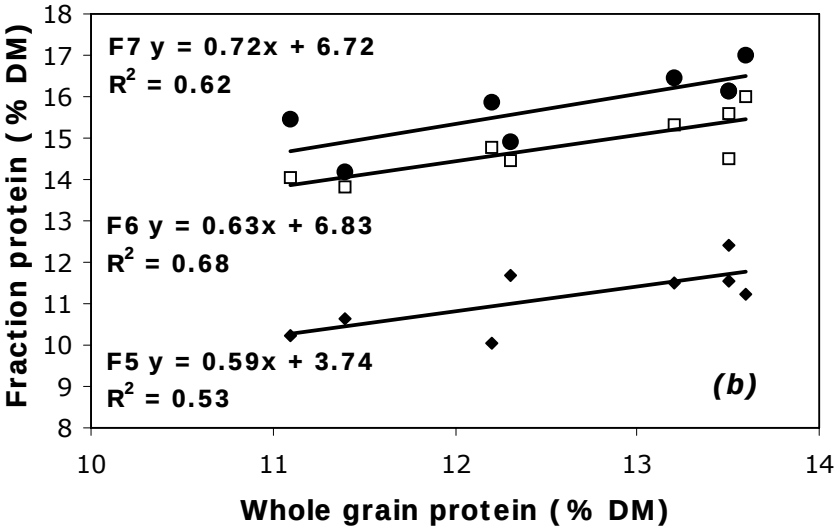


Figure 5. Distribution of protein in three milling fractions (F5, 4% pearlings ◆; F6, 8% pearlings □; F7, 12% pearlings ●), for either *(a)* all ten wheat studied or *(b)* eight hard wheats only (with data for two soft wheat samples removed).

5.5 Ash distributions in wheat milling fractions

Figure 6 shows the protein and ash contents for all the ten wheats and their milled fractions (data are also presented in the Annex, Table A2). For whole wheat samples (1), there was significant variation, with sample 104 (which was distinctive in having the highest length:width ratio) having the lowest ash content of 0.86%; this is perhaps surprising, as a high length:width ratio implies more bran, which should correspond to more ash. Sample 39, one of the soft wheats, had the highest ash content of 1.75%, probably reflecting that it had the smallest kernels and hence the highest surface area:volume ratio.

The flour fractions (both from pearled and unpearled wheat kernels) had the lowest ash contents, as expected. The bran fractions had high values of ash content, ranging from 3-5%, with the bran fractions from the unpearled wheat samples having higher ash contents than their pearled counterparts. The pearlings showed a trend whereby the higher the degree of pearling, the lower the ash content present, due to the greater incorporation of endosperm material; bran has a higher mineral content than endosperm. Samples 148 and 157 appeared to show an exception to this trend, which may reflect errors in the analysis, or may reflect variable mineral compositions of the bran layers in different wheats or variable interactions with the pearling process. Ash contents tended to be lower in flour produced from pearled kernels, compared with that from whole wheat, indicating less bran contamination in flour produced using pearling.

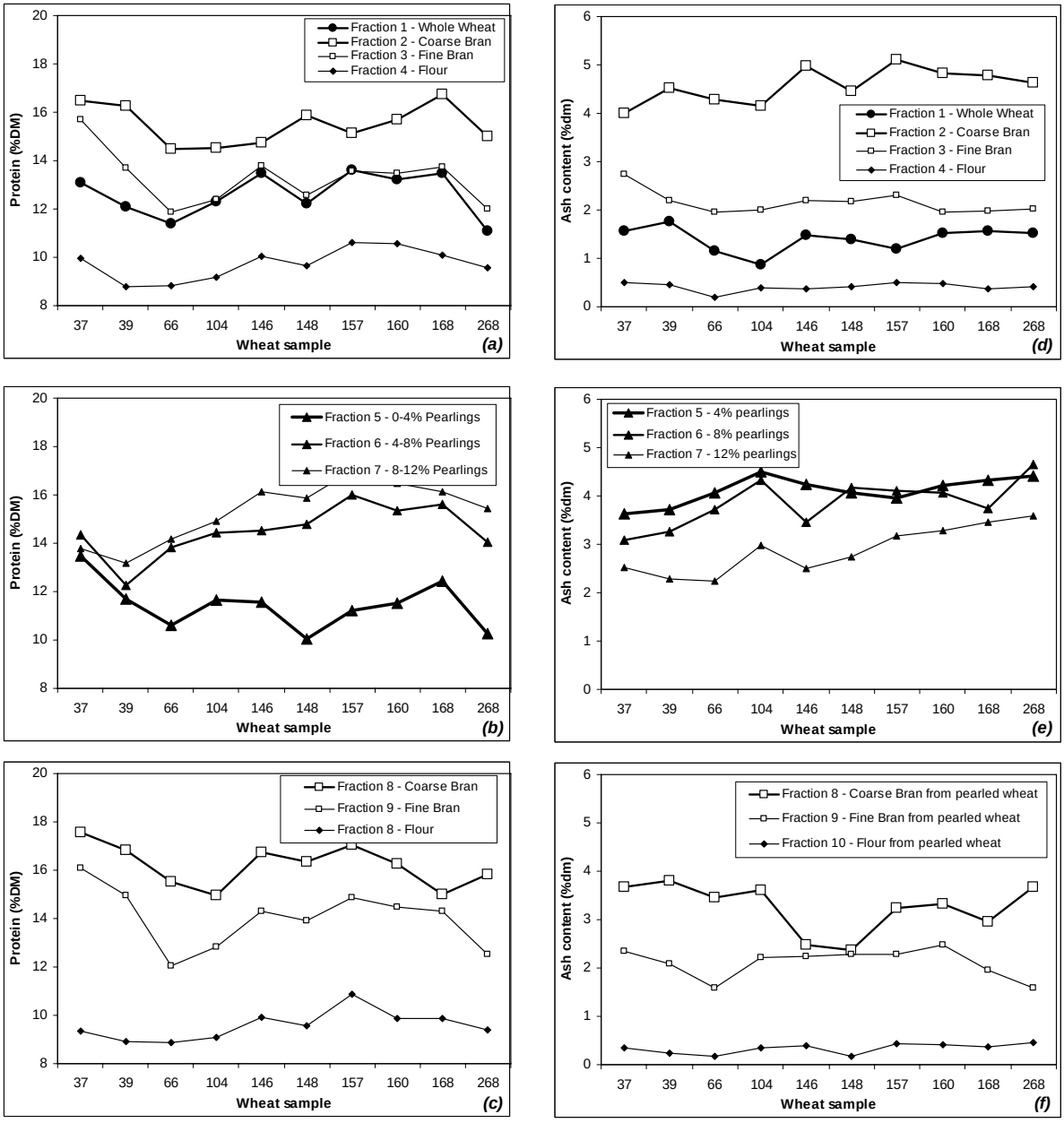


Figure 6. Protein (left) and Ash contents (right) for ten wheats and their milling and pearling fractions: Protein contents of (a) Whole Wheat (Fraction 1), Coarse Bran (Fraction 2), Fine Bran (Fraction 3) and Flour produced from Buhler milling of the Whole Wheat; (b) 4% (Fraction 5), 4-8% (Fraction 6) and 8-12% (Fraction 7) Pearlings; (c) Coarse Bran (Fraction 8), Fine Bran (Fraction 9) and Flour (Fraction 10) produced from Buhler milling of the Pearled Wheat; (d), (e) and (f), Ash contents of the fractions.

5.6 Economics of co-production of AX with bioethanol

Economic analyses were performed based on extraction of AX from the 4% pearlings, assuming 45% yield of the AX contained in the bran. The cost of AX depends on the yield of AX, but also on the yield (and hence cost) of ethanol, which was assumed (as a reasonable first approximation) to depend on the starch content of the whole wheat. In the analysis, the cost of ethanol was calculated as a function of starch content for each wheat, for the 'base-case' scenario of a bioethanol plant producing only ethanol and DDGS in which the cost of the wheat was assumed to be $200/tonne. The price of ethanol required in order to give a 17% Return on Investment (ROI) for this base case was calculated as a function of starch content. Then, the price at which the AX would need to be sold in order to give the same 17% Return on the additional investment required to extract AX was calculated, allowing for the different AX contents of the 4% pearling fractions from the different wheats. On this basis, the prices of ethanol and AX for each wheat were calculated, from which the most appropriate wheats for co-production of these two products were identified. The analysis was based on the assumption of a constant conversion of starch to ethanol for all wheats. Based on typical industrial yields as reported by Mortimer *et al*., (2004), a conversion of 79.9% of the maximum theoretical (stoichiometric) conversion was used, corresponding to 5.74 L of ethanol per 10 kg of starch.

Removal of 4% pearlings for AX extraction would reduce the viscosity of the fermentation broth. In principle, this would appear to allow less water to be used, reducing the subsequent cost of separating water, as well as reducing the size of tanks and the power requirement for mechanical agitation, and further enhancing the economics. In practice, however, the amount of water used in the fermentation is dictated by the maximum final ethanol concentration, not by viscosity issues; reducing the water level would increase the ethanol concentration beyond that tolerable by the yeast

Figure 7 shows the price of ethanol for the ten wheats, as a function of starch content. The slope of the fitted line indicates that for each 1% reduction in starch content, the price of the ethanol produced increases by $0.0055, which is 0.77% of the price based on wheat with a 71% starch content, i.e. each percentage point reduction in starch content increases the price of ethanol by a little under 0.8%, compared with this reference point, in part offset by the additional DDGS produced. It should be noted that the scatter in the data arises because of non-linear equipment costing functions

and because of the limited precision with which certain parameters can be set within the simulations. Table 8 lists the starch content of each wheat, the AX content of its corresponding 4% pearlings, the ethanol price, and the AX price.

Table 8. Starch contents of the ten wheats and AX contents of their 4% pearling fractions, and the corresponding ethanol and AX prices required to deliver a 17% ROI.

Sample*	37	39	66	104	146	148	157	160	168	268
Starch content (%DM)	68.9	68.2	70.8	68.9	70.5	69.9	67.9	71.0	68.7	67.4
AX content (%DM)	21.4	22.5	27.1	28.1	32.9	28.5	31.1	36.2	29.7	34.5
Ethanol price ($/kg)	0.726	0.729	0.714	0.722	0.715	0.719	0.729	0.712	0.724	0.732
AX price ($/kg)	11.38	11.08	9.36	9.14	7.77	8.86	8.19	7.04	8.58	7.33

* For explanation of sample codes see Table 4

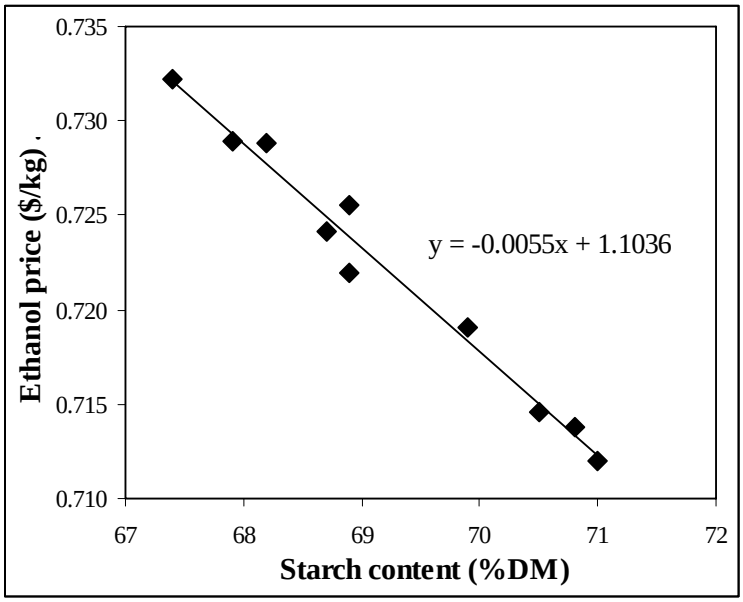


Figure 7. Price of ethanol as a function of starch content, for the 'base case' of production of ethanol and DDGS from wheat; price set to deliver a 17% Return on Investment.

Figure 8 plots the AX price against the ethanol price for each wheat sample. Clearly, some samples exhibit a positive combination of high starch content and high AX content in the pearlings, leading to a combination of low ethanol and AX prices; such wheats would be ideal for a biorefinery co-producing ethanol and AX. Wheats 160 (Hereward) and 146 (NSA02-1422) were in this category. Other wheats were better suited for either ethanol or AX production, but not both, while others such as 37 (Zebedee) and 39 (Glasgow) appeared, based solely on starch and AX contents but not allowing for variable yields (see below), to be suitable for neither.

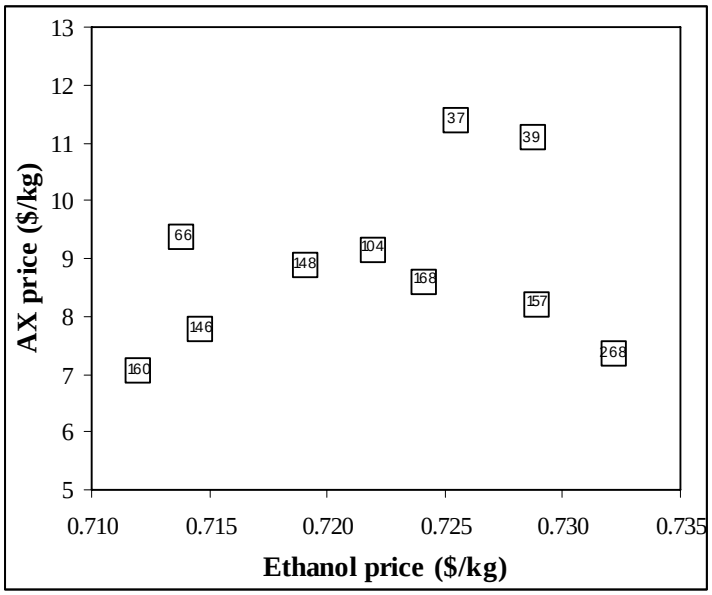


Figure 8. Prices of AX and ethanol for each of the ten wheats (for explanation of sample codes see Table 4).

5.7 Measured alcohol yield of selected flour fractions

Five samples showing a wide range of protein content (11.1-13.5% in the original grain) were selected for a final assessment of alcohol yield (AY), in order to compare the actual AY (Table 9) with that predicted from starch content alone in the simulation (see above; Table 8, Figures 7 and 8). Overall there was a very poor correlation between measured AY and starch content, and between AY and protein concentration (data not shown). In part this is due to the small dataset employed, and in part because some varieties tended to perform better than expected from their starch contents e.g. Zebedee and Glasgow (samples 37 and 39) which, for some unknown reason, both had high rates of conversion of starch to ethanol. The comparison of AY between the original grain and the flour of fraction 10 are shown in Figure 9. Fraction

10 was chosen for comparison with whole grain, as it represented the most 'refined' fraction available, *i.e.* that fraction most enriched in starch. For both milling fractions, the samples ranked in the same way with Zebedee (sample 37) always having the highest AY.

Note that the ethanol yields found by this method were on average higher than the 5.74 L/10 kg starch assumed for the economic analysis above (this latter value was based on the ethanol yields published by Mortimer *et al*., 2004). Multiplying the ethanol costs calculated above by the actual ethanol yield for each wheat, as reported in Table 9, could be used to give a revised estimate of the relative ethanol costs for each wheat (although these have not been calculated, because the AY data is only available for 5 of the 10 wheats). Moreover, the ethanol yields would still be estimates only, as they do not account for the reduction in DDGS production if ethanol yield is increased. Further work is required to refine this aspect of the simulations.

The removal of bran by pearling and milling concentrated the starch in the flour by 9.8% compared with that of the whole grain, and increased AY on average by 94 L/t across the five samples. This additional yield of ethanol, relative to starch content, implies a high rate of starch conversion, probably because of the smaller particle size of the flour, compared with that produced by hammer milling whole grain. The absolute yield is of course lower, as fermentable material was removed by the pearling and milling procedures; the flour yield for this process was on average 68.6% of the whole wheat.

Table 9. Starch content, alcohol yield and rate of conversion of starch to ethanol for five samples of wheat analysed either as original grain, or white flour after pearling.

Sample*	Original grain (Fraction 1)			Pearled flour (Fraction 10)		
	Starch (%)	AY (L/t)	EtOH/starch (L/10kg starch)	Starch (%)	AY (L/t)	EtOH/starch (L/10kg starch)
37	68.9	405	5.88	77.4	503	6.51
39	68.2	415	6.08	79.4	507	6.38
66	70.8	403	5.69	78.1	494	6.33
168	68.7	385	5.61	77.2	482	6.25
268	67.4	413	6.13	80.7	502	6.23
Average:	*68.8*	*404*	*5.88*	*78.6*	*498*	*6.34*

* For explanation of sample codes see Table 4

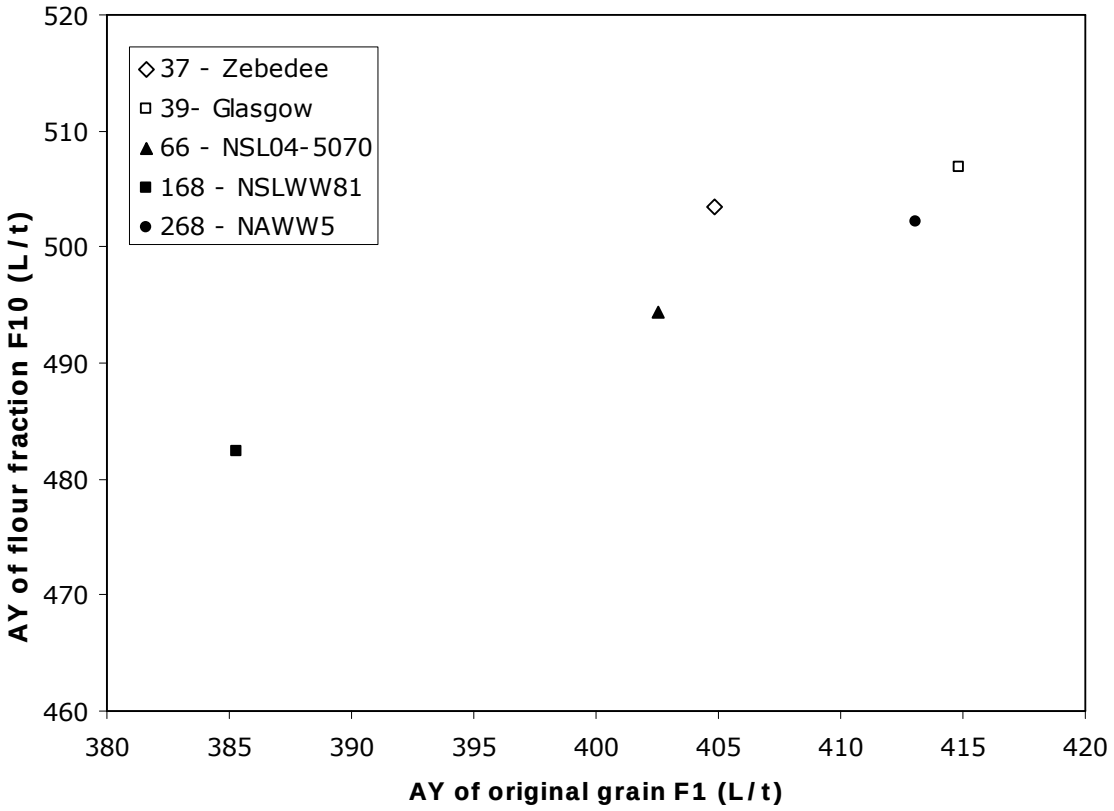


Figure 9. Alcohol yield (AY) of pearled flour plotted against AY of original grain (F1) determined in the laboratory, for five selected samples of wheat.

6 Discussion

6.1 Difference in grain composition between wheat samples

The samples studied were chosen to be broadly representative of UK wheats grown with standard agronomy at a single site, yet showing appreciable variation in grain size, shape and protein content. It was not possible within a study of this size to consider 'extremes' such as very low protein grain, which might be desirable for instance to maximise alcohol yield (Kindred *et al*., 2007). Rather, the aim was to focus on how grain size, shape and texture affect the way grain fractionates, and in turn to see how the distributions of a key component, in this case the AX fraction, vary between the different tissues within the grain, and how it is finally distributed upon milling and fractionation.

Prior to this study there were no published data on the AX content within UK wheats, an essential pre-requisite to optimising feedstocks for co-production of AX and ethanol. In the ten wheats analysed, AX contents ranged from 4.5% to 9.0% dry matter in the whole wheat, comparable and slightly higher than the 4.8-6.9% range reported for French wheats by Saulnier *et al*. (2007) and the 4.8-6.0% range for three durum wheats by Lempereur *et al*. (1997).

Of particular interest was the fact that the good distilling wheats Zebedee and Glasgow had the lowest AX contents. Anecdotal evidence would have predicted that these wheats had the lowest AX contents because they are preferred by the distilling industry partly due to their very low residue viscosities when processed, and wheat viscosity is to a large extent governed by the levels of soluble AX (Weightman *et al*., 2001).

6.2 Distribution of AX and protein in milling fractions

Milling the whole wheats gave Coarse Bran with AX contents in the range 11.5-22.5%, Fine Bran with AX contents of 6.4-11.4%, and Flour with much lower AX contents of around 1.6% (somewhat lower than the 2.2% average reported by Saulnier *et al*. (2007)). The 4% pearlings had substantially higher AX contents than any other

fraction, supporting the earlier suggestion that this fraction may be advantageous as the feedstock for AX extraction (Mustafa *et al*., 2007; Misailidis *et al*. 2009).

As well as the white flour (fractions F4 & F10) which contain principally water soluble AX, the 4% pearling fraction (F5) also yielded a material with a high A/X ratio, compared to the whole grain and to the other pearling fractions and brans. This observation appears to relate to the fact that the 4% pearlings are enriched in pericarp material, for which the AX is characterised by a high A/X ratio (Barron *et al*., 2007; Saulnier *et al*., 2007). This implies that its functionality might be unique, and considerably different from that of AX from elsewhere in the kernel. Further work is required to extract this form of AX in sufficient quantities to carry out functionality testing.

With increasing length of time, debranning broke deeper into the grain, and the AX content tended to reduce, giving marked differences in concentrations of AX between pearling fractions for the hard wheats. However, for the soft wheats (Zebedee and Glasgow) the differences in AX content between the pearling fractions was much less distinct (Figure 2). This suggested either that the AX was distributed differently in the soft wheats (i.e. that it was less concentrated in the outer bran layers in these wheats) or that debranning the soft wheats broke through more deeply into the inner bran layers and endosperm, diluting the AX in the resultant pearling fractions with starchy endosperm. On the basis solely of concentrating AX in a bran fraction, it would appear that the soft wheats are less useful for AX extraction, even though they tend to be good for bioethanol production.

Protein content varied differently between milling fractions, being concentrated in the inner bran layers – principally the aleurone cells which are rich in protein (Hemery *et al*., 2007). Since protein tends to co-extract with the AX under alkaline conditions, it causes problems during AX manufacture as the protein is a contaminant of the finished product (Weightman *et al*., 2002). This suggests that the outer bran layers (in the 4% pearling fraction) which would be lowest in protein, would again be most appropriate tissues for AX extraction.

The results in Figure 4 clearly show the distribution and characteristics of AX in different milling fractions, and would help to identify and produce fractions of high AX content combined with appropriate AX functionality. In particular, the 4% pearling

fraction appears promising, in respect of having high AX content and high A/X ratio, although the functionality of this AX relative to that from elsewhere in the kernel would need to be investigated. The results also suggest significant differences in the compositional and structural arrangements of the kernels from different wheat varieties, and hence differences in their responses to processing. Thus Sample 160, for example, although having only a moderate AX content in the whole wheat, appeared to give pearlings that were particularly enriched in AX. It is necessary to acknowledge the experimental error inherent both in the processing of the wheats and in the AX analysis, such that precise conclusions cannot be drawn about specific wheats, beyond the general conclusion that wheats vary significantly in their structure and composition and in the way these interact with fractionation processes, and that these differences and interactions would play key roles in the effective processing of wheats and the economics of a biorefinery and should therefore be investigated in more detail.

6.3 Consequences of distribution of AX between samples and milling fractions on economics of production

The economics of a biorefinery which fractionates wheat into a bran-rich stream, from which AX is extracted, and a starch-rich stream which is fermented into ethanol, depends on the starch and AX contents of the wheats and on the effectiveness of AX extraction in terms of yield, purity and functionality. In the current work, the effects of compositional variation represented by the ten wheats were illustrated by calculating, for each wheat, the cost of producing ethanol and an extracted AX product, based on the starch content of each wheat and the AX content of the 4% pearlings.

Misailidis *et al*. (2009) assessed the economics of ethanol and AX co-production from wheat in an integrated process, using two options for extraction of AX from bran: extraction from 'ordinary' bran particles obtained by simple hammer milling and sieving of the wheat, and extraction from bran particles obtained by pearling the wheat using a debranner. They concluded that both approaches could yield AX at a price sufficiently low to support the creation of a market, but that the latter approach, using pearling, was more expensive due to the additional capital and operating costs of the debranner and the additional costs of handling fine bran powder. However, although they suggested that the yield of AX from pearlings might be greater than

that from ordinary bran, they did not allow for this in their simulations, instead assuming the same yield of AX from both sources of bran.

The cost of AX (£/kg) produced is inversely proportional to the absolute amount of AX extracted, so the greater yield from pearlings could offset the higher costs calculated previously, thereby justifying investment in a debranner (in contrast to the conclusions of Misailidis *et al*., 2009). From the current work, it is now evident that the 4% pearlings are consistently more enriched in AX, so it is appropriate to allow for this in the simulations. From Figure 4, the AX content in the 4% pearlings is on average over 60% greater than in the Coarse Bran obtained from milling whole wheat. If this is translated into 60% more AX extracted from the same quantity of bran (i.e. if the AX is equally extractable from both sources), then this lowers the cost per kg by 37% and would make AX extracted from pearlings around 25% cheaper than AX extracted from Coarse Bran. Again, it is emphasised that the functionality of the AX from these different bran sources would need to be evaluated.

Thus, in the current work, economic analyses were performed based on extraction of AX from the 4% pearlings, assuming 45% yield of the AX contained in the bran. The cost of AX depends on the yield of AX, but also on the yield (and hence cost) of ethanol, which depends on the starch content of the whole wheat. In the analysis, the cost of ethanol was calculated as a function of starch content for each wheat, rather than being based on measured alcohol yield. The 'base-case' scenario was for a bioethanol plant producing only ethanol and DDGS in which the cost of the wheat was assumed to be 200 $/tonne. The price of ethanol required in order to give a 17% Return on Investment (ROI) for this base case was calculated as a function of starch content. Then, the price at which the AX would need to be sold in order to give the same 17% Return on the additional investment required to extract AX was calculated, allowing for the different AX contents of the 4% pearling fractions from the different wheats. On this basis, the prices of ethanol and AX for each wheat were calculated, from which the most appropriate wheats for co-production of these two products were identified.

The previous simulations of Misailidis *et al*. (2009) assumed a starch content of 71%, resulting in an ethanol price of 0.71 US$/kg. A starch content of 71% is the largest in the wheats used in the current work, and gives this same result (to two significant figures). The slope of the fitted line indicates that for each 1% reduction in starch

content, the price of the ethanol produced increases by $0.0055, which is 0.77% of the price based on wheat with a 71% starch content, i.e. each percentage point reduction in starch content increases the price of ethanol by a little under 0.8%, compared with this reference point, in part offset by the additional DDGS produced.

Clearly, some wheats exhibit a positive combination of high starch content and high AX content in the pearlings, leading to a combination of low ethanol and AX prices; such wheats would be ideal for a biorefinery co-producing ethanol and AX. Wheats 160 and 146 were in this category. Other wheats were better suited for either ethanol or AX production, but not both, while others such as 37 and 39 were suitable for neither. It would of course be conceivable that a biorefinery might take in several sources of wheats, some high in starch, others high in AX, and extract AX only from pearlings obtained from the latter, thereby enhancing their degrees of freedom for optimisation of the feedstock even further.

6.4 Differences between simulated and observed alcohol yields

The economic analysis above was based on a predicted ethanol yield based on the starch content of the wheat, and is consistent with the approach taken by Misailidis *et al*. (2009). As a final step in the current project, the AY of five selected samples were measured in the laboratory, using a protocol which would be applicable to a biofuel plant. The method uses only commercial enzymes and urea as additives (rather than barley malt as used by the distillers; Agu *et al*., 2006). The average AY of the grain (Fraction 1) was lower at 404 L/t than that predicted from protein content alone based on the barley malt method – the average AY would be 430 L/t predicted using equation: AY=519-(7.31 x protein) from Smith *et al*. (2006). The average AY of the flour fractions (Fraction 10) was higher at 498 L/t, in agreement with the higher starch and lower protein and bran contents, but importantly the different wheat samples ranked in the same way whichever fraction was analysed. Moreover, the rate of starch conversion of the flour fractions were similar to those reported for Riband and Option by Kindred *et al*. (2007), and for Zebedee in particular, measured AY was close to the theoretical value of 6.6 L/10 kg starch (Smith *et al*., 2006). This overall pattern of results and comparable rates of conversion to theoretical value give us further confidence that there is appreciable variation in alcohol yield which cannot be predicted from starch content alone. Thus, there will be an inherent error in the

economic simulation above when comparing different varieties of wheat based on their starch content alone. This indicates that more research is required into the sources of variation in alcohol yield particularly between varieties before definitive statements are made regarding their suitability for fractionation.

6.5 Implications for future studies in wheat biorefining

6.5.1 Maximising arabinoxylan content

The range of AX contents seen in the present study appeared larger than those reported by Saulnier *et al*. (2007) for French wheats. Even so, it may be possible to consider wheats with a still wider range of AX contents in future studies. For example, AX content is known to be influenced genetically by the 1BL/1RS translocation (Martinant *et al*., 1998), but none of the 10 wheats studied here possessed the translocation (the aim of the study being to focus on grain size and shape effects). Further data on AX and NSP contents in UK wheats are being measured in HGCA-funded project 3314 'Maximising bioethanol processing yield of UK wheat: Effects of non starch polysaccharides in grain' which will guide future work.

6.5.2 Minimising protein content

Protein content is a major determinant of wheat quality, particularly for distilling or biofuel production, where low grain protein is desirable (Smith *et al*., 2006). Broadly speaking whenever a wide range of wheat samples are analysed, protein is seen to be inversely related to starch content. This is most clearly seen where samples are taken from environments where N nutrition varies markedly, for example with samples from response trials with N fertiliser (Kindred *et al*., 2007). However, in the present study the wheats were taken from a single environment, where the major source of variation was genetic. In this situation with all the wheats fertilised at or near the economic optimum N rate, there was no relationship between protein and starch content (Figure 10).

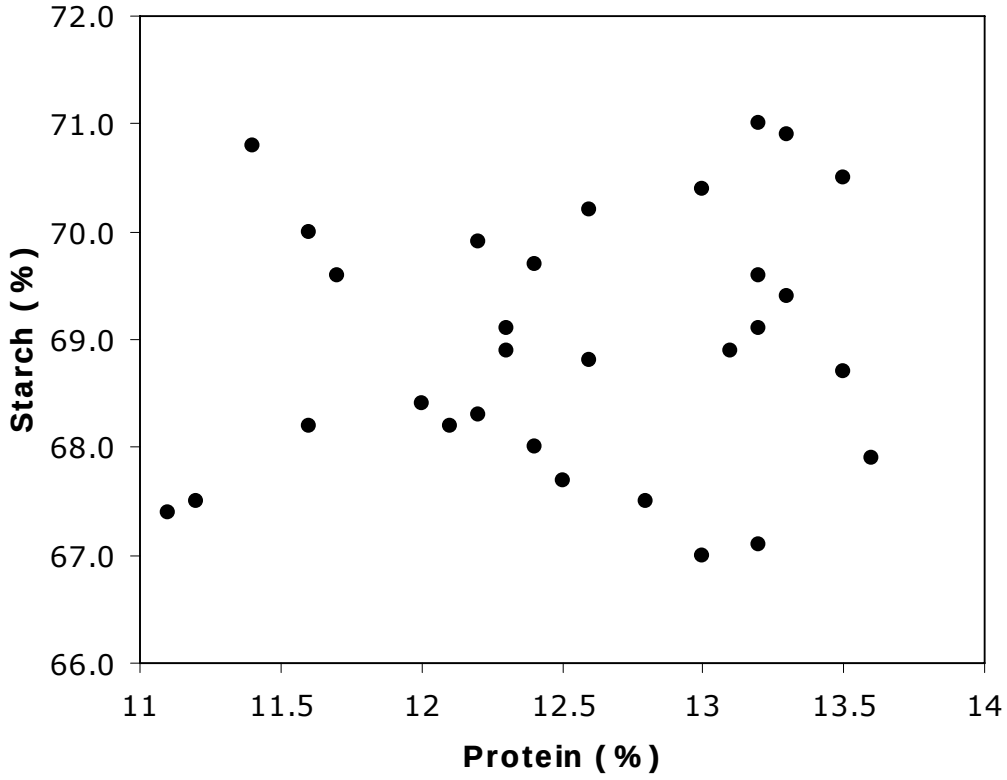


Figure 10. Relationship between starch and protein content of 30 samples of wheat grain from a NL trial at Maldon in Essex, UK in 2006.

It is important to consider further the relationship between starch and protein contents shown in Figure 10, because of its significance for planning future studies. In choosing samples for studies concerned with maximising yield of bioethanol, or AX (or any other aspect of wheat grain biorefining) there needs to be clarity over the aims of the study. In the present research, the aim was to gain insights regarding the effects of grain size and shape on their performance on fractionation and the potential as feedstocks for biorefining. However, another study might aim to look at the effect of grains varying widely in starch content for example, on potential for biorefining. In this case the grains should be of varying chemical composition, but care must be exercised to avoid simply taking a wide sample of grains from different sites (or of different varieties grown at different sites). It is clear that in such instances, the effects of genotype on starch, or protein, will be confounded with the effects of environment on these grain components. When attempting to stretch the range of variation in grain chemical composition, by far the best approach appears to be to take varieties from well-defined N response studies (as was the case with Kindred *et al*., 2007).

Smith *et al*. (2006) pointed out the composition of wheat in its entirety is poorly understood. Rarely is the composition of wheat measured where the fractions can be totalled to account for 100% of the dry matter. Therefore if starch, protein and AX are high in some samples (e.g. Hereward sample 160 in the present study), we still do not know which of the other components are low (lignin, cellulose, free sugars, lipid or ash). In the present study, there was no relationship between 'starch+AX' and protein in the 10 wheats studied. More work is needed to understand variation in the minor constituents before the ideal wheats for biorefining (in terms of combining protein, starch and AX at optimum levels) in wheat can be predicted.

6.5.3 Other grain fractions as sources of AX and alternative extraction methods

This work, and the previous simulation study reported by Mustafa *et al*. (2007), considered the extraction of AX from bran or pearling fractions using a protocol described by Hollmann and Lindhauer (2005). However it should be noted that there are other approaches to AX extraction which could be considered. For example Broekaert *et al*. (2008) have produced a patent for extracting AX oligosaccharides from the DDGS, rather than from a dry-milled fraction or component otherwise unmodified chemically, or through fermentation. We would not expect DDGS to yield functional polysaccharides of high molecular weight for use as thickeners (the context for this study) because degradation of the polysaccharides occurs during the mashing and fermentation process (due to the activity of the xylanase enzymes added to reduce viscosity). The end-use of Broekaert *et al*. (2008) is therefore somewhat different and specific – they are aiming to produce small chain oligosaccharides for specific nutritional uses. However, the fact that there are other options for processing wheat, or adding value to the DDGS fraction in particular in a biorefinery, should be noted.

6.6 Implications for breeding

Clearly, until a market develops for AX and other components of the wheat grain through biorefining, no recommendations can be made to breeders regarding appropriate targets. However, some themes emerge from the results of the present study.

6.6.1 Starch

Starch content is critical to maximising alcohol yield, and reducing the price per unit of ethanol produced by a distillery. Starch content is governed by different genes to those controlling protein synthesis, and so there is no reason to assume that these two traits are linked genetically. However, we have demonstrated in the present study that there is variation in alcohol yield per tonne of grain which is not explained by starch content alone, a conclusion which is being drawn from other parallel studies (Kindred *et al*., 2007, 2008). This was demonstrated in the present study where the soft wheats Zebedee and Glasgow both had alcohol yields higher than would have been predicted from their starch contents alone. Further work is required to understand the factors influencing rate of conversion of starch in these good distilling types, and identify these as traits for selection, with appropriate rapid screens.

6.6.2 Protein

High protein is generally undesirable in grain for bioethanol production and while breeders have been generally successful in selecting for high protein grain for breadmaking, there has been little effort on producing low protein grain with the exception of the Green Grain LINK project (HGCA project RD-2979). Breeding needs to be carried out in low fertility (i.e. low available N) situations in order to select for high yield with low grain protein. However, this study shows that selecting for total grain protein as a single trait, is perhaps simplistic: Much of the protein in the grain is stored in the aleurone layer, which becomes part of the bran fraction on milling and as these results show, the protein content of the flour is appreciably lower than that of the whole grain. In part, breeders have produced high quality breadmaking wheats through selection for specific high molecular weight gluten sub units in the endosperm. There has been very little focus on understanding the variation in the proteins in the aleurone layer (principally enzymes rather than storage proteins) partly because there is no quick and easy way of measuring them without carrying out milling studies. Milling performance is generally only assessed in the latter stages of a breeding programme, when the number of lines has been reduced through repeated selections for agronomic traits. However, reducing the amount of protein in the aleurone layer could be a very valuable trait in the context of a feedstock for AX production because of the problems caused by contamination of the AX with protein during extraction (Weightman *et al*., 2002).

6.6.3 Arabinoxylans

AX content is concentrated in the outer bran layers, and there is some evidence from the present study that different wheats fractionate differently and may produce pearlings with different concentrations of AX. However, as discussed earlier (see Section 6.2) it has to be concluded therefore that there are insufficient data on the variation of arabinoxylan concentration between different wheats and their milling fractions, on which to base decisions regarding the potential for genetic improvement. Further work is required in this area. It is possible that variation in bran thickness exists in wheat varieties, and this is being considered through ongoing work (Misailidis, PhD studentship sponsored by HGCA, RD-2007-3357 "Understanding and predicting the determination of alcohol yield from wheat"). Bran thickness is difficult to quantify on a routine basis, and very few previous studies of bran thickness have been reported; there is therefore practically no understanding of the genetic or environmental factors influencing bran thickness. However, bran thickness clearly must influence kernel composition. Bran thickness could be measured using image analysis techniques; however, modern versions of the SKCS allow Crush Response Profiles to be determined, which it is believed may relate to bran thickness. This is being investigated within the PhD work of Misailidis with a view to developing a practical basis for routine assessment of bran thickness in wheats in order to relate this to composition, fractionation and ultimately alcohol yield.

6.7 Conclusions

The work described here is the first to simulate the economics of co-production of AX with ethanol, based on measured starch and AX contents of UK wheats, and builds on the earlier work of Misailidis *et al*. (2009). The following conclusions are drawn from the work:

1. The present study has confirmed that the AX content of pearlings is higher than that of coarse bran, and that using pearlings can reduce the price of AX produced, implying that capital investment in debranning equipment may be a cost effective approach to integrating AX and bioethanol production.

2. Further practical work to extract and purify AX from milling fractions at pilot scale and to test functionality of the isolated AX in food products is required to fully test the concept.

3. The removal of AX from the co-product stream will result in differences in composition of the DDGS and at present this cannot be predicted accurately, because there are so little data on the variation in composition of wheat grain in terms of the non-protein components (starch, NSP, lignin, lipid, ash),.

4. Future work will be needed to take into account environmental as well as genetic variation in AX and starch content in wheat grain.

5. Simulation models will need better predictors of alcohol yield for different wheat varieties at a given starch content, otherwise false conclusions may be drawn.

6. Wheats for distilling may well have higher starch contents, and hence ethanol yields, than those studied here, therefore low protein grain should also be studied in the context of integrating AX and bioethanol production.

7. The implications of the distributions of grain components, particularly protein between bran/pearlings and endosperm/flour, need to be considered further in terms of breeding approaches to low protein grain, and in particular whether screens could be developed which specifically focus on reducing protein storage in the bran layer.

8. This study supports the earlier work of Misailidis *et al*. (2009) that AX can be produced economically at prices which are competitive in the marketplace, and has extended the earlier work to include actual concentrations of AX in the pearling fractions of UK wheats.

9. The study indicates that there is considerable scope for optimising the choice of wheat variety which can lead to reductions in the prices of both ethanol and AX produced in an integrated biorefinery.

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Appendix

Table A1. Protein contents of milling fractions (F1-10) of ten wheats.

Sample*	Protein (%)					
	Original grain	Unpearled wheat fractions*				
	F1	F2	F3	F4		
37	13.1	16.5	15.7	10.0		
39	12.1	16.2	13.7	8.8		
66	11.4	14.5	11.9	8.8		
104	12.3	14.5	12.4	9.2		
146	13.5	14.7	13.8	10.1		
148	12.2	15.9	12.6	9.6		
157	13.6	15.1	13.6	10.6		
160	13.2	15.7	13.5	10.6		
168	13.5	16.8	13.7	10.1		
268	11.1	15.0	12.0	9.6		
Average	*12.6*	*15.5*	*13.3*	*9.7*		
		Pearled wheat fractions*				
	F5	F6	F7	F8	F9	F10
37	13.5	14.3	13.8	17.6	16.1	9.4
39	11.7	12.3	13.2	16.8	14.9	8.9
66	10.6	13.8	14.2	15.5	12.0	8.9
104	11.7	14.4	14.9	15.0	12.8	9.1
146	11.6	14.5	16.1	16.8	14.3	9.9
148	10.1	14.8	15.9	16.4	13.9	9.6
157	11.2	16.0	17.0	17.0	14.9	10.9
160	11.5	15.3	16.5	16.3	14.5	9.8
168	12.4	15.6	16.1	15.0	14.3	9.9
268	10.2	14.1	15.4	15.8	12.5	9.4
Average	*11.4*	*14.5*	*15.3*	*16.2*	*14.0*	*9.6*

* For explanation of wheat sample codes see Tables 2 and 4

Table A2. Ash contents of milling fractions (F1-10) of ten wheats.

Sample*	Ash (%)					
	Original grain	Unpearled wheat fractions*				
	F1	F2	F3	F4		
37	1.57	4.00	2.74	0.51		
39	1.75	4.51	2.19	0.45		
66	1.16	4.28	1.95	0.19		
104	0.87	4.14	2.01	0.39		
146	1.47	4.98	2.19	0.38		
148	1.40	4.45	2.18	0.42		
157	1.21	5.11	2.30	0.50		
160	1.52	4.83	1.96	0.47		
168	1.56	4.78	1.98	0.38		
268	1.52	4.63	2.02	0.41		
Average	*1.40*	*4.57*	*2.15*	*0.41*		
	Pearled wheat fractions*					
	F5	F6	F7	F8	F9	F10
37	3.62	3.09	2.53	3.68	2.36	0.35
39	3.71	3.26	2.28	3.81	2.09	0.24
66	4.07	3.71	2.23	3.46	1.58	0.18
104	4.49	4.32	2.98	3.60	2.21	0.35
146	4.23	3.45	2.49	2.48	2.23	0.39
148	4.07	4.17	2.73	2.36	2.28	0.17
157	3.95	4.10	3.17	3.25	2.29	0.44
160	4.22	4.07	3.28	3.33	2.47	0.41
168	4.33	3.75	3.45	2.95	1.95	0.38
268	4.42	4.64	3.58	3.68	1.59	0.45
Average	*4.11*	*3.86*	*2.87*	*3.26*	*2.10*	*0.34*

* For explanation of wheat sample codes see Tables 2 and 4

Table A3. AX contents of milling fractions (F1-10) of ten wheats.

Sample*	AX (%)					
	Original grain	Unpearled wheat fractions*				
	F1	F2	F3	F4		
37	4.52	17.55	10.29	1.51		
39	4.73	11.56	6.40	1.28		
66	5.49	22.55	9.01	1.64		
104	6.36	16.85	10.06	1.82		
146	5.02	18.37	9.38	1.50		
148	7.08	16.61	7.45	1.40		
157	5.41	19.43	11.36	1.66		
160	6.51	17.83	9.25	1.33		
168	6.57	20.54	10.25	1.84		
268	9.03	19.43	9.55	1.76		
Average	*6.07*	*18.07*	*9.30*	*1.57*		
	Pearled wheat fractions*					
	F5	F6	F7	F8	F9	F10
37	21.40	12.96	7.93	13.25	8.93	1.37
39	22.46	13.30	8.00	15.73	6.87	1.40
66	27.14	16.35	11.17	14.34	7.82	1.59
104	28.10	16.14	12.65	16.66	7.99	1.65
146	32.94	17.52	11.19	14.52	9.01	1.53
148	28.46	19.77	13.46	13.40	8.14	1.55
157	31.09	19.32	12.81	16.45	11.76	1.82
160	36.24	17.67	10.62	15.36	8.53	1.56
168	29.67	17.47	12.02	17.97	13.12	1.88
268	34.52	15.47	10.39	11.86	11.83	1.78
Average	*29.20*	*16.60*	*11.02*	*14.96*	*9.40*	*1.61*

* For explanation of wheat sample codes see Tables 2 and 4

Table A4. A/X ratios of milling fractions (F1-10) of ten wheats.

Sample*	A/X ratio					
	Original grain	Unpearled wheat fractions*				
	F1	F2	F3	F4		
37	0.560	0.468	0.596	0.703		
39	0.625	0.542	0.635	0.780		
66	0.571	0.565	0.601	0.687		
104	0.588	0.539	0.584	0.705		
146	0.603	0.581	0.624	0.752		
148	0.613	0.574	0.670	0.763		
157	0.587	0.587	0.612	0.767		
160	0.584	0.566	0.644	0.755		
168	0.588	0.549	0.604	0.675		
268	0.613	0.548	0.610	0.681		
Average	*0.593*	*0.552*	*0.618*	*0.727*		
	Pearled wheat fractions*					
	F5	F6	F7	F8	F9	F10
37	0.647	0.546	0.497	0.443	0.560	0.717
39	0.661	0.570	0.520	0.421	0.565	0.597
66	0.714	0.607	0.571	0.495	0.561	0.709
104	0.644	0.567	0.509	0.448	0.547	0.737
146	0.695	0.633	0.572	0.506	0.592	0.769
148	0.722	0.660	0.551	0.475	0.574	0.757
157	0.728	0.617	0.552	0.479	0.571	0.764
160	0.676	0.611	0.553	0.490	0.585	0.748
168	0.658	0.584	0.525	0.456	0.535	0.680
268	0.696	0.593	0.522	0.493	0.547	0.702
Average	*0.684*	*0.599*	*0.537*	*0.471*	*0.564*	*0.718*

* For explanation of wheat sample codes see Tables 2 and 4

Table A5. Yields of milling fractions (F1-10) of ten wheats.

Sample*	Yield (5 of whole grain)					
	Original grain	Unpearled wheat fractions*				
	F1	F2	F3	F4		
37	100	19.9	6.8	73.3		
39	100	21.9	8.4	69.7		
66	100	17.3	10.8	71.9		
104	100	16.3	12.6	71.1		
146	100	18.4	8.9	72.7		
148	100	19.2	11.9	68.9		
157	100	17.3	8.0	74.7		
160	100	18.4	10.0	71.7		
168	100	17.8	11.4	70.8		
268	100	17.3	11.5	71.2		
Average	*100*	*18.4*	*10.0*	*71.6*		
		Pearled wheat fractions*				
	F5	F6	F7	F8	F9	F10
37	4.1	4.5	4.1	10.7	8.3	68.2
39	3.2	3.7	5.5	13.4	8.4	65.8
66	4.2	4.4	4.2	7.8	12.3	67.1
104	4.0	3.8	4.1	8.0	11.9	68.2
146	4.0	4.2	4.0	9.3	9.1	69.3
148	4.0	4.1	3.9	10.0	10.0	67.9
157	3.4	4.1	4.4	9.5	7.5	71.2
160	4.0	4.5	3.6	8.9	9.3	69.6
168	4.3	3.8	4.1	7.6	8.9	71.3
268	4.0	4.1	4.0	8.9	12.1	66.9
Average	*3.9*	*4.1*	*4.2*	*9.4*	*9.8*	*68.6*

* For explanation of wheat sample codes see Tables 2 and 4