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Effects of β-glucan fractions from barley on structure, texture, sensory characteristics and nutritional value of processed cereal foods

by

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Contents

Abstract

Much attention has been given recently to improving the nutritional value of foods. Notably, cereal foods have been negatively affected with the popularity of "nutritional" diets, such as the Atkins diet. These diets have linked carbohydrate consumption to obesity and as such, require the individual to reduce dietary intake of carbohydrates in order to optimise weight loss. However, grains are a good source of complex carbohydrates, of which β-glucans are one. These complex carbohydrates have been linked to a reduction in coronary heart disease, reduction in cholesterol and a possible control of blood glucose levels.

A major technical problem exists in both extracting these β-glucans from cereal materials, and applying them into cereal food systems. The current project examined the potential health claims associated with β-glucan inclusion into food systems and reviewed the available research regarding such issues, as well as examining the economic possibilities of β-glucan extraction and inclusion. The review clearly illustrated substantial data indicating the potential nutritional benefit of β-glucans in human foods. It also indicated that the potential bottleneck of such a use of these ingredients was in developing reliable and relatively inexpensive extraction processes.

Different extraction processes were analysed in terms of their effects on β-glucan yield, processing characteristics and cost effectiveness. This showed that the physical characteristics (potential processing behaviours) of β-glucans were dependent on the extraction process used. Although water extracted β-glucans provided a cost-effective way of extraction, combination of amylase purification of the isolates (to remove excess starch) produced a higher β-glucan return in terms of yield, and also the physical chemical properties of these isolates were more consistent and useful in terms of application to the food industry. Barley β-glucan fractions from water extracted barley flours were used in the production of bread samples. The results indicate that high levels of β-glucan (above 5%) may have negative effects on processability (loaf volume and crumb structure), which would again have negative effects on the sensorial properties of the breads.

However lower level inclusions had no significant effects in bread structure. From a nutritional point of view, inclusion of β-glucan fractions had a beneficial effect in reducing the rate of starch breakdown during in vitro digestion, and hence in reducing sugar release. This appeared to be because the β-glucan may inhibit (or reduce) the degree of starch gelatinisation. Such a reduction in reducing sugar release shows a great potential for the use of such additives in improving the nutritional quality of cereal foods. In a separate experiment, a commercially available form of β-glucan (Glucogel) was obtained from the supplier and similar processing and nutritional analysis performed. Similar results were obtained for this commercial sample, indicating that commercial processing of such glucan fractions is a possibility.

Further research on pasta products showed a similar trend with regards to the inclusion of purified β-glucans being of nutritional benefit to the potential consumer. Research was also conducted on the possibility of increasing the β-glucan composition of breads and pastas by using barley flour as opposed to β-glucan extracts. However the research illustrated that the use of barley flour, at high enough levels to obtain a significant amount of β-glucan in the food product, had major negative effects on bread and pasta quality, As such, the use of β-glucan extracts are the most process friendly way to incorporate β-glucan material into cereal foods.

The results illustrate the possible use of β-glucan extracts from UK-grown barley, in the human food system. Barley β-glucan inclusions can significantly improve the nutritional quality of breads and pasta without significantly affecting the textural, physical and sensory characteristics of such foods. As such there is a possible novel ingredient market which could be explored for the use of high β-glucan barley varieties in being a source of such food ingredients.

1:- **Summary**

Barley, *Hordeum vulgaris,* is an ancient crop plant, and is also one of the world's most cultivated cereal crops. World production in 2000/2003 has been evaluated at approximately 134 million metric tonnes. Europe is one of the leading barley producers (51.659Mt) followed by the Former Soviet Union (25.013Mt), and Canada (13.172Mt). In the UK barley has a particular importance as being the second most important crop, with approximately 6.2 million metric tonnes produced in 2002 (HGCA 2003).

UK barley is principally used as feed for animals, grain material for malting and brewing in the manufacture of beer and whisky, and a minor role in human food stuffs. Research into barley quality has traditionally centered around the role of endosperm components on malting potential, and hence brewing quality (Bathgate et al 1974; Bamforth et al 1979; Henry & Blackeney 1986; Palmer 1987; Brennan et al, 1996a, 1997, 1999). As such there has been relatively little research concentrating on the potential use of barley (or barley fractions) as an ingredient in processed foods.

Recent research attention however, has focussed on the potential nutritional use of β-glucan as a functional food ingredient. This interest has developed from the role of soluble and insoluble fibres in human nutrition. The influence of β-glucans in cereal food products has been linked to malting potential and brewing yield in barley, regulating the rate of endosperm modification (Brennan et al, 1999). Actual levels of β-glucan can vary dramatically between barley varieties. However the normal range is between 1-6%. Although β-glucans are relatively small components of the barley grain they have a disproportionate impact on the technology of barley utilisation and on the nutritional value of the grain.

Barley β-glucan can be regarded as a non-starch polysaccharide, which is a linear molecule with approximately 30% β-(1→3) and 70% β-(1→4) linkages randomly dispersed and are associated with firmly linked peptide sequences in the barley endosperm cell wall (Fleming & Kawakami 1977; Forrest & Wainwright 1977). Differences have been observed in cell wall composition between the starchy endosperm and the aleurone (Bacic & Stone 1981a, b; Wood et al 1983; Woodward et al 1983, 1988).

The current research project focussed on two main areas:

The evaluation of possible extraction processes for β-glucans from barley, and the effects of these process conditions on the physical and chemical characteristics of recovered β-glucan glucan rich fractions (BBG fractions).

The potential use of these fractions in cereal foods, with regard to the effect these inclusions have on the structure, texture and nutritional characteristic of the food.

1.1:- Evaluation of potential extraction processes

Extraction procedures for β-glucans (from both oats and also barley) have been investigated for the last 30 years or more (Fincher 1975; Woodward et al 1983; Klopfenstein & Hoseney 1987; Woodward et al 1988; Bhatty 1993, 1995; Temelli 1997; Burkus & Temelli 1998). A simplified extraction technique from cereal grains involves three steps: deactivation of native enzymes, isolation of the β-glucan, and then recovery of the β-glucan. Generally, enzymes such as β-glucanases, need to be deactivated to prevent enzymic degradation of the isolated glucan fraction.

The research of this project investigated the potential use of different protocols in obtaining homogeneous and process capable glucan extracts. The research was also aimed to examine the effect of extraction conditions on the process characteristics of such glucan rich extracts.

BBG fibre fractions were prepared from barley flour using the method of Wood *et al* (1978), with some modifications as used by Temelli (1997). This generalised extraction process is illustrated in Figure 1.

Extraction: 1:10 flour-to-water ratio with vigorous agitation (55 °C, pH 7, 0.5 h)

↓

Centrifuge 15 min, (15,000xg, 4 °C)

↓

Supernatant to pH 4.5 (centrifuge 20 min, 21,000xg, 4 °C)

↓

99% EtOH to 50% concentration (12 h,4 °C)

↓

Recover precipitate (centrifuge, 10 min, 3000xg)

↓

Homogenize with 99.9% EtOH, filter, and wash (99.9% EtOH)

↓

Dry under constant flow 1.5 h

Fig water, pH

Figure 1. Generalized BBG fibre extraction procedure (Wood *et al* 1978; Temelli 1997)

Five extraction treatments were used,

(a) a simple water extraction of soluble BBG material (water fraction)
(b) barley flours refluxed once with ethanol (99.9%) for 10 min (refluxed fraction)
(c) BBG fractions from refluxed flours (as of b) followed by treatment with thermostable alpha amylase (Termamyl, National Center for Biotechnology Education, Reading, UK) at levels of 1 mL enzyme to 100 mL extraction buffer for 1 h at 98 °C to eliminate starch impurities (purified fractions)
(d) barley flour extraction at pH 10 achieved by the addition of a few mL of 1 M NaOH (alkali fractions)
(e) untreated barley flour extracts from flour which was boiled for 1 h immediately after total solids separation (boiled fractions).

All treatments were performed at 55 °C and pH 7 with the exception of treatment d, where pH 10 was used.

Moisture, starch, protein, and β-glucan contents of whole flour and BBG fibre fractions were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000). Total starch, total dietary fibre, and β-glucan were determined using the total starch assay kit (Approved Method 76.13; AACC 2000), total dietary fibre assay kit (Approved Method 32-07; AACC 2000), and β-glucan enzymatic assay kit (Approved Method 32-23; AACC 2000), respectively. All assay kits were supplied by Megazyme^{TM} International Ireland Ltd, Wicklow, Ireland. Nitrogen was determined using a nitrogen analyzer (Model FP-2000; Leco Instruments Ltd, St Joseph, MI) and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis.

Water retention capacity (WRC) of BBG fibre fractions was determined by the procedure of Robertson *et al* (2000) with some modifications. Each fibre fraction (1 g) was hydrated in tubes containing 30 mL of distilled water for 18 h at room temperature. Following hydration, samples were centrifuged (3,000 x *g* for 20 min). The supernatant was decanted and the sample left to drain. Sample fresh weight was recorded before drying (120 ^{o}C for 2 h). WRC was calculated as the amount of water retained by the pellet (g/g dry weight) after draining.

Pasting characteristics of wheat starch was determined. Peak viscosity (PV), final viscosity (FV), and breakdown (BD) development of wheat starch substituted with 1 and 5% BBG fibre fraction were determined using a Rapid Visco Analyser (RVA-4 Newport Scientific PTY, Australia). An RVA Standard One Profile was used with heating and cooling rates of 12 ^{o}C per min, over a temperature range of 50-95 ^{o}C, and paddle speed of 160 rpm. Samples were prepared by mixing 3.5 g ($\pm$0.1) in 25 mL ($\pm$0.1) distilled water in an aluminium canister.

Thermal properties of wheat starch were also investigated using a differential scanning calorimetry (DSC 12E; Mettler Toledo, Leicester, UK) to record onset of gelatinization, T_{onset}; gelatinization peak temperature, T_p; gelatinization end point, T_{endset}; and total enthalpy, (j/g) of wheat starch substituted with 1% and 5% BBG fibre fraction. The starch-to-distilled water ratio was 1:4. Nominal scan rate was 5 ºC/min over a 20-100 ºC heating rate.

Results of evaluation of extraction processes and addition to wheat starch mixes.

The yield of barley β-glucan (BBG) fibre fraction, and β-glucan recovery efficiency from barley flours was significantly affected ($p<0.05$) by varying the extraction conditions (Table 1). Actual recovery rates for BBG fibre fraction ranged from 4.5-6.2 % of the grain weight (Figure 2). Out

of this the purified BBG fraction recovered the highest amount of dietary fibre material (91.62 %) and the most β-glucan rich BBG fraction (73.05). This was mainly due to the significantly lower starch contamination, together with a reduced protein content, of the BBG fraction (Table 1). However, it should be noted that the simple water extraction procedure resulted in total dietary fibre and β-glucan composition values which were not significantly different to those of the purified fraction. This indicated that if the aim of the process is to efficiently recover a fraction of high dietary fibre and β-glucan values, the economically inexpensive route of water extraction may be suitable.

Table 1. Composition of BBG fibre fractions extracted at 55 ^{o}C with differing treatments

BBG	Extraction pH	Total dietary fibre (%)	β-Glucan (%)	Protein (%)	Starch (%)
Water	7	88.60^{a,b,c}	69.75^{a}	3.74^{a}	4.04
Refluxed	7	88.06^{a,b,c}	71.53^{a}	4.55	2.68^{a}
Purified	7	91.62^{b}	73.05^{a}	3.75^{a}	1.18
Alkali	10	87.60^{a,c}	70.06^{a}	6.90	2.99^{a}
Boiled	7	84.80^{c}	63.53	5.01	6.95

^{a}means in the same column followed by the same letter are not significantly different ($p>0.05$)

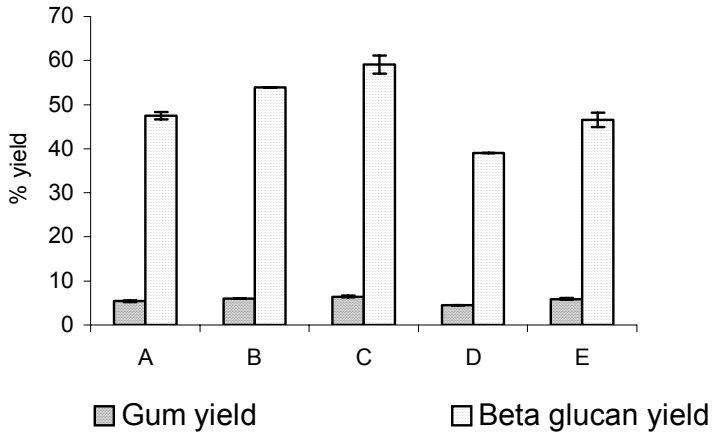


Figure 2. Percentage BBG fraction yield from original flour, and β-glucan content of such fractions from:- water (A), refluxed (B), purified (C), alkali (D), and (E) boiled treatments. Standard errors are included on the figure as error bars.

In order to determine the possible effects of extraction procedure on the processing characteristics of the BBG fractions, further experiments were conducted on these BBG fractions to determine water retention capacities and flour pasting and starch gelatinisation characteristics of BBG fraction and flour mixes. Once again the purified fraction showed a greater water retention capacity when compares to the other extraction techniques (Figure 3). This greater water retention capacity is almost certainly due to the increased β-glucan content of the BBG fraction. β-glucan has the potential to absorb large quantities of water (possibly illustrating a potential use in the food industry as a thickener and stabiliser), and the ranking of BBG fractions in order of water retention capacity (Figure 3) closely resembles the ranking of the BBG fractions in terms of actual β-glucan content (Table 1).

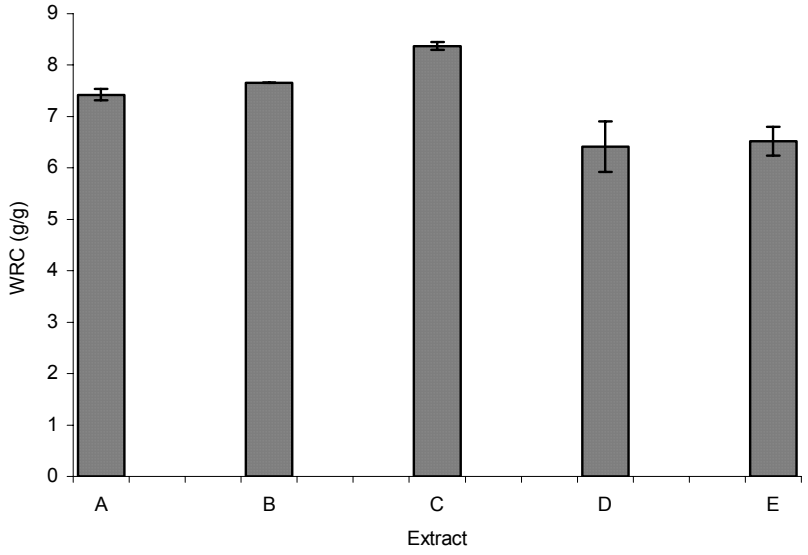


Figure 3. Water retention capacity of the extracted BBG fibre fractions from:-water (A), refluxed (B), purified (C), alkali (D) and boiled (E) treatments. Standard errors are included on the figure as error bars.

Surprisingly pasting characteristics of β-glucan starch mixes showed no significant variations between a control starch sample and samples with 1 % BBG fractions (Table 2). Although there was variability in the pasting characteristics (some fractions yielding elevated final viscosities

and peak viscosities) no firm correlation could be made between the composition of the BBG fraction and pasting characteristics at a 1% replacement level.

Use of the BBG fractions at a 5% replacement level (Table 3) showed a general reduction in peak viscosity, final viscosity and also the breakdown of the paste, when comparing the control sample to the samples containing the BBG fractions. This reduction in pasting characteristics is likely to be due to the β-glucan competing with the starch in the sample for water. As the viscosity of a paste is directly related to the degree and extent of starch gelatinisation and hence realignment during subsequent cooling, any material that aggressively competes for water will restrict the amount of water available for the starch granules during starch gelatinisation. This in turn will limit the degree of starch gelatinisation. Hence the results clearly indicate the role of β-glucan material in reducing the amount of available water in the system for the starch to gelatinise.

Table 2. Thermal parameters (T_{onset}, T_{peak}, T_{endset}; and enthalpy (J/g)) of wheat starch substituted with 1 and 5% water, refluxed, purified, alkali, and boiled BBG fibre fractions

	T_{onset} (^{o}C)	T_{endset} (^{o}C)	T_{peak} (^{o}C)	Enthalpy (J/g)
Control	52.00	69.50	58.80	8.84[a]
1% Water fraction	51.05	70.55	59.00	8.44[b]
5% Water fraction	52.10	69.65	59.40	8.40[b]
1% Refluxed fraction	52.05	70.00	59.01	9.07[a]
5% Refluxed fraction	52.05	69.60	59.00	7.43[c]
1% Purified fraction	51.75	69.65	59.25	8.98[a]
5% Purified fraction	52.10	71.90	59.4	8.19[b,d]
1% Alkali fraction	51.95	69.95	59.35	8.37[b]
5% Alkali fraction	51.99	70.20	59.29	7.89[d,e]
1% Boiled fraction	51.83	70.21	59.26	8.66[b]
5% Boiled fraction	52.05	69.65	59.10	7.64[c,e]

[a]means of J/g values followed by same letter are not significantly different ($p>0.05$)

Table 3. Peak (PV), breakdown (BD), and final viscosity (FV) of wheat starch substituted with 1% water, refluxed, purified, alkali, and boiled (E) BBG fibre fractions

	PV	BD	FV
Control	4046[a]	1163[a]	4622[a]
Water fraction	4161[a]	1185[a]	4724[a]
Refluxed fraction	4476[a,]	1186[a]	5153[a]
Purified fraction	4393[a]	1205[a]	4959[a]
Alkali fraction	4428[a,]	1160[a]	4990[a]
Boiled fraction	4508[a,]	1255[a]	5120[a]

[a]means values in same column followed by same letter are not significantly different ($p>0.05$)

When the thermal properties of starch gelatinisation were examined using the DSC, the results showed a significant reduction in the degree of starch gelatinisation, with the replacement of starch with β-glucan (Table 4). This difference was more than would be expected by taking into account the reduction of available starch on a replacement – loss basis. The enthalpy of the system reveals that in real energy terms a 5% replacement of starch with any of the BBG fractions impairs starch gelatinisation. There was no significant difference between the BBG fractions used, thus illustrating that either the purified or the water extracted BBG fractions may be of commercial use in the food industry. Although the purified sample appeared to yield higher amounts of dietary fibre and hence β-glucan, the process is more expensive in terms of both handling time and ingredients, hence the water extraction process may be more viable.

Table 4. Peak (PV), breakdown (BD), and final viscosity (FV) of wheat starch substituted with 5% water, refluxed, purified, alkali, and boiled (E) BBG fibre fractions

	PV	BD	FV
Control	4046[a]	1163	4622
Water fraction	3294[b]	874[a,b,c]	3543[a]
Refluxed fraction	3451[a,b]	773[b]	3882[a]
Purified fraction	3416[b]	767[a,b,c]	3627[a]
Alkali fraction	3866[a]	986[c]	4024[a]
Boiled fraction	3671[a,b]	805[a,b]	4048[a]

[a]means value, in same column followed by same letter are not significantly different ($p>0.05$)

1.2:- BBG fraction in breads (as a model cereal food product)

For the next part of the research the water extracted fraction was selected, as being the most stable and economical β-glucan containing fraction, for inclusion into bread products (using bread as a model cereal system). The effect of such inclusion on loaf volume, bread textural and hence sensorial, and nutritional characteristic (*in vitro* digestion) were evaluated.

A fresh BBG fraction, of approximately 70% β–glucan (db), was extracted. Milled barley flour was suspended in pre-heated distilled water (55 ^{o}C, pH 7) and stirred with vigorous agitation for 30 min. The mixture was centrifuged (15 min 15,000 *x g*, 4 ^{o}C) and the supernatant recovered and reduced to pH 4.5 with the addition of 2M HCL. The acidified supernatant was centrifuged (20 min, 21,000 *x g*, 4 ^{o}C). Ethanol (99%) was added to 50% concentration, and the resulting mix held at 4 ^{o}C for 12 h. The precipitate was recovered by centrifugation (10 min, 3000 *x g*). The precipitate was homogenized with 99.9% ethanol, followed by washing and filtering with 99.9% ethanol. Extracts were dried in an air cabinet for 1.5.

Pasting characteristics (peak viscosity, PV; breakdown, BD; and development of final viscosity, FV) of bread wheat flour substituted with 2.5 and 5% BBG fraction dispersions were determined using a Rapid Visco Analyser (model RVA-4; Newport Scientific PTY, Warriewood, Australia) as previously described.

Bread making was also conducted. 6.25 g (2.5%) and 12.5 g (5%) of BBG fraction were hydrated in 140 ml of boiling water with vigorous stirring for 5 min. Gums were subsequently stirred for 2 h. Breads, containing 0, 2.5, and 5% BBG fraction, were manufactured according to Approved Method 10-09 (AACC 2000), using 250 g (control), 243.75 g (2.5% BBG fraction) and 237.5 g (5% BBG fraction) strong white bread flour, 12.5 g vegetable fat, 6 g dried yeast, 6 g salt, 2 g sugar, and 140 ml of water. Doughs were divided into 70 g portions and baked in test bakery tins (length 85 mm, width 50 mm). Baked breads were cooled for 1 h prior to analysis.

Extensibility of the bread doughs was measured using a texture analyzer (model TA-XT2, Stable Micro Systems, Reading, UK) equipped with a Kiefer dough and extensibility rig (A/KIE) using a 5 kg load cell. Dough (15 g) was placed in an oiled teflon dough former for 20 min, removed with the aid of a spatula, and subjected to the tensile test. The rig extended the sample by 75 mm, at a pre-test, test, and post-test speed of 2, 3.3, and 10 mm/s, respectively.

The maximum peak force in tension was regarded as the degree of elasticity of the dough, whereas the distance travelled for dough break was regarded as the extensibility of the dough.

Loaf height was determined using calibrated callipers and reported in centimetres. Measurements were taken from the centre of each loaf. Loaf volume was measured using Approved Method 10-05, volume by rapeseed displacement (AACC 2000).

A TA-XT2 texture analyzer (Stable Micro Systems, Reading, UK) was used to measure bread firmness. An AACC 36-mm-cylinder probe with radius (model P/36R) and a 5 kg load cell was used. The probe compressed the sample by 40% at a pre-test, test, and post-test speed of 1, 1.7, and 10 mm/s, respectively. The maximum peak force in compression was recorded as the firmness value in gram units. Measurements were taken on 1-cm slices. Only 1 measurement was taken from the slices; samples were discarded after the test.

For analysis of crumb firmness over storage (1, 2, and 4 days) slices were placed in polythene bags, sealed, and stored at ambient temperature and on the appropriate day subjected to texture analysis as the samples at day 0.

Moisture, starch, protein, and β-glucan contents of the breads were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000). Total starch was determined using the total starch assay kit, Approved Method 76.13 (AACC 2000); β-glucan content was measured using the enzymatic assay kit, Approved Method 32-23 (AACC 2000). All assay kits were supplied by Megazyme^{TM} International Ireland Ltd (Wicklow, Ireland). Nitrogen was determined using a nitrogen analyzer (model FP-2000; Leco Instruments Ltd, St Joseph, USA) and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis.

Bread samples were subjected to an *in vitro* digestion according to the following methodology (Tudorica *et al* 2002). Samples of bread, equivalent to 3 g available carbohydrate, were crumbed (using a food processor) to a size of approx 1 cm^3, placed in sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (with HCl), and digested with pepsin (Sigma-Aldrich, Pool, UK) (575 units/g starch) for 30 min at 37 ^oC. The pH of the mixture was subsequently re-adjusted to pH 6.9 (with NaOH), and the volume of the liquid made up to 50 mL with a solution of sodium phosphate buffer to which porcine pancreatic alpha amylase (Sigma-Aldrich, Pool, UK) (110 units/g starch) had been added. The mixture was transferred to dialysis tubing (Medicell

International Ltd, Reading, UK), and placed in 450 ml of sodium phosphate buffer for 5 h at 37 ^{o}C. Aliquots (1 ml) duplicate were taken every 30 min.

Dialysate was analyzed for total sugars by the 3,5-dinitrosalicylic acid method. Reducing sugars released (RSR) was expressed in maltose equivalents as percentage of total available carbohydrates present in the sample using the following calculation:
$RSR = A_{sample} \times 500 \times 0.95 / A_{maltose} \times SS \times 100$

where: A_{sample} is value of absorbance at 540 nm, $A_{maltose}$ is value of absorbance of a solution containing 1 mg of pure maltose per ml/phosphate buffer, SS is the amount of starch plus sugars (in milligrams) contained within the sample, 500 is the total volume, and 0.95 is the conversion from maltose to starch. .

Results from BBG* fraction *inclusion into breads

A similar pattern of reduced pasting characteristics were observed in the samples of flour / BBG mixtures to that of the starch / BBG mixtures previously discussed (Table 5). This illustrates that the BBG fraction behaves in a similar way both in pure starch and flour systems. In both instances the BBG fraction appears to compete for the water within the mixture and as such inhibits starch gelatinisation and pasting.

Table 5. Wheat flour pasting characteristics peak viscosity (PV), breakdown (BD) and final viscosity (FV) of bread wheat flour substituted with 2.5 and 5% BBG fraction

	Control	2.5% BBG FRACTION	5% BBG FRACTION
PV (RVU)	175.28^{a}	126.92^{b}	131.28^{ba}
BD (RVU)	82.95^{a}	65.78^{b}	73.17^{b}
FV (RVU)	233.22^{a}	73.17^{b}	165.8^{b}

^{a} means in the same row followed by a different letter are significantly different ($p<0.05$).

PV-peak viscosity; BD-breakdown; FV-final viscosity.

Addition of BBG into dough systems had a significant effect on both the extensibility and extendibility of the dough (Table 6). Increasing BBG content increased the force required to stretch the dough pieces to breaking point, and at the same time increased the distance required to stretch the dough strip. This indicates that the dough itself has become increasingly elastic in nature and resilient to deformation.

Such an increase in the elastic nature of the dough could explain why both loaf volume and also loaf height decreased with increasing BBG inclusion. Both at 2.5 and 5% levels, the BBG fraction had a negative effect on loaf size. At the 2.5% level it may be possible to negate this effect by altering mixing speed and also by the addition of dough improvers; however this preliminary research project did not investigate this possibility.

Table 6. Dough and bread physicochemical characteristics

Evaluation parameters	Control bread	2.5% BBG FRACTION bread	5% BBG FRACTION bread
Dough (force g) extensibility (n=9)	19.09[a]	22.62[a]	37.86[b]
Distance (mm)	10.07[a]	10.18[a]	13.84[a]
Loaf volume (cm3)	184[a]	165[b]	133[c]
Loaf height (cm)	5.48[a]	5[a]	4.5[b]

[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

The firmness of the bread was also affected by the inclusion of BBG fractions, with bread firmness increasing with increasing levels of BBG (Table 7). Although this effect was largely insignificant at the 2.5% level of BBG, at 5% such changes were significant and appeared to increase during storage. The increase of firmness could be due to a lightly more compact loaf being formed (as observed in the reduced loaf volume and height) and also by the formation of a glucan-gel network within the bread. Such a gel-network would result in a product with a firmer and denser sensory characteristic.

Table 7. Crumb firmness (g) of breads at days 0, 1, 2, and 4.

	Control bread	2.5% BBG	5% BBG
Day 0	5.33[a]	5.47[a]	5.6[a]
Day 1	5.46[a]	5.47[a]	5.6[a]
Day 2	5.55[a]	5.6[a]	6.23[b]
Day 4	5.67[a]	5.71[a]	6.28[b]

[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

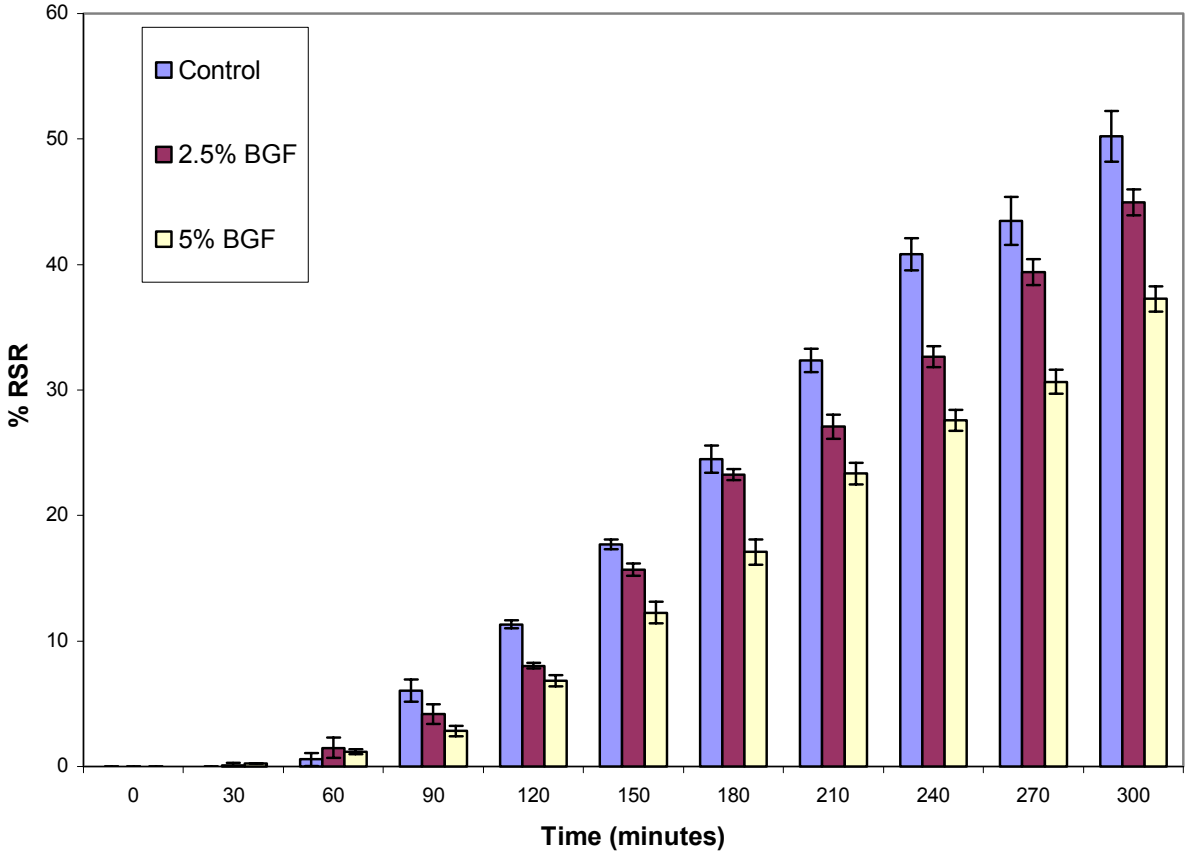
Thus from an applications point of view the inclusion of BBG fractions may have a slight negative effect on bread quality (consumer perceived quality attributes). However the *in vitro* determination of sugar release from starch shows that the BBG fractions have a potential nutritional benefit.

Figure 4 clearly shows that increasing levels of BBG in the bread product leads to increasing reductions in sugar release from eth product. As this sugar release is expressed in terms of available starch content in eth sample, the results indicate that the BBG fraction is having a significant inhibitory effect on starch degradation.

The nutritional implications of this are great. For instance, in terms of reducing the potential sugars released from bread, an inclusion of 2.5% BBG has the potential to reduce sugar release by up to 7.5%, whereas a 5% BBG level has a potential 14% reduction effect. Again it is important to note that these effects are on a g/g carbohydrate comparison and exclude any additional effect the inclusion of BBG may have solely from a replacement term. The BBG fraction appears to make the carbohydrate, a slowly digestible carbohydrate.

Thus, it is distinctly possible to reduce the overall glycaemic loading of bread by the addition of BBG fractions, opening a potential application in low glycaemic food products.

Figure 4. Reducing sugar released (RSR), expressed as maltose equivalents, as percentage total available carbohydrates following *in vitro* digestion of control bread and breads with added BBG fraction.



[a] points at same time interval with different letters are significantly different ($p<0.05$), data are mean $\pm$ SD. Values at 0, 30, and 60 minutes are not significantly different.

Separate research on a commercially available beta-glucan extract showed similar characteristics to the water extract obtained in our study, with regards to pasting, processing and nutritional characteristics (results not shown). Illustrating that the commercial scale up of beta-glucan extraction is a distinct possibility.

Additional work conducted on pasta revealed that BBG fractions did not significantly affect the quality of enriched pasta (compared to the control) but did significantly increase the nutritional quality of the pasta.

1.3:- Conclusions

The observations from our research have importance in the application of BBG fibre fractions in foods and in human nutrition, particularly where starch is a primary ingredient, for example bread and pasta, as summarised below:

- β-glucan can be extracted from UK grown barley using either water or enzyme treatment and isolation techniques
- different extraction techniques have an effect on fibre recovery and β-glucan yield
- β-glucan inclusion into starch and flour model systems can reduce pasting characteristics of the cooked mixtures
- β-glucan inclusion into starch and flour model systems may reduce the degree of starch gelatinisation in the pastes
- inclusion of β-glucan into model food systems (breads and pasta) can be obtained at levels up to and including 5% without significant loss of structure or texture
- similar levels of barley flour inclusion have significant effects on food structure
- inclusion of β-glucan into foods reduces the rate of starch degradation during an *in vitro* digestion process
- the reduction in starch degradation leads to a reduction in sugar release from the carbohydrate food and hence lowers the glycaemic index of such foods
- there is a real potential to use barley β-glucan as a functional food ingredient

The high WRC exhibited by BBG fibre fractions and the associated reduction in gelatinisation of starch has relevance to human nutrition and the regulation of *in vitro* and *in vivo* glycaemic response in carbohydrate-rich diets where the degree of starch gelatinization can affect the postprandial sugar availability of foods.

Results also indicate that in the processing of β-glucan-enriched foods, considerable alterations to formulations may have to be introduced. This would be particularly important in bread products where water absorption can significantly influence processing and final product quality.

Prior to β-glucan incorporation the choice of extract treatment would have to be carefully assessed, as this may have an influence on the functional behaviour of β-glucans in a food system. Our results illustrate that refluxing and purification treatments yield fractions with the highest β-glucan purity and, therefore, from all treatments were the most efficient treatments that might be considered in large-scale BBG fibre fraction production.

The aim of further research will be to incorporate extracted BBG fibre in food models and assess the physicochemical changes that occur and the necessary modifications in food formulations that come about.

References

AACC. 2000. AACC Board holds mid-year meeting. *Cereal Foods World*, 45, 325.

AACC. 2001. AACC Dietary Fibre Technical Committee. The definition of dietary fibre. *Cereal Foods World*, 46, 112.

AACC. 2003. AACC Dietary Fibre Technical Committee. All dietary fire is fundamentally functional. *Cereal Foods World*, 48, 128-131.

Bacic, A., & Stone, B.A. 1981a. Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology*, 8, 453-474.

Bacic, A., & Stone, B.A. 1981b. Chemistry and organisation of aleurone cell wall components from wheat and barley. *Australian Journal of Plant Physiology*, 8, 475-495.

Bamforth, C.W., Martin, H.L., & Wainwright, T. 1979. A role for carboxypeptidase in the solubilisation of barley β-glucan. *Journal of the Institute of Brewing*. 89, 34-37.

Bathgate, G.N., Palmer, G.N., & Wilson, G. 1974. The action of endo-β-glucanases on barley and malt β-glucans. *Journal of the Institute of Brewing*, 80, 278-285.

Beer, M.U., Arrigoni, E., & Amado, R. 1995. Effect of oat gum on blood cholesterol levels in healthy young men. *European Journal of Clinical Nutrition*, 49, 517-522.

Bhatty, R.S. 1993. Extraction and enrichment of (1→3) (1→4) -β-D-glucan from barley and oat brans. *Cereal Chemistry*, 70, 73-77.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. *Journal of Cereal Science*, 22, 163-170.

Brennan, C.S., Harris, N., Smith, D. & Shewry, P.R. (1996a). Structural differences in the mature endosperms of good and poor malting barley cultivars. *Journal of Cereal Science* 24:171-178.

Brennan, C.S., Blake, D.E., Ellis, P.R. & Schofield, J.D. (1996b). Effects of guar galactomannan on wheat bread microstructure and on the in vitro and in vivo digestibility of starch in bread. *Journal of Cereal Science* 24:151-160.

Brennan, C.S., Amor, M.A., Harris, N., Smith, D., Cantrell, I. Griggs, D. & Shewry, P.R. (1997). Cultivar differences in modification patterns of protein and carbohydrate reserves during malting of barley. *Journal of Cereal Science*. 26 83-93.

Brennan, C.S., Smith, D.B., Harris, N. and Shewry, P.R. (1998) The production and characterisation of *Hor* 3 null lines of barley provides new information on the relationship of D hordein to malting performance. *Journal of Cereal Science* 28:291-301.

Burkus, Z., & Temelli, F. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum, *Cereal Chemistry*, 75, 805-809.

Fincher, G.B. 1975. Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brew*ing, 82, 347-349.

Forrest, I.S., & Wainwright, T. 1977. The mode of binding of β-glucans and pentosans in barley endosperm cell walls. *Journal of the Institute of Brewing*, 83, 279-286.

Henry, R.J., & Blankeney, A.B. 1986. Determination of total β-glucan in malt. *Journal of the Institute of Brewing,* 94, 354-356.

HGCA 2003 Recommended Lists for cereals and oilseeds. Home-Grown Cereals Authority, London, UK.

Klopfenstein, C.F. & Hoseney, R.C. 1987. Cholesterol lowering effect of β-glucan enriched bread. *Nutrition Reports International*, 36, 1091-1098.

Palmer, G.H. 1987. Influence of cell wall structure on enzyme breakdown of the endosperm of germinated barley. *Journal of the Institute of Brewing*, 93, 105-107.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. *Journal of Food Science*, 62, 1192-1201

Tudorica C.M., Kuri, V. & Brennan, C.S. (2002) Nutritional and physicochemical characteristics of dietary fibre enriched pasta. *Journal of Agricultural and Food Chemistry,* 50*,* 347-356.

Wood, P.J., Paton, D., & Siddiqui, I.R. 1978. Determination of β-glucan in oats and barley. *Cereal Chemistry*, 54, 524-533.

Wood, P.J., Fulcher, R.G., & Stone, B.A. 1983. Studies on the specifity of interaction of cereal cell wall components with Congo Red and Calcoflour: Specific detection and histochemistry of (1→3) (1→4) -β-D-glucan(1→3) (1→4) -β-D-glucan. *Journal of Cereal Science*, 1. 95-110.

Woodward, J.R. Ficher, G.B. & Stone, B.A. 1983. Water soluble (1-3) (1-4) β-D-Glucan from barley (*Hordeum vulgaris* endosperm. II. Fine structure. *Carbohydrate Polymers*, 3, 207-225.

Woodward, J.R., Philips, D.R., & Ficher, G.B. 1988. Water soluble (1-3) (1-4) β-D-Glucan from barley (*Hordeum vulgare(ditto above)*) endosperm. IV. Comparison of 40^{o}C and 65^{o}C soluble fractions. *Carbohydrate Polymers*, 8, 85-97.

2 Technical Detail Report.

The research project was conducted at University of Plymouth, Seale-Hayne Campus, Newton Abbot, Devon, TQ12 1QR. Miss Louise Symons was employed as the research assistant conducting the investigations during the 12 month period of the project.

For ease of reading the report, it will be written in the form of individual papers which explore the individual elements of the project. Information form each of these sections has been used for the production of actual research publications (some of which have been accepted for publication).

The current publication output from this project include:-

1. Symons, L.J. & Brennan, C.S. (2004) The influence of (1→3) (1→4)-β-D-glucan rich fractions from barley on the physicochemical properties and *in vitro* reducing sugar release of white wheat breads *Food Science* (IN PRESS).
2. Brennan, C.S. & Symons, L.J. (2004) The potential use of barley β-glucan as a functional food ingredient *Applied Biotechnology, Food Science and Policy* (IN PRESS).
3. Symons, L.J. & Brennan, C.S. (2004) The effect of barley β-glucan fibre fractions on starch gelatinisation and pasting characteristics. *Food Science* **69**: 257-261

Proceedings of Conferences

4. Brennan, C.S. (to be presented) Low GI cereal foods: the role of dietary fibre and food structure. IN: Proceedings of the 12th ICC Cereal & Bread Congress Harrogate 2004. Woodhead Publishers.
5. Symons, L.J., Jones, T.E.R., Moates, R. & Brennan, C.S. (2003) Evaluation of extraction techniques on barley β-glucan behaviour in starch gel systems: effects on starch gelatinisation, pasting rheology and degradability. (paper in the Cereals 2003 Proceedings of The Royal Australian Chemical Institute September 2003 Adelaide).
6. Symons, L.J., & Brennan, C.S. (2003) Pasting behaviour of β-glucan / starch gels: the effect of barley β-glucan extracts on starch gelatinisation and degradation. Proceedings of Dietary Fibre 2003 (AACC / ICC / TNO, Netherlands) p79-80.

The support of the HGCA has been / will be acknowledged in each of these publications.

2.1- The potential use of barley β-glucan as a functional food ingredient.

Introduction

Over recent years there has been an increased focus on the importance of dietary fibre as part of a balanced and nutritious diet. In particular soluble fibres such as β-glucans have been shown to have effects on the glycaemic and cholestromic nature of foods. Barley is a good source for these functional ingredients. The rheological characteristics and hence potential nutritional benefits may be related to β-glucan extraction procedures. Additionally, the inclusion of β-glucans have potential uses in low-fat food products and in optimising dairy product formulations.

Barley as a source of beat-glucan

Barley belongs to the genus *Hordeum* and can be considered as one of the most ancient crop plants, with its cultivation is mentioned in the Bible. Archaeological studies have revealed two-rowed barley cultivation by about 8000 B.C. in Iran, with six-rowed barley appearing approximately 6000 B.C. (Bothmer & Jacobsen 1985). World production of barley in 2000/2003 was approximately 134 million metric tonnes. By far the leading barley producer is the EU (51.659Mt) followed by the Former Soviet Union (25.013Mt), and Canada (13.172Mt). In terms of the UK, Barley is the second most important crop, with approximately 6.2 million metric tonnes produced in 2002 (HGCA 2003).

The principal uses for barley are as feed for animals, in the form of barley meal, and as a grain material for malting and brewing in the manufacture of beer and whisky. Much research has been conducted on the role of endosperm components on the malting potential of barley (Bathgate et al 1974; Bamforth et al 1979; Henry & Blackeney 1986; Palmer 1987; Brennan et al, 1996a, 1997, 1999). However the barley crop may be considered relatively under-utilised with regards to its potential use as an ingredient in processed foods. Recent attention has focussed on the potential use of β-glucan as a functional food ingredient.

Occurrence of barley β-glucan in the grain

β-Glucan has long been regarded as one of the most influential characteristics in relation to malting potential and brewing yield in barley, regulating the rate of endosperm modification (Brennan et al, 1999). Levels of β-glucan can vary dramatically between varieties but usually range from 2-6%. Despite their relatively small contribution to the total weight of the grain, it is clear that β-glucans have a disproportionate impact on the technology of barley utilisation and on the nutritional value of the grain. There have been several studies on the dependence of β-glucan content on genetic and environmental factors (Knuckles et al 1992; Yoon et al 1995; Zhang et al 2002). Although the relative contributions of these factors cannot be precisely quantified, there is a general agreement that the genetic background of the barley is more important than environmental conditions as a determinant of the final β-glucan content of the grain (Gill et al 1982, Morgan et al 1983, Henry 1986, Stuart et al 1988). For instance research conducted by Lehtonen et al (1987) reported that 2 row barley genotypes had higher β-glucan content than 6 row barley. Studies have also indicated that waxy (up to 100% amylopectin) barley cultivars have higher levels of β-glucan in the endosperm than no-waxy varieties (Ulrich et al 1986, Yoon et al 1995).

One of the major environmental factors that influences β-glucan levels appears to be the availability of water during grain maturation. Dry conditions before harvest result in high β-glucan levels (Bendelow 1975). Conversely, moist conditions cause a decrease in β-glucan levels (Stuart et al 1988, Aman et al 1989). This may either be related to the fact that final grain filling is adversely affected in drought conditions through impairment of starch synthesis and deposition, or because β-glucan synthesis is enhanced in dry conditions.

Non-starch polysaccharides of the barley grain

The non-starch polysaccharides found in mature barley grains include fructans, β-(1-4)-D-glucan (cellulose), β-glucan, arabinoxylans and glucomannans. The β-glucan components are linear molecules with approximately 30% β-(1→3) and 70% β-(1→4) linkages randomly dispersed and are associated with firmly linked peptide sequences in the barley endosperm cell wall (Fleming & Kawakami 1977; Forrest & Wainwright 1977). Differences can be observed in the composition of cell walls of the starchy endosperm and the aleurone. Cell walls of the starchy endosperm consist of about 70% β-glucan and 20% arabinoxylan whereas the aleurone

cells contains 26% β-glucan and 67% arabionxyaln, both contain similar amounts of glucomannan and cellulose (Bacic & Stone 1981a,b; Wood et al 1983; Woodward et al 1983, 1988).

Extraction procedures

Barley β-glucan has been subject to much investigation regarding the isolation, purification, and the effects that these processing conditions have upon their physicochemical, structural and physiological effects (Fincher 1975; Woodward et al 1983; Klopfenstein & Hoseney 1987; Woodward et al 1988; Bhatty 1993, 1995; Temelli 1997; Burkus & Temelli 1998).

The extraction of β-glucan from cereal grains generally involves three basic steps: deactivation of native enzymes, extraction of the β-glucan, and then precipitation of the β-glucan. Enzymes, namely the endogenous β-glucanases, need to be deactivated in the cereal grains since they are responsible for decreasing the molecular weight and thereby the functional properties of the extracted β-glucan. Deactivation is usually achieved by refluxing the barley with aqueous ethanol or treating the barley flour with dilute aqueous ethanol.

Key extraction methodologies for barley and oat β-glucan were developed by Wood et al (1977, 1978), who assessed particle size, temperature, pH and ionic strength on β-glucan yield at laboratory scale, and prepared an oat gum containing 78% β-glucan from oat bran at the pilot plant scale using hot alcohol deactivated oat bran (75% ethanol/4 hours) and a sodium carbonate extraction at pH 10 (Wood et al 1989). McCleary (1988) increased the extraction rate of barley β-glucan to 90% using sequential water extractions at 40, 65, and 95^{o}C. Bhatty (1993) investigated the influence of different solvents on the recovery and viscosity of barley and oat gums and achieved the highest enrichment of β-glucan and pentosans from barley bran using 1M NaOH as solvent and applied the same approach as Wood et al (1989). However this procedure solubilised considerable quantities of starch and protein, resulting in a contaminated extract. This problem can be negated using a hot water extraction procedure in the presence of thermostable α-amylase (Sauliner et al 1994).

The cost of these extraction techniques can be regarded as a major limiting factor in the use of β-glucan as a functional food ingredient. Additionally, the use of organic solvents may affect the solubility of the precipitated β-glucan gums (Beer 1996; Morgan and Ofman 1998). To

counteract these negative factors, whilst endeavouring to produce a more cost effective extraction process, Morgan and Ofman (1998) developed a hot water extraction procedure of the β-glucan from the grain followed by a freeze and thaw of the extract. The resulting product (Glucagel) contained between 89-94% β-glucan, depending on the length of the initial extraction.

The temperature and pH of extraction process also affects the recovery of β-glucan. Temelli (1997) illustrated that β-glucan content increased with temperature, but not pH. Burkus & Temelli (1998) further evaluated the effect of extraction conditions upon yield, composition, and viscosity stability of barley gum from a regular barley (Condor) and a waxy cultivar blend. In their research, extraction conditions were evaluated including an extraction with:- no additional treatment, boiling of extract, prior refluxing of flour with 70% ethanol, and treatment of extract with thermostable alpha-amylase for purification. Highest β-glucan purity achieved was with a boiled Condor extract at pH 7 (81.3% yield), closely followed by refluxed waxy barley extracted at pH 8 and treated with an amylase (79.3% yield). Refluxed gums followed by purification treatment (pH 7), produced the highest stable viscosity. Symons and Brennan (2004a) examining extraction procedures have also shown that extraction with thermostable alpha-amylase yielded the purest β-glucan fraction.

The nature of extraction can also have a profound effect upon β-glucan molecular weight, which in turn affects the functional behaviour of β-glucan. Carr et al (1990) observed that the use of NaOH for complete extraction results in depolymerisation of the β-glucan, whilst Ahluwahila & Ellis 1984) found similar effects with acids. Beer et al (1997a,b) later observed loss in molecular weight during extraction of oats and barley in NaOH, whereas Knuckles et al (1997a) found that sequential extractions resulted in a decrease in extracted β-glucan molecular weight. Thus care must be taken to optimise the yield and rheological characteristics of Beer et al (1997a) observed loss in molecular weight during extraction of β-glucan extracts.

The role of β-glucan as a source of dietary fibre

Much of the more recent attention with regards to β-glucan use in food systems has stemmed from its use as a functional dietary fibre. The term dietary fibre is used to collectively describe a group of substances in plant material which resist human digestive enzymes. Official definitions of dietary fibre have been made by both the Dietary Fibre Technical Committee of the American Association of Cereal Chemists AACC (AACC 2000, 2001, 2003) and the Food Nutrition Board (FNB) of the Institute of Medicine of the National Academics.

Potential health benefits of dietary fibre include; manipulation of bowel transit time (Feldheim et al 2000), prevention of constipation, reduction in risk of colorectal cancer (Hill 1997; Bingham et al 1990; Faivre et al 1999), lowering of cholesterol and regulation of blood glucose levels for diabetes management (Bornet et al 1981; Gallagher et al 1993; German et al 1996; Frost et al 1999), production of short chain fatty acids (Velasquez et al 2000: Wisker et al 2000; Karppinen et al 2000) and promotion of colonic health, stimulating the growth of beneficial gut microflora (prebiotic).

Traditionally dietary fibres have been divided into soluble and insoluble fractions. Foods high in soluble dietary fibre have been shown to have a positive effect on reducing hyperglycaemia and hyperinsulinaemia, with reference to the control of diabetes (Brennan & Tudorica 2003) and in the reduction of the possibility of developing risk factors for degenerative diseases, such as obesity (Burley et al 1987), hyperlipidaemia (Jenkins et al 1985) and hypertension (Anderson, 1983). High fibre foods have been correlated to the modulation of glycaemic response, on the basis of studies by Jenkins et al (1976, 1977, 1978) with purified fibres and with fibre rich foods (Jenkins et al 1980; Truswell 2002; Tudorica et al 2002a).

Many attempts have been made to clarify the mechanisms by which dietary fibres behave. With regard to the reduction of glycaemic response, proposed mechanisms include the amount and quality of fibre (Wolever 1990; Nishimune et al 1991), increased viscosity (Mourot et al 1988), maintenance of physical integrity (O.Dea, et al 1980) and incomplete starch gelatinisation (Ross et al 1987; Brennan et al 1996b; Tudorica et al 2002a). The cholesterol lowering potential of cereal fibres is considered as a result of activity in the upper gastrointestinal tract and are related in part to the network formation capability and viscosity of the dietary fibre. However there is also evidence to suggest that fermentation products in the lower gastrointestinal tract may influence the physiological outcome (Throburn et al 1983; Reimer et al 2000)

Physiological effects of β-glucan enrichment in cereal food

The most widely known nutritional benefits of β-glucans are the attenuation of blood glucose and insulin (Wood et al 1990; Wood et al 1994a), and hypocholestrolemic properties (Braaten 1994; Beer 1995), and the prevention of colorectal cancer (Thorburn et al 1983; Lebet 1996; Reimer et al 2000). Other notable but perhaps less documented effects of β-glucan in the form of oat bran and gum include gastric emptying (Johansen et al 1996, 1997), diminished absorption of nutrients (Edwards et al 1988; Lund et al 1989), prolonged postprandial satiety, through secretion of cholecystokinin (Anderson 1990; Bourdon et al 1999), and increased stool bulk and relief of constipation (Hojgaard et al 1980; Valle-Jones 1985; Odes et al 1993).

Part of these physiological properties appear to be related to the rheological characteristics of β-glucan. Wood et al (1994a) demonstrated an inverse linear relationship between log (viscosity) of oat β-glucan in a drink model (varying MW/dose) and the magnitude of 50g oral load. Although individual comparisons with controls were insignificant, observations from regressional analysis revealed that viscosity accounted for 79-96% of the modifications in glucose and insulin response. *Inter alial* viscosity also appears to be controlled by solution concentration and the molecular weight of β-glucans. Further studies Wood et al (1994b) proposed that the glycaemic response of fibre rich foods was inversely related to viscosity (concentration and molecular weight).

Potential use of β-glucan in cereal food products

The potential benefits of β-glucan inclusion in food systems has been illustrated by a number of cereal food commodities. Hallfrisch et al (1997) reported that an oat β-glucan concentrate (oattrim) reduced glycaemic responses in men and women. More recent studies by Hallfrisch et al (2003) have evaluated the use of β-glucans isolated from oats and barley (NutrimX), and their corresponding affects upon plasma glucose, insulin, and glucagons responses in non-diabetic adults, concluding that barley β-glucan were of greater use in the regulation of glucose and insulin responses.

Research specifically examining the effect of barley β-glucan additions to durum wheat pasta has shown that post prandial blood glucose and insulin responses were reduced following

ingestion of pasta enriched with barley flour substituted for durum wheat flour, resulting in a reduced glycaemic response (Knuckles et al 1997b; Yokoyama et al 1997).

Cavallero et al (2002) incorporated β-glucan rich fractions into wheat bread. Four breads were produced 100% bread wheat (total β-glucan 0.1: soluble β-glucan 0.1), 50% bread wheat flour and 50% barley flour, 50% bread wheat flour and 50 % sieved barley fraction and 50% bread wheat flour and 50% water soluble barley fraction. Eight adults were fed test meals of each of the four breads and glycaemic indexes calculated from finger prick capillary tests. Results revealed a linear decrease in glycaemic index associated with increasing β-glucan concentrations. Similar reductions in starch degradation and sugar release have been demonstrated using *in vitro* digestion of breads enriched with purified barley β-glucan fractions (Symons and Brennan 2004b).

Jenkins et al (2001) observed the depression of glycaemic index by high levels of β-glucan fibre in two functional foods tested in type 2 diabetic outpatients. Volunteers were randomly given 50g portions of white bread, commercial oat bran breakfast cereal (4.4% β-glucan) a prototype β-glucan enriched breakfast cereal and a β-glucan breakfast bar (8.1% and 6.5% β-glucan respectively). Glycaemic indexes of prototype β-glucan enriched cereal (52) and bar (43) were significantly lower than commercial oat bran breakfast cereal (80) and white bread (100).

Hypocholestrolemic properties of barley β-glucan

As well as the documented effect of β-glucan on the glycaemic index of foods, research has indicated a potential benefit of β-glucan in reducing cholesterol levels. Kerckhoffs et al (2003) investigated the effects of β-glucan from oat bran in bread and cookies and in orange juice in mildly hypercholestrolemic subjects. Although consumption of the β-glucan enriched bread and cookies (daily intake β-glucan 5.9g) there was no significant change in LDL cholesterol, consumption of the orange juice (5g β-glucan) resulted in decreased LDL cholesterol (6.7%) and the ratio of total to HDL cholesterol reduced (5.4%) compared with other drinks. Similarly Keogh et al (2003) investigating the possible effect of β-glucan on the incidence of serum cholesterol levels, however their results failed to demonstrate any significant response directly associated to β-glucan inclusion. This non-significant response may in part be due to a reduction in the efficacy of β-glucan following processing.

The mechanisms by which β-glucans lower cholesterol are still not clearly defined (Kritchevsky 1997) although it is believed that viscosity is the predominant mechanism (Jensen et al 1993). One thought is that β-glucan increases intestinal viscosity and thus decreases absorption of cholesterol and the reabsorption of bile acids. Studies on the hypocholestrolemic effects of barley have tended to be conducted on model systems. Examples of such research includes studies investigating the effects of waxy hull-less barley in chicks by Fadel et al (1987), Martinez et al (1991), Newman et al (1991), Newman et al (1992) and Wang et al (1992), all reporting significant reductions in HDL or LDL cholesterol. Similarly, Ranhorta et al (1991) reported significantly lower serum cholesterol with rats fed diets containing bran or flour of from hull-less waxy barley compared with meal from the same material with reductions apparently related to the ratio of soluble fibre in each fraction.

Potential use of β-glucan in dairy food products

The increased attention with regards to the use of β-glucans in foods may not be solely to beneficial nutritional properties, but also in processing optimisation. A good example of this is the potential use of both cereal and non-cereal β-glucans in the diary industry. Recent research has focussed on the use of soluble dietary fibres, and in particular β-glucans, in the manufacture of low-fat ice creams and yoghurts (Brennan *et al*, 2002). Such research has shown that by combining the use of β-glucans with other soluble dietary fibres the mouthfeel, scoopability and sensory properties of fibre enriched low-fat dairy products can be made to resemble those of full-fat products. Similarly, research examining the role of β-glucans on the glelation and rheological characteristics of cheese curds (Tudorica et al 2002 b) has indicated a beneficial effect of β-glucan inclusion in low fat products. The addition of β-glucan solutions to milk has also been shown have beneficial effects on curd formation, including a potential reduction in curd cutting time and increased curd yields (Tudorica et al 2002 b,c). Such results appear to be related to the gelling capacity of β-glucans and their ability to form a highly structured, and elastic, caseien-protein-glucan matrix.

Effect of processing on nutritional and rheological characteristics of β-glucan

Relatively little work has been conducted on the role of food processing on the rheological or nutritional characteristics of β-glucans. Changes during processing may include molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding and solubility). These in turn may have a negative influence upon the sensory, physiological and ultimately the health benefits of β-glucan. Alterations in the properties of β-glucan may arise from shearing damage due to mechanical processing (Wood et al 1989), or by excessive heat treatment of food products. Unfavourable structural changes may also occur during commercial purification, such as the depolymerisation of the linear structure (Wursh et al 1997), resulting in decreased molecular weight and reduced viscosity. Furthermore, mild extraction conditions (50-60°c) may not deactivate endogenous β-glucananses, which in turn may lead to increased depolymerisation β-glucan (Fastnaught 2001; McCleary 2001). Thus, prior to processing of β-glucan rich and enriched foods, there is a need for consideration and manipulation of processing practices in order to optimise the nutritional quality of foods and minimise the degradation of the properties of β-glucan.

Conclusion

There is little doubt that β-glucans offer many nutritional and rheological advantages to the food industry. The challenge now exists to optimise extraction procedures so as to produce consistent raw materials for the food industry. Additionally more research is needed to investigate potential effects of β-glucan inclusion in both dairy and cereal based food systems. Within this research, work is required to determine the effect of process parameters on the rheological characteristics and molecular weight profiles of β-glucan extracts, and determine if processing affects the efficacy of β-glucan inclusions. Such research is vital in broadening our understanding of how β-glucans can affect the nutritional characteristics of foods by altering the structure, texture and viscosity of food products.

References

AACC. 2000. AACC Board holds midyear meeting. *Cereal Foods World*, 45, 325.

AACC. 2001. AACC Dietary Fibre Technical Committee. The definition of dietary fibre. *Cereal Foods World*, 46, 112.

AACC. 2003. AACC Dietary Fibre Technical Committee. All dietary fire is fundamentally functional. *Cereal Foods World*, 48, 128-131.

Ahluwahlia, B., & Ellis, E.E. 1984. A rapid and simple method for the determination of starch and β-glucan in barley and malt. *Journal of the Institute of Brewing*, 90, 254-259.

Aman, P., Graham, H., & Tilly, A.C. 1989. Content and solubility of mixed (1→3) (1→4) -β-D-glucan in barley and oats during kernel development and storage. *Journal of Cereal Science*, 10, 45-50.

Anderson, J.W. 1983. Plant fibre and blood pressure. *Annals of Internal Medicine,* 98, 842-846.

Anderson, J.W. 1990. Dietary fibre and human health. *Horticultural Science*, 25, 1488-1495.

Bacic, A., & Stone, B.A. 1981a. Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology*, 8, 453-474.

Bacic, A., & Stone, B.A. 1981b. Chemistry and organisation of aleurone cell wall components from wheat and barley. *Australian Journal of Plant Physiology*, 8, 475-495.

Bamforth, C.W., Martin, H.L., & Wainwright, T. 1979. A role for carboxypeptidase in the solubilisation of barley β-glucan. *Journal of the Institute of Brewing*. 89, 34-37.

Bathgate, G.N., Palmer, G.N., & Wilson, G. 1974. The action of endo-β-glucanases on barley and malt β-glucans. *Journal of the Institute of Brewing*, 80, 278-285.

Beer, M.U., Arrigoni, E., & Amado, R. 1995. Effect of oat gum on blood cholesterol levels in healthy young men. *European Journal of Clinical Nutrition*, 49, 517-522.

Beer, M.U., Arrigoni, E., & Amado, R. 1996. Extraction of oat gum from oat bran: effects of process on yield, molecular weight distribution, viscosity and (1→3) (1→4) -β-D-glucan content of gum. *Cereal Chemistry*, 73, 58-62.

Beer, M.U., Wood, P.J. & Weisz, J. 1997a. Molecular weight distribution and (1→3) (1→4) -β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chemistry*. 74, 476-480.

Beer, M.U., Wood, P.J. Weisz, J. & Fillion, N. 1997b. Effect of cooking and storage on the amount and molecular weight of (1→3) (1→4) -β-D-glucan extracted from oat products by an in vitro digestion system. *Cereal Chemistry*, 74, 705-709.

Bendelow, V.M. 1975. Determination of non-starchy polysaccharides in barley breeding programs. *Journal of the Institute of Brew*ing, 81, 127-130.

Bhatty, R.S. 1993. Extraction and enrichment of (1→3) (1→4) -β-D-glucan from barley and oat brans. *Cereal Chemistry*, 70, 73-77.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. *Journal of Cereal Science*, 22, 163-170.

Bingham, S.A. 1990. Mechanisms experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proceedings of the Nutritional Society*, 49, 153-171.

Bornet, F.R.J., Costagliola, D., Rizkalla, S.W., Blayo, A., Fontvieille, A.M., Haardt, M.J., Letanoux, M., Tchobroutsky, G., & Slama, G. 1987. Insulinemic and glycaemic indexes of six starch rich foods taken alone and in a mixed meal by type 2 diabetics. *American Journal of Clinical Nutrition*, 45, 588-95.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., & Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in

men fed barley pasta enriched with β-glucan. *American Journal of Clinical Nutrition*, 69, 55-63.

Bothmer., R.V. & Jacobsen, N. 1985. Origin, taxonomy and related species. In: D.C. Rasmusson. ed. *Barley*. American Society of Agronomy, Madison, WI, 19-56.

Brennan, C.S., Harris, N., Smith, D. & Shewry, P.R. (1996a). Structural differences in the mature endosperms of good and poor malting barley cultivars. *Journal of Cereal Science* 24:171-178.

Brennan, C.S., Blake, D.E., Ellis, P.R. & Schofield, J.D. (1996b). Effects of guar galactomannan on wheat bread microstructure and on the in vitro and in vivo digestibility of starch in bread. *Journal of Cereal Science* 24:151-160.

Brennan, C.S., Amor, M.A., Harris, N., Smith, D., Cantrell, I. Griggs, D. & Shewry, P.R. (1997). Cultivar differences in modification patterns of protein and carbohydrate reserves during malting of barley. *Journal of Cereal Science*. 26 83-93.

Brennan, C.S., Smith, D.B., Harris, N. and Shewry, P.R. (1998) The production and characterisation of *Hor* 3 null lines of barley provides new information on the relationship of D hordein to malting performance. *Journal of Cereal Science* 28:291-301.

Brennan, C.S., Tudorica, C.M. & Kuri, V. (2002) Soluble and Insoluble Dietary Fibres (Non-Starch Polysaccharides) and Their Effects on Food Structure and Nutritional. *Food Industry Journal* 5 261-272.

Brennan, C.S. & Tudorica, C.M. (2003) The role of carbohydrates and non-starch polysaccharides in the regulation of postprandrial glucose and insulin responses in cereal foods. *Journal of Nutraceuticals, Functional and Medical Foods.*4, 49-55.

Braaten, J.T., Wood, P.J., & Scott, F.W. 1994. Oat β-glucan reduces serum cholesterol concentration in hypercholestrolemic subjects. *European Journal of Clinical Nutrition*, 48, 465-474.

Burkus, Z., & Temelli, F. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum, *Cereal Chemistry*, 75, 805-809.

Burley, V.J., Leeds, A.R., & Bundell, J.E. 1987. The effect of high and low fibre breakfast in hunger satiety, & food intake in a subsequent meal. *International Journal of Obesity 11*, Suppl. 1, 87-93.

Carr, J.M., Glatter, J.L., Jeraci, J.L. & Lewis, B.A. 1990. Enzymic determination of β-glucan in cereal-based food products. *Cereal Chemistry*, 67, 226-229.

Cavallero, A., Empilli, S., Brighenti, F., & Stanca, A.M. 2002. High (1→3) (1→4) -β-D-glucan fractions in bread making and their effect on human glycaemic response. *Journal of Cereal Science*, 36, 59-66.

Edwards, C.A., Johnson, I.T., & Read, N.W. 1988. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? *European Journal of Clinical Nutrition*, 42, 307-312.

Fadel, J.G., Newman, R.K., Newman, C.W., & Barnes, A.E. 1987. Hypocholestrolemic effects of beta glucans in different barley diets fed to broiler chicks. *Nutrition Rep Int*, 35, 1049.

Faivre, J., Bonithon-Kopp, C. 1999. Diets, fibres and colon cancer. *Advanced Experiments in Medical Biology*, 472, 199-206.

Fastnaught, C. 2001. Barley fibre. In: Cho, S.S., & Drecher, M.L., eds. *Handbook of dietary fibre*. New York, Marcel Dekker, 519-422.

Feldheim, W., Wisker E. 2000. Studies on the improvement of dietary fibre intake. *Dtsch Lebensm-Rundsch*, 96, 327-330.

Fincher, G.B. 1975. Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brew*ing, 82, 347-349.

Forrest, I.S., & Wainwright, T. 1977. The mode of binding of β-glucans and pentosans in barley endosperm cell walls. *Journal of the Institute of Brewing*, 83, 279-286.

Frost, G., Leeds, A.A., Dore, C.J. Maderios, S., Brading, S., & Dornhurst, A. 1999. Glycaemic index as a determinant of serum, cholesterol concentration. *Lancet*, 353, 1045-1048.

Gallagher, D.D., Hassel, C.A., Lee, K.J., Gallagher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholestrolemic effects in hamsters. *Journal of Nutrition*, 123, 244-252.

German, J.B., Xu., R. Walzem, R. Kinsella, J.E., Knuckles, B., Nakamura, M., &Yokoyama, W.H. 1996. Effect on dietary fats and barley fibre on total cholesterol and lipoprotein cholesterol distribution in plasmas of hamsters. *Nutrition Research*, 16, 1239-1249.

Gill, A.A., Morgan, A.G., and Smith, D.B. 1982. Total β-glucan content of some barley cultivars. *Journal of the Institute of Brewing*, 88, 317-319.

Hallfrisch, J., & Behall, K.M. 1997. Evaluation of foods and physiological responses to menus in which fat content was lowered by replacement with Oatrim. *Cereal Foods World,* 43, 100-103.

Hallfrisch, J., Schofield, D.J., & Behall, K.M. 2003. Physiological responses of men and women to barley and oat extracts (NutrimX). II. Comparison of glucose and insulin responses. *Cereal Chemistry*, 80, 80-83.

Henry, R.J., & Blankeney, A.B. 1986. Determination of total β-glucan in malt. *Journal of the Institute of Brewing,* 94, 354-356.

HGCA 2003 Recommended Lists for cereals and oilseeds, Home-Grown Cereals Authority, London, UK.

Hill, M.J. 1997. Cereals, cereal fibre and colorectal cancer risk: a review of the epidemiological literature. *European Journal of Cancer Prevention*, 6, 219-225.

Hojgaard, L., Balslov, S, & Krag, E. 1980. The effects of oat bran on the intestinal transit time, faecal output and the output of faecal bile acids in healthy volunteers: A double blind investigation. *Ugeskrift for loger*, 142, 2625-2626.

Jenkins, D.J.A, Geoff, D.V., Leeds, A.R. Wolever, T.M.S., Gassull, M.A. &., Hockaday, T.D.R. 1976. Unabsorbable carbohydrates and diabetes: Decreased postprandial hyperglycaemia. *Lancet,* I, 172-174.

Jenkins, D.J.A., Leeds, R. Gassul, M.A., Cochet, R. & Alberti, K.G.M.M. 1977. Decreased post prandial insulin and glucose concentration by guar and pectin. *Annals of Internal Medicine*, 86, 20-23.

Jenkins, D.J.A. Goff, D.V., Leeds, Gassul, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., & Alberti, K.G.M.M. 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *British Medical Journal,* 1, 1392-1394.

Jenkins, D.J.A., Wolever, T.M.S., Taylor, R.H., Barker, H.M., & Fielden, H. 1980. Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *British Medical Journal,* 281, 578-570.

Jenkins, D.J.A., Wolever, T.M.S., Kalmusky, J., Giudici, S, Giordano, C., Wong, G.S., Bird, J.N., Patten, R., Hall, M., Buckley, G., & Little, J.A. 1985. Low glycaemic index carbohydrate foods in the management of hyperlipidaemia.. *American Journal of Clinical Nutrition,* 42, 604-617.

Jenkins, A.L., Jenkins, D.J., Zdravkkovic, U., Wursch, P., & Vuskan, V. 2001. Depression of the glycaemic index by high levels of β-glucan fibre in two functional foods tested in type 2 diabetes. *European Journal of Clinical Nutrition*, 56, 622-628.

Jensen, C.D., Spiller, G.A. Gates, J.E., Miller, A.F., & Whittam, J.H. 1993. The effect of acacia gum and a water soluble dietary fibre mix on blood lipids in humans. *Journal of American College of Nutrition*, 12, 147-154.

Johansen, H.N., Bach Knudsen, K.E., Sandstrom, B., & Skjoth, F. 1996. Effects of varying content of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs. *British Journal of Nutrition*, 75, 339-351.

Johansen, H.N., Bach Knudsen, K.E., Wood, P.J., & Fulcher, R.G. 1997. Physicochemical properties and the degradation of oat bran polysaccharides in the gut of pigs. *Journal of the Science of Food and Agriculture*, 73, 81-92.

Karppinen, S., Liukkonen, K; Aura, A.M., Forsell, P., Poutanen, K. 2000. *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *Journal of the Science Food Agriculture*, 80, 1469-1476.

Keogh, G.F., Cooper, G.J.S., Mulvey, T.B., McArdle, B.H., Coles, G.D., Monro, J.A., & Poppitt, S.D. 2003. Randomised controlled crossover study of the effect of a highly β-glucan enriched barley on cardiovascular disease risk factors in mildly hypercholestrolemic men. *American Journal of Clinical Nutrition*. 78, 711-718.

Kerckhoffs, D.A.J.M., Hornstra, G. & Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucan from oat bran in mildly hypercholersterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. *American Journal of Clinical Nutrition*, 78, 221-227.

Knuckles, B.E., Chiu, M.M., & Betschart, A.A. 1992. β-glucan enriched fractions from laboratory dry scale milling and sieving of barley and oats. *Cereal Chemistry*, 69, 198-202.

Knuckles, B.E., Yokoyama, W.H., & Chiu, M.M. 1997a. Molecular characterisation of barley β-glucan by size exclusion chromatography with multiple angle laser light scattering and other detectors. *Cereal Chemistry*, 74, 599-604.

Knuckles, B.E., Hudson, C.A., Chiu, M.M. 1997b. Effect of Beta glucan barley fractions in high fibre bread and pasta. *Cereal Food World*, 42, 94-99.

Klopfenstein, C.F. & Hoseney, R.C. 1987. Cholesterol lowering effect of β-glucan enriched bread. *Nutrition Reports International*, 36, 1091-1098.

Kritchevsky, D. 1997. Cereal fibre and lipidemia. *Cereal Foods World*, 42, 81-85.

Lehtonen, M, & Ailasalo, R. 1987. β-glucan in two- and six-rowed barley. *Cereal Chemistry,* 64, 191-193.

Lund, E.K., Gee, J.M., Brown, J.C., Wood, P.J., & Johnson, I.T. 1989. Effect of oat gum on the physical properties of the gastrointestinal contents on the uptake of D-galactose and cholesterol by rat small intestine *in vitro*. *British Journal of Nutrition*, 62, 91-101.

Martinez, V.A., Newman, R.K., & Newman C.W. 1991. Barley diets with different fat sources have hypocholestrolemic effects in chicks. *Journal of Nutrition*, 122, 1070-1076.

McCleary, B.V. 1988. In: W.A. Wood & S.T. Kellogg (eds). *Methods in Enzymology*. Academic Press, San Diego, USA. pp. 511-514.

McCleary, B.V. 2001. Measurement of dietary fibre components: the importance of enzyme purity, activity and specifity. In: McCleary, B.V., & Prosky, L. eds. *Advanced dietary fibre technology*. London, Blackwell Science Ltd, 89-105.

Morgan, A.G., Gill, A.A., & Smith, D.B. 1983. Some barley grain and green malt properties and their influence on malt hot water extract. I. β-glucan solubilase and endo- β-glucanase. *Journal of the Institute of Brew*ing. 89, 283-291.

Morgan, K.R., & Ofman, D.J. 1998. Glucagel, a gelling β-glucan from barley. *Cereal Chemistry*, 75, 879-881.

Mourot, J., Thouvenot, P., Couet, C., Antoine, J.M., Krobicka, A., & Debry, G. 1988. The relationship between the rate of gastric emptying and glucose and insulin responses to starchy foods in healthy young adults. *American Journal of Clinical Nutr*ition, 48, 1035-1040.

Newman, R.K., Newman, C.W., Hofer, P.J., & Barnes, A.E. 1991. Growth and lipid metabolism as affected by feeding of hull-less barleys with and without supplemented β-glucanase. *Plant Foods and Human Nutrition*, 41, 371-380.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., & Hofer, P.J. 1992. Comparison of the cholesterol lowering properties of whole barley, oat bran and wheat red dog in chicks and rats. *Cereal Chemistry*, 69, 240-244.

Nishimune, T., Yakushiji, T., Sumimoto, T., Taguchi, S., Konchi, Y., Nakahara., S., Ichikawa, T., & Kunita, N. 1991. Glycaemic response and fibre contents of some foods. *American Journal of Clinical Nutrition,* 54, 414-419.

O.Dea, K., Nestel, P.J., Antonoff, L. 1980. Physical factors influencing postprandial glucose and insulin responses to starch. *American Journal of Clinical Nutr*ition, 33, 760-765.

Odes, H.S., Lazovski, H., Stern, I., & Madar, Z. 1993. Double blind trial of high dietary fibre, mixed grain cereal in patients with chronic constipation and hyperlipidaemia. *Nutrition Research*, 13, 979-985.

Palmer, G.H. 1987. Influence of cell wall structure on enzyme breakdown of the endosperm of germinated barley. *Journal of the Institute of Brewing*, 93, 105-107.

Preece, I.A., & MacKenzie, K.G. 1952. Non-starchy polysaccharides of cereal grains II. Distribution of water-soluble gum like materials in cereals. *Journal of the Institute of Brewing*. 58, 353-362.

Ranhotra, G.S., Gelroth, J.A., Astroth, K., & Bhatty, R.S. 1991. Relative lipidemic responses in rats fed barley and oat meals and their fractions. *Cereal Chemistry*, 68, 548-551.

Reimer, A.R., Thomson, A.B.R., Rajotte, R.V. Basu, T.K., Ooraikul, B., & McBurney, M.I. 2000. Proglucagon messenger ribonucleic acid and intestinal glucose uptake are modulated by fermentable fibre and food intake in diabetic rats. *Nutrition Research*, 20, 851-864.

Ross, S.W. Brand, J.C., Thorburn, A.W., & Truswell, A.S. 1987. Glycaemic index of processed wheat products. *American Journal of Clinical Nutrition.* 46, 631-635.

Saulnier, L., Gevaudan, S., & Thibault, J.F. 1994. Extraction and partial characterisation of β-glucan from the endosperms of two barley cultivars. *Journal of Cereal Science*, 19, 171-178.

Stuart, I.M., Loi, L., and Fincher, G. B. 1988. Varietal and environmental variation in 1→3) (1→4) -β-glucan levels and (→3) (1→4) -β-glucanase potential barley: Relationships to malting quality. *Journal of Cereal Sci*ence, 7, 61-71.

Symons, L.J. & Brennan, C.S. (2004a) The effect of barley β-glucan fibre fractions on starch gelatinisation and pasting characteristics. *Journal of Food Science* (In Press).

Symons, L.J. & Brennan, C.S. (2004b) The physicochemical and nutritional evaluation of wheat breads supplemented with (1-3) (1-4)-β-D-glucan rich fractions from barley. *Journal of Food Science* (In Press).

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. *Journal of Food Science*, 62, 1192-1201

Thorburn, A., Muir, J., & Proietto, J. 1983. Carbohydrate fermentation decrease hepatic glucose output in healthy subjects. *Metabolism*, 42, 780-785.

Truswell, A.S. (2002) Cereal grains and coronary heart disease. *European Journal of Clinical Nutrition*. 56,1-14.

Tudorica C.M., Kuri, V. & Brennan, C.S. (2002a) Nutritional and physicochemical characteristics of dietary fibre enriched pasta. *Journal of Agricultural and Food Chemistry,* 50*,* 347-356.

Tudorica, C.M., E. Jones, Kuri , V. & Brennan, C.S. (2002b) Evaluation of milk coagulation and curd rheological and structural characteristics as influenced by beta glucan incorporation. In *Progress in Rheology: Theory and Applied.* (Ed F.J. Martinez Boza, A. Guerrero, P. Partal, J.M. Franco and J. Munoz) Publidisa, Sevilla, Spain. pp405-408.

Tudorica, C.M., Brennan, C.S., Kuri, V., & E. Jones (2002c) Yoghurt rheology and microstructure as affected by barley beta glucan inclusion. In *Progress in Rheology: Theory and Applied.* (Ed F.J. Martinez Boza, A. Guerrero, P. Partal, J.M. Franco and J. Munoz) Publidisa, Sevilla, Spain. pp425-427.

Ulrich, S.E., Clancy, J.A., Eslick, R.F., and Lance, R.C.M. 1986. β-glucan content and viscosity of extracts from waxy barley. J*ournal of Cereal Sci*ence, 4, 11-18.

Valle-Jones, J.C. 1985. An open study of oat bran meal biscuits (Lejifibre) in the treatment of constipation in the elderly. *Current Medical Research and Opinion*, 9, 716-720.

Velasquez, M., Davies, C., Marret, R., Slavin, J.L., Feirtag, J. M. 2000. Effect of oligosaccharides and fibre substitutes on short chain fatty acid production by human microflora. *Anaerobe*, 6, 87-92.

Wang, L., Newman, R.K., Newman, C.W., & Hofer, P.J. 1992. Barley β-glucan alters intestinal viscosity and reduce plasma cholesterol concentrations in chicks. *Journal of Nutrition*, 122, 2292-2297.

Wisker, E., Daniel, M., Rave, G., Feldeim, W. 2000. Short chain fatty acids produced by *in vitro* from fibre residues obtained from mixed diets containing different breads and human faeces during ingestion of diets. *British Journal of Nutrition*, 84, 31-37.

Wolever, T.M.S. 1990. Relationship between dietary fibre content and composition in foods and the glycaemic index. *American Journal of Clinical Nutrition,* 51, 72-75.

Wood, P.J., Paton, D., & Siddiqui, I.R. 1977. Determination of β-glucan in oats and barley. *Cereal Chemistry*, 54, 524-533.

Wood, P.J., Fulcher, R.G., & Stone, B.A. 1983. Studies on the specifity of interaction of cereal cell wall components with Congo Red and Calcoflour: Specific detection and histochemistry of (1→3) (1→4) -β-D-glucan(1→3) (1→4) -β-D-glucan. *Journal of Cereal Science*, 1. 95-110.

Wood, P.J., Weisz, J., Fedec, P., & Burrows, V.D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3) (1→4) -β-D-glucan. *Cereal Chemistry*, 66, 97-103.

Wood, P.J. Braaten, J.T. Fraser, W.S. Riedel, D. Poslel, M. 1990. Comparisons of viscous properties of oat and guar gum and effects of these and oat bran on glycaemic index. *Journal of Agricultural and Food Chemistry*, 38, 753-757.

Wood, P.J. 1993. Physicochemical characteristics and physiological properties of oat (1-3) (1-4) β-D-Glucan. In: Wood P.J. ed. *Oat Bran*. AOAC, St Paul, MN, 83-112

Wood, P.J., Braaten, J.T., Scott, F.W. Riedel, K.D. Wolynetz, M.S. & Collins, M.W. 1994a. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*, 72, 731-743.

Wood, P.J., Weisz, J., & Blackwell, B.A. 1994b. Structural studies of (1→3) (1→4) -β-D-glucans by 13C-NMR by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chemistry*, 71, 301-307.

Woodward, J.R. Ficher, G.B. & Stone, B.A. 1983. Water soluble (1-3) (1-4) β-D-Glucan from barley (*Hor~~e~~deum vulgare*) endosperm. II. Fine structure. *Carbohydrate Polymers*, 3, 207-225.

Woodward, J.R., Philips, D.R., & Ficher, G.B. 1988. Water soluble (1-3) (1-4) β-D-Glucan from barley (*Hor~~e~~deum vulgare*) endosperm. IV. Comparison of 40^{o}C and 65^{o}C soluble fractions. *Carbohydrate Polymers*, 8, 85-97.

Wursch, P., Pi-Sunyer, F.X. 1997. The role of viscous soluble fibre in the metabolic control of diabetes-a review with special emphasis on cereals rich in β-glucan. *Diabetes Care*, 20, 1774-1780.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnland, J.R., & Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycaemic response. *Cereal Chemistry*, 74, 293-296.

Yoon, S.H., Berglund, P.T. & Fastnaught, C.E. 1995. Evaluation of selected barley cultivars and their fractions for β-glucan enrichment and viscosity. *Cereal Chemistry*, 72, 187-190.

Zhang, G., Chen, J. and Wang, J. 2002. Variation in barley endosperm β-glucan content in three barley cultivars as a function of spike number and within spike. *Journal of Cereal Science*, 35, 99-101.

2.2:- The extraction procedures of barley β-Glucan fibre fractions, and their effects on starch gelatinization and pasting characteristics

Introduction

(1→3) (1→4)-β-D-Glucan, referred to as β-glucan, is a cell wall polysaccharide of cereal grains. Barley β-glucan (BBG) additions in human diets have been associated with lowered plasma cholesterol and postprandial serum glucose and insulin responses (Anderson *et al* 1990; Hallfrisch *et al* 1995; Wood *et al* 1990, 1993). Such effects of β-glucan are apparently related to its ability to form highly viscous solutions and their solubility, which is controlled by molecular weight. These beneficial properties have led to a demand for the incorporation of β-glucan into various food systems.

Key extraction methodologies of β-glucan from barley and oat were developed by Wood *et al* (1977, 1978) and, recently, by Bhatty (1993, 1995), who investigated the influence of different solvents on recovery and viscosity of barley and oat β-glucan. More recent studies by Temelli (1997) and by Burkus and Temelli (1998) investigated the effects of concentration, temperature, and pH on the rheological properties of BBG gums concluding that extraction conditions have an influence upon the molecular weight and functional behaviour of β-glucan. This is supported by Knuckles *et al* (1997) who found sequential extraction of β-glucan resulted in a decrease in molecular weight and an alteration in molecular weight.

Starch-hydrocolloid interactions have been investigated by many workers for more than two decades. Studies have examined a variety of hydrocolloids, including guar gum, locust bean gum, xanthan gum, pectin, aliginate, kappa-carrageenan, hydroxypropylmethylcellulose (HPMC), arabinoxylan, konjac flour and gellan in a wide spectra of cereal flours starches (maize starch, wheat flour, waxy maize, wheat starch, corn, waxy corn, tapioca)(Shi and BeMiller 2002; Alloncle and Doublier 1991; Rojas *et al* 1999; Biliaderis *et al* 1997; Bahnassey and Breene 1994; Cameron *et al* 1993; Christianson *et al* 1981; Alloncle *et al* 1989). The effect these hydrocolloids at low concentrations (0.1-0.2% (w/w)) on starch during pasting/gelatinisation have been measured using a number of methods (viscometer amylographic analysis, differential scanning calorimetry, dynamic rheometry, rapid visco

analysis, optical microscopy and ultra violet spectrophotometry). These studies have revealed that variations in starch pasting characteristics (increase or decrease, greatly or slightly, or no significant effect) are dependent upon hydrocolloid type, starch source, concentration and method of measurement.

The food industry has the potential to be an important user of BBG; however, there is a lack of information on the functionality and behaviour of β-glucan in food systems. The objectives of this study were to evaluate the effect of (a) extraction condition on yield and composition of BBG fibre fractions from barley and (b) the subsequent effects of these fractions on the thermal properties and pasting characteristics of wheat starch.

Materials and Methods

Whole kernels of Riviera, a nonwaxy feed variety barley (4.01% β-glucan, db) were finely ground in a laboratory mill (Glen Creston, Stanmore, UK) to pass through a 500-µm-mesh screen. Unmodified wheat starch (S5127) was used in thermal processing experiments (Sigma-Aldrich, Dorset, UK).

Extraction of BBG fibre fraction*s*

BBG fibre fractions were prepared from barley flour using the method of Wood *et al* (1978), with some modifications as used by Temelli (1997). Five extraction treatments were used, (a) water extraction (water fraction), (b) flours refluxed once with ethanol (99.9%) for 10 min (refluxed fraction), (c) extracts of refluxed flours were treated with thermostable alpha amylase (Termamyl, National Center for Biotechnology Education, Reading, UK) at levels of 1 mL enzyme to 100 mL extraction buffer for 1 h at 98 ^{o}C to eliminate starch impurities (purified fractions); (d) extraction at pH 10 achieved by the addition of a few mL of 1 M NaOH (alkali fractions), and (e) extracts from untreated flour were boiled for 1 h immediately after total solids separation (boiled fractions). All treatments were performed at 55 ^{o}C and pH 7 with the exception of treatment d, where pH 10 was used. Figure 1 illustrates the basic extraction treatment.

Chemical composition of BBG fibre fraction*s*

Moisture, starch, protein, and β-glucan contents of whole flour and BBG fibre fractions were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000). Total starch, total dietary fibre, and β-glucan were determined using the total starch assay kit (Approved Method 76.13; AACC 2000), total dietary fibre assay kit (Approved Method 32-07; AACC 2000), and β-glucan enzymatic assay kit (Approved Method 32-23; AACC 2000), respectively. All assay kits were supplied by Megazyme^{TM} International Ireland Ltd, Wicklow, Ireland. Nitrogen was determined using a nitrogen analyzer (Model FP-2000; Leco Instruments Ltd, St Joseph, MI) and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis.

Water retention capacity (WRC) of BBG fibre fractions

WRC was determined by the procedure of Robertson *et al* (2000) with some modifications. Each fibre fraction (1 g) was hydrated in tubes containing 30 mL of distilled water for 18 h at room temperature. Following hydration, samples were centrifuged (3,000 x *g* for 20 min). The supernatant was decanted and the sample left to drain. Sample fresh weight was recorded before drying (120 ^{o}C for 2 h). WRC was calculated as the amount of water retained by the pellet (g/g dry weight) after draining.

Pasting characteristics of wheat starch

Peak viscosity (PV), final viscosity (FV), and breakdown (BD) development of wheat starch substituted with 1 and 5% BBG fibre fraction were determined using a Rapid Visco Analyser (RVA-4 Newport Scientific PTY, Australia). An RVA Standard One Profile was used with heating and cooling rates of 12 ^{o}C per min, over a temperature range of 50-95 ^{o}C, and paddle speed of 160 rpm. Samples were prepared by mixing 3.5 g ($\pm$0.1) in 25 mL ($\pm$0.1) distilled water in an aluminum canister.

Thermal properties of wheat starch

Differential scanning calorimetry (DSC 12E; Mettler Toledo, Leicester, UK) was used to measure thermal parameters (onset of gelatinization, T_{onset}; gelatinization peak temperature, T_p; gelatinization end point, T_{endset}; and total enthalpy, (j/g) of wheat starch substituted with 1% and 5% BBG fibre fraction. The starch-to-distilled water ratio was 1:4. Nominal scan rate was 5 ºC/min over a 20-100 ºC heating rate.

Statistical analysis

Sample extractions, chemical analyses, and RVA and DSC measurements of each starch mixture were performed in duplicate. Analysis of variance of the results (ANOVA) were performed using the Minitab 13 statistical software package. Significance was defined as $p<0.05$.

Results and Discussion

Effect of treatment on yield of BBG fibre fraction and β–glucan recovery

Figure 2 illustrates the effect of extraction treatment on the yield of BBG fibre fraction and β–glucan extraction efficiency. Yield of BBG fibre fraction (wt of fraction/wt 50 g flour) from the different extraction treatments ranged from 4.48-6.05%. Significantly higher yields were achieved with purification (6.05%) and refluxing treatments (6.04%), whereas alkali extraction produced the lowest yields (4.48%).

Efficiency of β–glucan extraction was determined by dividing, g β–glucan in 100 g fraction by g β–glucan in 100 g flour. β–Glucan recovery was higher in purified fractions (59%) than all other fractions, followed by the refluxed fraction which had a β–glucan recovery efficiency of 53%. The lower extraction efficiency achieved with a water treatment (pH 7/55 ^{o}C) (47%) may be a result of β–glucan cleavage by β–glucanase enzymes, while in the boiled (47.1%) and alkali (39%) fractions lower extraction efficiency may be attributed to thermal degradation and/or starch contamination, or alkali depolymerisation, respectively. Beer *et al* (1996), Temelli (1997), and Burkus and Temelli (1998) reported loss of β–glucan under alkali conditions. Enzymic cleavage, thermal degradation, and acid/alkali depolymerisation that result in loss of β–glucan also cause a loss in molecular weight (Beer *et al* 1997), reducing the functional properties of β–glucan.

Optimum conditions for β-glucanase activity appear to be at neutral pH. Temelli 1997 found a significant reduction in apparent viscosity of solutions containing 1% β-glucan extracts recovered at pH 7, and concluded that since extracts recovered at a higher pH (but with lower β-glucan contents) had higher apparent viscosity, β-glucanase had an important role in reducing viscosity. Popular methods for inhibiting β-glucanase activity include autoclaving and treatment with hot aqueous ethanol (Forrest and Wainwright 1977; Balance and Manners 1978; Wood *et al* 1983, 1990, 1991; Carr *et al* 1990). Beer *et al* (1996) observed a 12% reduction in β-glucanase (U / kg) in oat bran found treated with 75% ethanol for 4 h at 80 ^{o}C, compared to non-treated oat bran. Extracts from the treated oat bran were higher in β-glucans and exhibited increased solution viscosity, although yield was lower. Knuckles and Chiu (1999) observed similar results with barley flours treated with 70% ethanol.

Effect of extraction condition on composition of BBG fibre fractions

β–Glucan, protein, total dietary fibre and starch contents of BBG fibre fractions, from different extraction treatments, are compared in Table 1. The β–glucan contents of the fractions lay between 63.6-73.1%. The purified treatment gave the highest yield of β–glucan and total dietary fibre in the extracted fraction; this coupled with the lower starch and protein content within this fraction may help to explain the higher water holding capacity observed. Similar β–glucan levels were achieved with fractions extracted with water, refluxed, and alkali treatments. Boiled fractions had significantly lower β–glucan contents. It is possible that β–glucan was degraded by thermal treatment. The starch contents of the BBG fibre fractions were between 1.18-6.95%. Purified fractions, as expected, had the lowest level of starch contamination, whilst boiled fractions had the highest level of starch contamination (6.95%). Similar effects of starch contamination of oat gums at high concentrations have been reported by Dawkins and Nnanna (1993). There is evidence to suggest that high temperatures result in degradation of β-glucans. Burkus and Temelli (1998) found that a β-glucan extracted at 86 ^{o}C, and subjected to a 90 ^{o}C heat treatment for 30 min, had an apparent viscosity 45% higher than an extract that had been boiled for a similar time period.

Water retention capacity (WRC) of BBG fibre fraction*s*

WRC is defined as the amount of water retained by a known weight of fibre under the conditions used (Robertson *et al* 2000). Extraction treatment significantly affected the WRC of the BBG fibre fractions (Figure 3). Purified fractions exhibited the highest WRC (8.37 g/g dry weight). The lowest (WRC) was exhibited by the alkali fractions (6.41 g/g dry weight). This difference in water retention capacity has been shown to be related to the amount of soluble and insoluble fibre within the gel matrix (Robertson *et al* 2000). In this particular case, both the total amount of dietary fibre and also the BBG content of these fractions appear to be related to increased WRC.

Thermal properties of wheat starch substituted 1 and 5% BBG fibre fraction*s*

The thermal properties (T_{onset}, T_{endset}, T_{peak}, and enthalpy (J/g)) of wheat starch substituted with 1 and 5% BBG fibre fractions are illustrated in Table 2. BBG fibre fraction addition did not

significantly affect T_{onset}, T_{endset}, and T_{peak} ($p>0.05$), however, enthalpy was altered in comparison to the control wheat starch ($p<0.05$). Addition of 5% BBG fibre fraction universally resulted in a decrease in the total enthalpy value of wheat starch compared against the value of control wheat starch ($p<0.05$). Lowest enthalpies were observed in starch substituted with 5% refluxed fraction (7.43 J/g) and boiled fraction (7.64 J/g). These reduced values do not appear to be related solely to total fibre β-glucan contents, but may also be a result of protein and starch interactions within the starch and fibre matrix or as a result of an alteration in the thermal properties of starches within the extracted fractions.

Addition of 1% water, alkali, and boiled BBG fibre fractions also resulted in a significant decrease in enthalpy. Again, these observations do not appear to be related to β-glucan content, but again maybe as a result of functional behaviour of the starches within the extraction. Addition of 1% boiled and purified fractions did not have any effect on the enthalpy value of starch against the control ($p>0.05$).

Pasting properties of wheat starch substituted 1 and 5% BBG fibre fraction*s*

The pasting characteristics (peak viscosity (PV), breakdown (BD) and final viscosity (FV)) of wheat starch pastes with 1 and 5% BBG fibre fraction substitutions are illustrated in Table 3 and 4, respectively. Values were compared against the control.
The peak viscosity of wheat starch was not significantly affected by the addition of 1% BBG fibre fractions ($p>0.05$), although there was a general increase in peak and final viscosity of samples with 1% BBG. Samples with 5% water gum and 5% purified fractions showed a decrease in peak viscosity ($p<0.05$) when compared against the control. Breakdown values of wheat starch substituted with 5% BBG fibre fractions were lower in comparison to the control ($p<0.05$). The addition of 1% BBG had no influence on breakdown ($p>0.05$). Substitution of wheat starch with 5% fraction resulted in a decrease in final viscosity. Lowering was independent of fraction type. These lower values are an indication of a reduction in starch available for gelatinization. This reduction is likely to be due to water being withheld from the starch granules by the β-glucan, and from a general reduction in the starch content of the pastes due to replacement with BBG.

Bahnassey *et al* (1994) used Rapid Visco Analyser to assess the effects of different levels of hydrocolloids (konjac, guar, gellan, xanthan and locust bean gum) on various starch cooking properties. A noticeable increase in viscosity was observed with 0.4% locust bean gum addition.

The increase in peak viscosity of starch/hydrocolloid systems was believed to be associated with the release of amylose and low molecular weight amylopectin, and the subsequent formation of polymer complexes which significantly altered the viscosity of the system. A similar explanation was also been proposed by Shi and BeMiller (2002) with analogous hydrocolloids.

In the studies of Biliaderis *et al* (1997) the interactions of starch hydrocolloids were evaluated using DSC and dynamic rheometry. Xanthan, beta glucan, arabinoxylan, guar gum were incorporated at (1-2%, w/w) in concentrated aqueous dispersions (40%, w/w) of maize and wheat starch. The gums did not affect the gelatinisation temperature of the maize or the wheat starch, and did not influence the rheology of wheat starch gel. However the gelation of maize starch was retarded by hydrocolloid addition. The mechanism by which this decrease in the development of amylopectin-hydrocolloid gels occurred was believed to be attributed to the interference of intermolecular associations among amylopectin molecules by the polysaccharide. It is plausible that similar factor may be affecting the pasting and gelatinisation characteristics of the glucan-starch gel systems reported in this study.

Conclusion

The observations from our research have importance in the application of BBG fibre fractions in foods and in human nutrition, particularly where starch is a primary ingredient, for example bread and pasta. The high WRC exhibited by BBG fibre fractions and the associated reduction in gelatinisation of starch has relevance to human nutrition and the regulation of *in vitro* and *in vivo* glycaemic response in carbohydrate-rich diets where the degree of starch gelatinization can affect the postprandial sugar availability of foods.

Results also indicate that in the processing of β-glucan-enriched foods, considerable alterations to formulations may have to be introduced. This would be particularly important in bread products where water absorption can significantly influence processing and final product quality.

Prior to β-glucan incorporation the choice of extract treatment would have to be carefully assessed, as this may have an influence on the functional behaviour of β-glucans in a food system. Our results illustrate that refluxing and purification treatments yield fractions with the highest β-glucan purity and, therefore, from all treatments was the most efficient treatment that

might be considered in large-scale BBG fibre fraction production. The aim of further research will be to incorporate extracted BBG fibre in food models and assess the physicochemical changes that occur and the necessary modifications in food formulations that come about.

References

AACC. 2000. 10th Edition. Approved Methods. St. Paul. Minnesota. The American Association of Cereal Chemists.

Alloncle M, Lefebure J, Llamas G, Doublier JL. 1989. A rheological characterisation of cereal starch galactomannan mixtures. Cereal Chem 66:90-93.

Alloncle M, Doublier JL. 1991. Viscoelastic properties of maize starch hydrocolloid pastes and gels. Food Hydrocolloids 5:455-467.

Anderson JW, Deakins DA, Floore TL, Smith BM, Whitis SE. 1990. Dietary fibre and coronary heart disease. Critical Reviews in Food Science & Nutrition 29:95-147.

Bahnassey YA, Breene WM. 1994. Rapid Visco-Analyser (RVA) pasting profiles of wheat, corn, waxy corn, tapioca, and amaranth starches (*A. hypochondriacus and A. cruentus*), in the presence of konjac flour, gellan, guar, xanthan, and locust bean gums. Starch-Starke 46:134-141.

Ballance GM, Manners DJ. 1978. Structural analysis and enzymic solubilsation of barley endosperm cell walls. Carbohydrate Research 61:107-118.

Beer MU, Arrigoni E, Amado R. 1996. Extraction of oat gum from oat bran: effects of process on yield, molecular weight distribution, viscosity and (1→3) (1→4) -β-D-glucan content of gum. Cereal Chem 73:58-62.

Beer MU, Wood PJ, Weisz J, Fillion N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3) (1→4) -β-D-glucan extracted from oat products by an in vitro digestion system. Cereal Chem 74:705-709.

Bhatty RS. 1993. Extraction and enrichment of (1→3) (1→4) -β-D-glucan from barley and oat brans. Cereal Chem 70:73-77.

Bhatty R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J Cereal Sci 22:163-170.

Biliaderis CG, Arvanitoyannis I, Izydorczyk MS, Prokopowich DJ. 1997. Effect of hydrocolloids on gelatinisation and structure formation in concentrated waxy maize and wheat starch gels. Starch-Starke 49:278-283.

Burkus Z, Temelli F. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum. Cereal Chem 75:805-809.

Carr JM, Glatter JL, Jeraci JL, Lewis BA. 1990. Enzymic determination of β-glucan in cereal-based food products. Cereal Chem 67:226-229.

Cameron RE, Sansom CM, Donald AM. 1993. The interactions between hydoxypropylcellulose and starch during gelatinisation. Food Hydrocolloids 7:181-193.

Christianson DD, Hodge JE, Osborne D, Detroy RW. 1981. Gelatinisation of wheat starch as modified by xanthan gum, guar gum and cellulose gum. Cereal Chem 58:513-517.

Dawkins NL, Nnanna IA. 1993. Studies on oat gum and β-glucan extraction from oat bran and rolled oats: Temperature & pH effects. J Food Sci 58:562-566.

Forrest IS, Wainwright T. 1977. The mode of binding of β-glucans and pentosans in barley endosperm cell walls. Journal of the Institute of Brewing 83:279-286

Hallfrisch J, Schofield DJ, Behall KM. 1995. Diets containing soluble oat extracts improve glucose and insulin response of moderately hypocholestrolemic men and women. Am J Clin Nutr 61:379-384.

Knuckles BE, Yokoyama WH, Chiu MM. 1997. Molecular characterisation of barley β-glucan by size exclusion chromatography with multiple angle laser light scattering and other detectors. Cereal Chem 74:599-604.

Knuckles BE, Chiu MCM. 1999. β-glucanase activity and molecular weight of β-glucans in barley after various treatments. Cereal Chem 76:92-95.

Robertson JA, Monredon DF, Dysler P, Guillon F, Amado R, Thibault JF. 2000. Hydration properties of dietary fibre and resistant starch: a European Collaborative study. Lebensm-Wiss Technol 33:72-79.

Rojas JA, Rosell CM, de Barber CB. 1999. Pasting properties of different wheat-flour hydrocolloid systems. Food Hydrocolloids 13:27-33.

Shi XH, BeMiller JN. 2002. Effects of food gums on viscosities of starch suspensions during pasting. Carbohydrate Polymers 50:7-18.

Temelli F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J Food Sci 62:1192-1201

Wood PJ, Paton D, Siddiqui IR. 1977. Determination of β-glucan in oats and barley. Cereal Chem 54:524-533

Wood PJ, Siddiqui IR, Paton D. 1978. Extraction of high-viscosity gums from oats. Cereal Chem 54:524-533

Wood PJ, Fulcher RG, Stone BA. 1983. Studies on the specifity of interaction of cereal cell wall components with Congo Red and Calcoflour: Specific detection and histochemistry of (1→3) (1→4) -β-D-glucan(1→3) (1→4) -β-D-glucan. J of Cereal Sci 1:95-110.

Wood PJ, Braaten JT, Fraser WS, Riedel D, Poslel M. 1990. Comparisons of viscous properties of oat and guar gum and effects of these and oat bran on glycaemic index. J Ag Food Chem 38:753-757.

Wood PJ, Weisz J, Blackwell B. 1991. Molecular characterisation of cereal β-glucans. Structural analysis of oat- β-D-glucan and rapid structural evaluation of β-D-glucans from different sources by high-performance liquid chromatography of oligosacchardies released by lichenase. Cereal Chem 68:31-39.

Wood PJ. 1993. Physicochemical characteristics and physiological properties of oat (1-3) (1-4) β-D-glucan. Pages 83-112 in Oat Bran. Wood PJ editor. St. Paul. Minnesota. The American Association of Cereal Chemists.

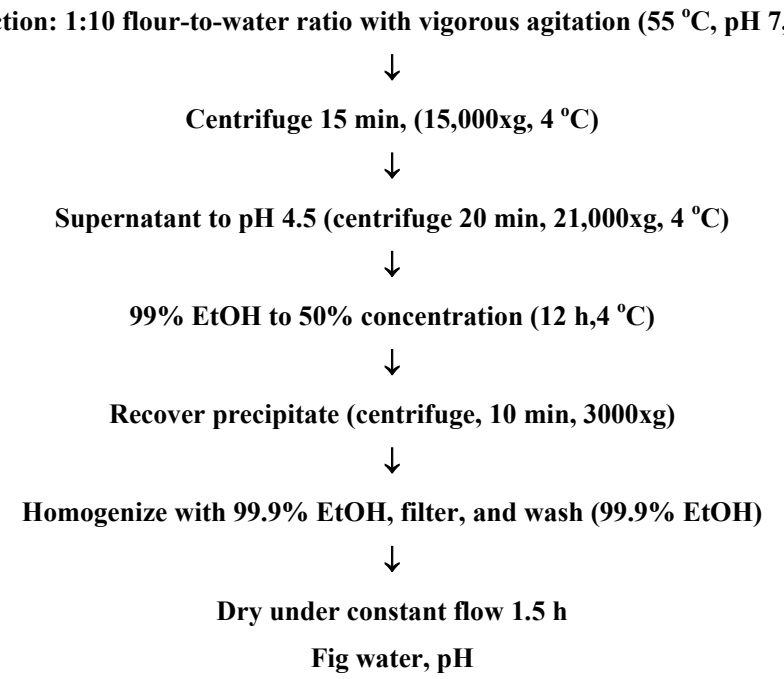


Figure 1. Generalized BBG fibre extraction procedure (Wood *et al* 1978; Temelli 1997)

Table 1. Composition of BBG fibre fractions extracted at 55 °C with differing treatments

BBG	Extraction pH	Total dietary fibre (%)	β-Glucan (%)	Protein (%)	Starch (%)
Water	7	88.60[a,b,c]	69.75[a]	3.74 [a]	4.04
Refluxed	7	88.06[a,b,c]	71.53[a]	4.55	2.68 [a]
Purified	7	91.62[b]	73.05[a]	3.75 [a]	1.18
Alkali	10	87.60[a,c]	70.06[a]	6.90	2.99 [a]
Boiled	7	84.80[c]	63.53	5.01	6.95

[a]means in the same column followed by the same letter are not significantly different (p>0.05)

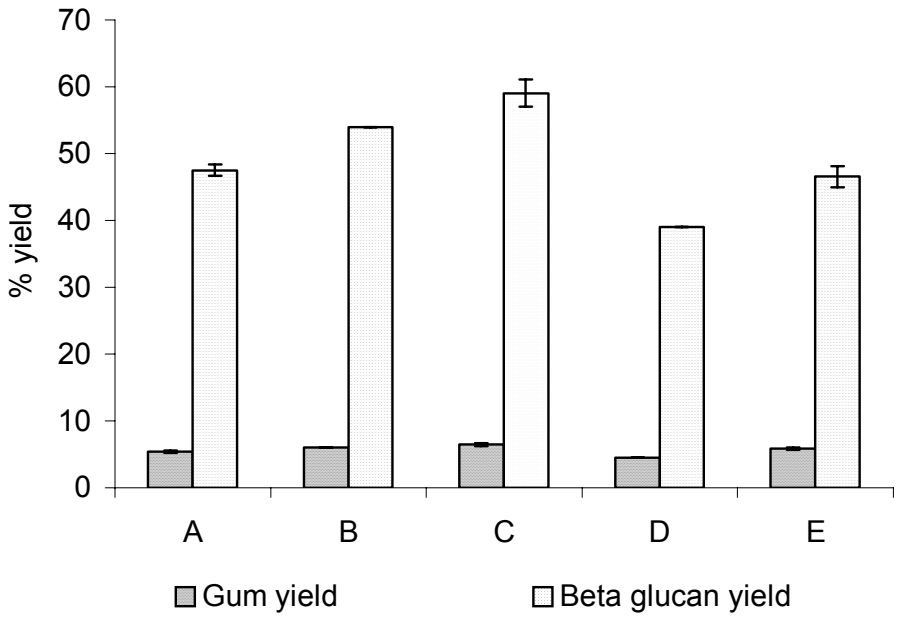


Figure 2. Percentage BBG fraction yield from original flour, and β-glucan content of such fractions from:- water (A), refluxed (B), purified (C), alkali (D), and (E) boiled treatments. Standard errors are included on the figure as error bars.

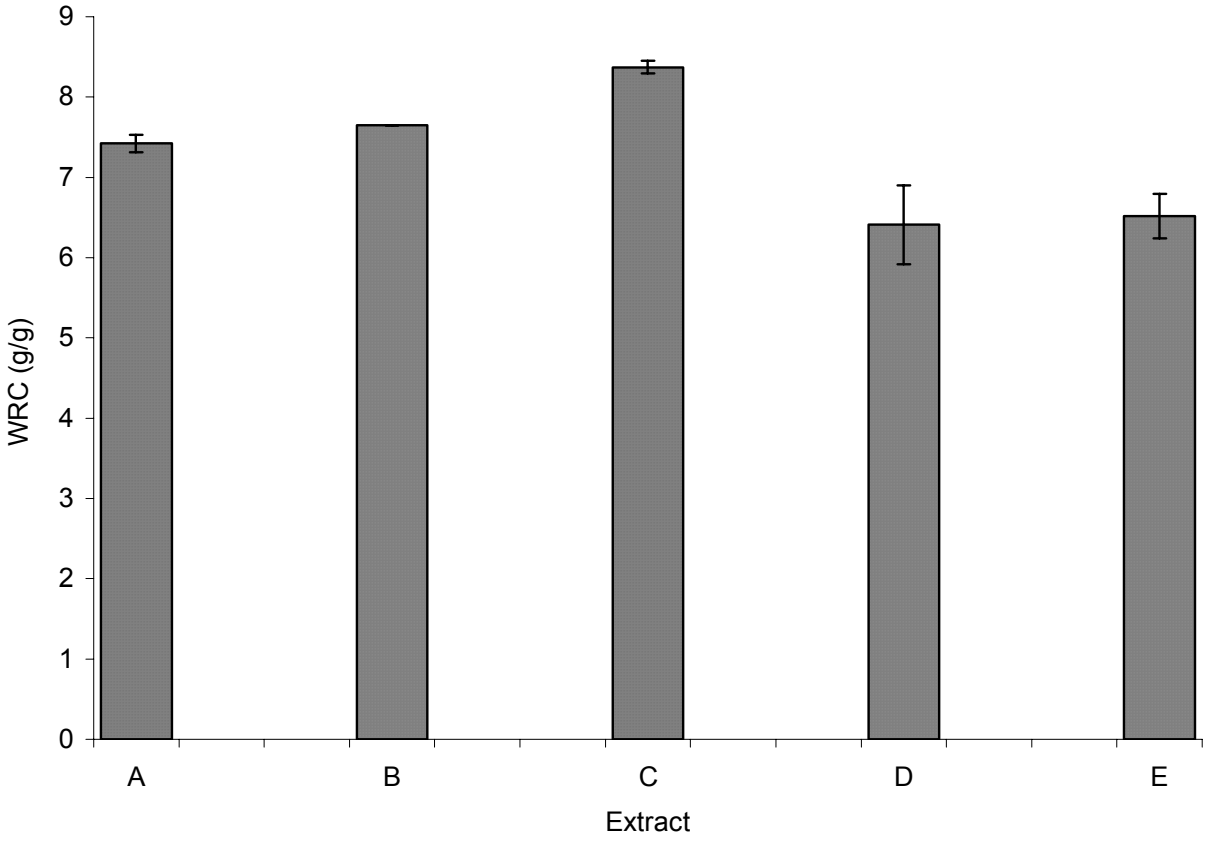


Figure 3. Water retention capacity of the extracted BBG fibre fractions from:-water (A), refluxed (B), purified (C), alkali (D) and boiled (E) treatments. Standard errors are included on the figure as error bars.

Table 2. Thermal parameters (T_{onset}, T_{peak}, T_{endset}; and enthalpy (J/g)) of wheat starch substituted with 1 and 5% water, refluxed, purified, alkali, and boiled BBG fibre fractions

	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)	Enthalpy (J/g)
Control	52.00	69.50	58.80	8.84[a]
1% Water fraction	51.05	70.55	59.00	8.44[b]
5% Water fraction	52.10	69.65	59.40	8.40[b]
1% Refluxed fraction	52.05	70.00	59.01	9.07[a]
5% Refluxed fraction	52.05	69.60	59.00	7.43[c]
1% Purified fraction	51.75	69.65	59.25	8.98[a]
5% Purified fraction	52.10	71.90	59.4	8.19[b,d]
1% Alkali fraction	51.95	69.95	59.35	8.37[b]
5% Alkali fraction	51.99	70.20	59.29	7.89[d,e]
1% Boiled fraction	51.83	70.21	59.26	8.66[b]
5% Boiled fraction	52.05	69.65	59.10	7.64[c,e]

[a]means of J/g values followed by same letter are not significantly different ($p>0.05$)

Table 3. Peak (PV), breakdown (BD), and final viscosity (FV) of wheat starch substituted with 1% water, refluxed, purified, alkali, and boiled (E) BBG fibre fractions

	PV	BD	FV
Control	4046[a]	1163 [a]	4622 [a]
Water fraction	4161[a]	1185 [a]	4724 [a]
Refluxed fraction	4476[a,]	1186 [a]	5153 [a]
Purified fraction	4393[a]	1205 [a]	4959 [a]
Alkali fraction	4428 [a,]	1160 [a]	4990 [a]
Boiled fraction	4508 [a,]	1255[a]	5120 [a]

[a]means values in same column followed by same letter are not significantly different ($p>0.05$)

Table 4. Peak (PV), breakdown (BD), and final viscosity (FV) of wheat starch substituted with 5% water, refluxed, purified, alkali, and boiled (E) BBG fibre fractions

	PV	BD	FV
Control	4046[a]	1163	4622
Water fraction	3294[b]	874[a,b,c]	3543[a]
Refluxed fraction	3451[a,b]	773[b]	3882[a]
Purified fraction	3416[b]	767[a,b,c]	3627[a]
Alkali fraction	3866[a]	986[c]	4024[a]
Boiled fraction	3671[a,b]	805[a,b]	4048[a]

[a]means value, in same column followed by same letter are not significantly different ($p>0.05$)

2.3:- The influence of (1→3) (1→4)-β-D-glucan rich fractions from barley on the physicochemical properties and *in vitro* reducing sugar release of white wheat breads

Introduction

Diets high in fat and high in calories are considered as one of the key causes of coronary heart disease (CHD), obesity, and non insulin dependent diabetes mellitus (NIDDM). Dietary changes that reduce the incidence of these diseases include a reduction in fat intake and salt, and an increased intake of dietary fibre and cereals, fruits and vegetables. Cereal products are a diverse group of foods ranging from relatively unrefined whole grains to processed ready-to-eat products. Although cereal foods are rich in complex carbohydrates and are low in fat, recent media reports and the advent of diets that restrict carbohydrate intake have led to a decrease in their consumption. A prime example of his has been the negative change in the consumer perception of the nutritional characteristics of bread.

Bread, in its various forms, is considered to be one of the oldest processed cereal foods and also one of the most popular foods consumed globally. In a UK study by the Federation of Bakers (Anon 2003), 97% of consumers were found to consume bread, with 75% claiming to eat pre-packed bread at least once a week. However, in a UK report covering the perception and understanding of the health components of bread, 23% of individuals 25-34 years old regarded their consumption of starch and carbohydrates as unhealthy and were actively limiting the intake of these nutrients (Anon 2003). Additionally, whilst bread can be considered low in fat and a source of complex carbohydrates, bread (namely white, the most popular variety in the UK) is a poor source of dietary fibre, containing typically less than 2.5% (Anon 2003).

The impact of dietary fibre on the maintenance of human health has attracted considerable scientific interest in the past 3 decades. Potential health benefits of dietary fibre include bowel transit time (Feldheim and Wisker 2000), prevention of constipation, reduction in risk of colorectal cancer (Faivre and Bonithon-Kopp 1999), enhanced methanogenesis (Fernandes *et al* 2000), production of short-chain fatty acids (Velasquez *et al* 2000: Wisker *et al* 2000; Karppinen *et al* 2000), and promotion of colonic health, stimulating the growth of beneficial gut microflora (prebiotic property).

Foods high in soluble dietary fibre, are considered as low glycaemic index (GI) foods. Low GI foods are differentiated from other foods by the reduced rate at which they are digested and

release glucose to the blood (Bjorck *et al* 2000). Soluble dietary fibres are believed slow the release of reducing sugars from the food and hence lower postprandial blood glucose level by several mechanisms, including reduced amylolysis, but more specifically at the gastrointestinal level through delayed gastric emptying (Rainbird and Low 1986; Cherburt 1995) and reduced nutrient motility (Braaten *et al* 1991).

Epidemiological data strongly correlates low GI diets to reduced insulin resistance (Bjorck *et al* 2000). The potential consequences of a reduced insulin resistance are the control of diabetes and reduction of the possibility of developing risk factors for degenerative diseases, such as obesity (Burley *et al* 1987), hyperlipidemia (Jenkins *et al* 1985), and hypertension (Anderson 1983).

The β–glucans from both barley and oat grains have been shown to have an important influence on human glycaemic control. When β–glucan was isolated from oats and consumed as oat porridge it was reported to lower postprandial blood glucose (Wood *et al* 1990; Wood 1993). Additionally, barley flour enriched with β–glucans has been shown to have physiological effects comparable to other isolated fibres, such as guar gum, psyllium, and oat β–glucan (Knuckles *et al* 1997).

With their low fibre contents, breads are considered as high glycaemic foods. Several workers have examined the glycaemic response of bread as affected by fibre inclusion and reported no or variable effects on sugar release (Bhatty 1986, Hudson *et al* 1992; Holm *et al* 1992; Malki *et al* 1992). However in more recent studies, a strong correlation between the addition of soluble dietary fibre addition to bread and improved glycaemic control has been found. Pick *et al* (1998) and Cavallero *et al* (2002) found that barley β–glucan rich breads elicit a lower glycaemic response, compared to reference white wheat bread.

In European countries where white bread is commonly consumed it is clear that a demand exists for the supplementation of wheat bread with dietary fibre. However this must be achieved without compromising palatability, a problem often encountered with fibre enrichment, particularly soluble fibre addition (Jenkins *et al* 2002).

The aim of this part of the study was to evaluate the effect of adding a barley β–glucan-rich fraction (BBG FRACTION) on the physicochemical and nutritional properties of white wheat

bread, with the aim of obtaining a cereal product with improved nutritional status that can be successfully manufactured and that retains the internal, external, and textural eating qualities that make bread so acceptable to the consumer.

MATERIALS AND METHODS

Extraction of BBG FRACTION

BBG FRACTION containing approximately 70% β–glucan (db) was extracted from the milled barley flour (5.77% β–glucan) according to methodology used by Symons and Brennan (2004). Milled barley flour was suspended in pre-heated distilled water (55 ^{o}C, pH 7) and stirred with vigorous agitation for 30 min. The mixture was centrifuged (15 min 15,000 *x g*, 4 ^{o}C) and the supernatant recovered and reduced to pH 4.5 with the addition of 2M HCL. The acidified supernatant was centrifuged (20 min, 21,000 *x g*, 4 ^{o}C). Ethanol (99%) was added to 50% concentration, and the resulting mix held at 4 ^{o}C for 12 h. The precipitate was recovered by centrifugation (10 min, 3000 *x g*). The precipitate was homogenized with 99.9% ethanol, followed by washing and filtering with 99.9% ethanol. Extracts were dried in an air cabinet for 1.5 h, milled to pass 500µm screen and stored at 4 ^{o}C.

Pasting characteristics

Pasting characteristics (peak viscosity, PV; breakdown, BD; and development of final viscosity, FV) of bread wheat flour substituted with 2.5 and 5% BBG FRACTION dispersions were determined using a Rapid Visco Analyser (model RVA-4; Newport Scientific PTY, Warriewood, Australia). Test parameters included heating and cooling rates of 12 ^{o}C per minute, over a temperature range of 50-95 ^{o}C, and paddle speed of 160 rpm. Samples were prepared by mixing 3.5 g ($\pm$0.1) in 25 mL ($\pm$0.1) distilled water in an aluminium canister. Analysis were performed in triplicate.

Bread making

6.25 g (2.5%) and 12.5 g (5%) of BBG FRACTION were hydrated in 140 ml of boiling water with vigorous stirring for 5 min. Gums were subsequently stirred for 2 h. Breads, containing 0, 2.5, and 5% BBG FRACTION, were manufactured according to Approved Method 10-09 (AACC 2000), using 250 g (control), 243.75 g (2.5% BBG FRACTION) and 237.5 g (5% BBG FRACTION) strong white bread flour, 12.5 g vegetable fat, 6 g dried yeast, 6 g salt, 2 g sugar, and 140 mL of water. Doughs were divided into 70 g portions and baked in test bakery tins (length 85 mm, width 50 mm). Baked breads were cooled for 1 h prior to analysis.

Dough extensibility

Extensibility of the bread doughs was measured using a texture analyzer (model TA-XT2, Stable Micro Systems, Reading, UK) equipped with a Kiefer dough and extensibility rig (A/KIE) using a 5 kg load cell. Dough (15 g) was placed in an oiled teflon dough former for 20 min, removed with the aid of a spatula, and subjected to the tensile test. The rig extended the sample by 75 mm, at a pre-test, test, and post-test speed of 2, 3.3, and 10 mm/s, respectively. The maximum peak force in tension was regarded as the degree of elasticity of the dough, whereas the distance travelled for dough break was regarded as the extensibility of the dough.

Loaf height and volume

Loaf height was determined using calibrated callipers and reported in centimetres. Measurements were taken from the centre of each loaf. Loaf volume was measured using Approved Method 10-05, volume by rapeseed displacement (AACC 2000).

Crumb firmness

A TA-XT2 texture analyzer (Stable Micro Systems, Reading, UK) was used to measure bread firmness. An AACC 36-mm-cylinder probe with radius (model P/36R) and a 5 kg load cell was used. The probe compressed the sample by 40% at a pre-test, test, and post-test speed of 1, 1.7,

and 10 mm/s, respectively. The maximum peak force in compression was recorded as the firmness value in gram units. Measurements were taken on 1-cm slices, Only 1 measurement was taken from the slices; samples were discarded after the test.

For analysis of crumb firmness over storage (1, 2, and 4 days) slices were placed in polythene bags, sealed, and stored at ambient temperature and on the appropriate day subjected to texture analysis as the samples at day 0.

Chemical composition

Moisture, starch, protein, and β-glucan contents of the breads were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000). Total starch was determined using the total starch assay kit, Approved Method 76.13 (AACC 2000); β-glucan content was measured using the enzymatic assay kit, Approved Method 32-23 (AACC 2000). All assay kits were supplied by Megazyme^{TM} International Ireland Ltd (Wicklow, Ireland). Nitrogen was determined using a nitrogen analyzer (model FP-2000; Leco Instruments Ltd, St Joseph, USA) and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis.

Reducing sugars (In vitro digestion)

Bread samples were subjected to an *in vitro* digestion according to the methodology of Tudorica *et al* (2002a). Samples of bread, equivalent to 3 g available carbohydrate, were crumbed (using a food processor) to a size of approx 1 cm^3, placed in sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (with HCl), and digested with pepsin (Sigma-Aldrich, Pool, UK) (575 units/g starch) for 30 min at 37 ^oC. The pH of the mixture was subsequently re-adjusted to pH 6.9 (with NaOH), and the volume of the liquid made up to 50 mL with a solution of sodium phosphate buffer to which porcine pancreatic alpha amylase (Sigma-Aldrich, Pool, UK) (110 units/g starch) had been added. The mixture was transferred to dialysis tubing (Medicell International Ltd, Reading, UK), and placed in 450 ml of sodium phosphate buffer for 5 h at 37 ^oC. Aliquots (1 ml) duplicate were taken every 30 min.

Dialysate was analyzed for total sugars by the 3,5-dinitrosalicylic acid method (James 1999). Reducing sugars released (RSR) was expressed in maltose equivalents as percentage of total

available carbohydrates present in the sample using the following calculation (Brighneti *et al* 1995):

$RSR = A_{sample} \times 500 \times 0.95 / A_{maltose} \times SS \times 100$

where: A_{sample} is value of absorbance at 540 nm, $A_{maltose}$ is value of absorbance of a solution containing 1 mg of pure maltose per ml/phosphate buffer, SS is the amount of starch plus sugars (in milligrams) contained within the sample, 500 is the total volume, and 0.95 is the conversion from maltose to starch. .

Statistical analysis

Unless otherwise stated the results of analyses were reported as mean obtained from triplicate samplings of duplicate production runs and duplicate analysis determination. Analysis of variance of the results (ANOVA) was performed using the Minitab 13 statistical software package. Significance was defined as $p<0.05$.

Results and discussion

Pasting characteristics of bread wheat flour supplemented with BBG FRACTION

The pasting characteristics of bread wheat flour substituted with BBG FRACTION are illustrated in Table 1. Results illustrate that the use of both 2.5 and 5% BBG FRACTION decreased the PV, BD, and FV development compared to the control ($p<0.05$). There were no significant differences between the PV, BD, and FV values of 2.5 and 5% substitutions ($p>0.05$).

Pasting properties of wheat flours are generally correlated to the starch characteristics of the flours such as swelling potential, degree of gelatinization, and the subsequent reassociation of amylose and amylopectin after granule disruption. Although the reduction in pasting

characteristics observed between BBG FRACTION-supplemented flours and control pastes may occur as a result of replacing available starch with BBG FRACTION, it is also likely that preferential hydration of the BBG FRACTION fraction would limit the available water within the paste mixture, and hence, limit the swelling characteristics of the starch granules within the pastes, in turn leading to greater retention of starch granule integrity and a reduction in gelatinization. In our previous studies with a BBG FRACTION, reductions in PV, FV and BD were experienced in wheat starches substituted with 5% BBG FRACTION (Symons and Brennan 2004).

Effect of BBG FRACTION *supplementation on the rheological properties of doughs*

The inclusion of BBG FRACTION had a significant effect on the rheological characteristics of doughs (Table 2). Elasticity of bread doughs containing 5% BBG FRACTION was significantly higher ($p<0.05$) than either the control bread dough or dough containing 2.5% BBG FRACTION. Such changes in the rheological properties of β–glucan doughs may be related to the gelling effects of the β–glucan-enriched doughs, and the elastic nature of β–glucan polymers. No significant differences could be seen between the elasticity of the control dough and 2.5% BBG FRACTION enriched dough.

The effects of fibre inclusion on dough rheology have been reported by previous workers. Jankiewicz and Michniewicz (1986) found that the inclusion of soluble pentosans in breads resulted in stiffer doughs with a high water absorption. Gomez *et al* (2003) found that in wheat dough supplemented with various fibres, water absorption increased, and extensibility decreased. The magnitude of both these effects increased with elevated fibre concentrations.

Effect of BBG FRACTION *supplementation on the composition of wheat flour mixes and wheat breads*

Table 3 shows the starch, protein, and β–glucan percentages of the flour mixes and breads with 2.5 and 5% β–glucan substitution. The results show an expected decline in starch content (nonsignificant) associated with an increase in β–glucan content of both the flour mixes and the final breads. Similarly, protein levels of the flour mixes and the breads were not significantly different between the treatments. It is interesting to note that both the starch and β–glucan

contents of the breads were reduced compared to the original composition of the flour mixes. This reduction in starch and β–glucan may be associated with the increase in protein content of the samples, which, may in turn, be related to the fermentation process. Additionally it is probable that the thermal processing during bread making converts some of the starch of the flour into resistant starch, and may also cause a degree of β–glucan depolymerization.

Loaf volume, height, and firmness

Generally, an increase in the elastic and extendable nature of doughs is related to increased loaf height and overall loaf volume. However, inclusion of BBG FRACTION in breads resulted in a decrease in loaf volume and height (Table 2). The reduction in volume and height increased with a higher addition of BBG FRACTION. Additionally, breads containing BBG FRACTION exhibited firmer crumb texture compared to the control breads (Table 4).

Reduced loaf volumes as a consequence of barley β-glucan addition have been experienced by Knuckles *et al* (1997) Cavallero *et al* (2002) and Gill *et al* 2002. A volume reduction and a firmer crumb texture may be attributed to gluten dilution as reported by Pomeranz *et al* (1977) and Dubois (1978. The physicochemical properties of β-glucans, appear to affect dough texture by the formation of an elastic, semi-rigid gel-like matrix similar to the glucan gels known to occur in dairy-based systems (Tudorica *et al* 2002b). It is possible that when added to wheat during bread making the glucan component could tightly bind appreciable amounts of water, thus making it less available for the development of the gluten network, resulting in an underdeveloped gluten network. This, in turn, would limit the extent of dough inflation and gas cell stability during proving, hence leading to a more compact loaf with a reduced loaf volume and increased firmness. Gill *et al* (2002) proposed that β-glucan, due to its high water affinity (Gaosong and Vasanthan 2000) may bind water (otherwise used in steam generation) in the dough. This suppression of steam generation, could lead to a reduced volume and firmer crumb.

Table 4 reports changes in crumb firmness over storage time, a phenomenon which may be related to bread staling. Crumb firmness levels for BBG FRACTION breads over day 0 and day 1 were consistent, indicating crumb stability and a reduced staling over a 24 h storage period compared to the control bread sample (Table 2). BBG FRACTION breads exhibited bread firming characteristics between day 2 and 4. Bread containing 5% BBG FRACTION was significantly firmer ($p<0.05$) than the control bread at days 2 and 4.

Starch retrogradation is the principal factor involved in the firming of bread, via the time dependent recrystallization of amylopectin from the completely amorphous state of freshly heated product to the partially crystalline state of a stale product. Other flour and bread components impart subtle effects (Kim and D'Appolonia 1977). These results illustrate BBG FRACTION gels may retrograde with aging as previously suggested by Schwarz and Lee (1995).

The effect of BBG FRACTION *inclusion on the reducing sugar release of breads*

Figure 1 illustrates the effect of BBG FRACTION inclusion on the release of reducing sugars from the bread matrix during an in vitro digestion process. The results show a decrease in reducing sugar release related to the amount of BBG FRACTION used in the bread formulation. This difference is not only related to the total sugar release of the system but also the rate of sugar release. For instance, the evolution of reducing sugars from 60 to 210 min is significantly slower in the 5% BBG FRACTION bread samples compared to the control bread sample.

Reductions in sugar release could arise as a consequence of flour and starch replacement by the inclusion of BBG FRACTION into the breads. However previous research conducted on the effect of non starch polysaccharides on the sugar release of pastas (Yokoyama *et al* 1997; Tudorica *et al* 2002a) illustrates that such a reduction in sugar release and starch digestion may occur due to alterations to the structural arrangement of the food matrix and therefore the decrease in sugar release maybe linked to a reduction in starch availability for degradation. This, in turn, may be related to the ability of starch within the flour matrix to gelatinize during baking, and is consistent with the reduction in pasting characteristics observed in Table 1. It remains unclear as to whether this reduction is a consequence of altered rheological properties of pastes and doughs resulting in a limitation of available water for starch hydration due to β-glucan hydration and gelation, or whether the formation of a glucan gel matrix inhibits enzyme accessibility to partially gelatinized starch granules. It is anticipated that in addition to a reduction in starch available for degradation, attenuation of reducing sugar release, may also be attributed to the increased digesta viscosity and reduced motility as a result of the presence of the β-glucan, (Braaten *et al* 1991; Wood *et al* 2000).

CONCLUSIONS

This part of the study illustrates that the incorporation of 5% barley β–glucan rich fraction, in white wheat breads significantly reduces the rate at which reducing sugars are released.

This in turn may have a potential to regulate *in vivo* sugar release from, white wheat bread, a traditionally high glycaemic foods. The aim of further studies will be to correlate this *in vitro* digestion data with that gathered from *in vivo* glucose responses to β–glucan addition to bread.

REFERENCES

AACC. 2000. Approved methods 10th Edition. St. Paul, Minnesota. The American Association of Cereal Chemists.

Anderson JW. 1983. Plant fibre and blood pressure. Ann Int Med 98:842-846.

Anon. 2003. Perception and understanding of health components of bread. Federation of Bakers, London. UK.

Bhatty RS. 1986. Physicochemical and functional (bread making) properties of hull-less barley fractions. Cereal Chem 63:31.

Bjorck I, Liljeberg H, Ostman E. 2000. Low glycaemic-index foods. Brit J Nut 83: S149-S155.

Braaten JT, Wood P, Scott FW, Riedel KD, Poste L, Collins W. 1991. Oat gum lowers glucose and insulin responses after an oral glucose load. Am J Clin Nutr 53: 1425-1430.

Brighneti F, Pellengrini N, Casiraghi MC, Testolin G. 1995. *In vitro* studies to predict physiological effects of dietary fibre. Eur J Clin Nutr 49: S81-88.

Burley VJ, Leeds AR, Bundell JE. 1987. The effect of high and low fibre breakfast in hunger satiety, and food intake in a subsequent meal. Int J Obesity 1: 187-93.

Cavallero A, Empilli S, Brighenti F, Stanca, AM. 2002. High (1 →3, 1→4)- β-glucan barley fractions in bread making and their effects on human glycaemic response. J Cereal Sci 36: 59-66.

Cherburt C. 1995. Role of gastrointestinal motility in the delay of absorption by dietary fibre. E J Clin Nutr 49: S74-S80.

Dubois DK. 1978. The practical applications of fibre materials in bread production. Bakers Dig 52: 30-33

Faivre J, Bonithon-Kopp C. 1999. Diets, fibres and colon cancer. Adv Exp Med Biol 472: 199-206.

Feldheim W, Wisker E. 2000. Studies on the improvement of dietary fibre intake. Dtsch Lebensm-Rundsch 96: 327-330.

Fernandes J, Wolever TMS, Rao AV. 2000. Interrelationships between age, total dietary fibre intake and breath methane in humans. Nutr Res 20: 929-940.

Gaosong J, Vasanthan T. 2000. Effect of extrusion cooking on the primary structure and water solubility of β-glucan from regular and waxy barley. Cereal Chem 77: 396-400.

Gill S, Vasanthan T, Ooraikul B, Rossnagel B. 2002. Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. J Cereal Sci 36: 219-237.

Gomez M, Randa F, Blanco CA, Caballero PA, Apestegula A. 2003. Effect of dietary fibre on dough rheology and bread quality. Eur J Food Res Technol 216: 51-56.

Holm J, Koellreutter B, Wursch P. 1992. Influence of sterilisation, drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on the rate and extent of in vitro starch digestion. Euro J Clin Nutr 46: 629.

Hudson CA., Chiu MM., Knuckles BE. 1992. Development and characteristics of high fibre muffins with oat bran, rice bran or barley fibre fractions. Cereal Food World. 37: 373.

James CS. 1999. Analytical chemistry of foods. Aspen Publishers, USA, 123-5.

Jankiewicz M, Michniewicz J. 1987. The effect of soluble pentosans isolated from rye grain on staling of bread. Food Chem 25: 241-249.

Jenkins DJA, Wolever TMS, Kalmusky J, Giudici S, Giordano C, Wong GS, Bird JN, Patten R, Hall M, Buckley G, Little JA. 1985. Low glycaemic index carbohydrate foods in the management of hyperlipidaemia. Am J Clin Nutr 42: 604-617.

Karppinen S, Liukkonen K, Aura AM, Forsell P, Poutanen K. 2000. *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. J Food Sci 80:1469-1476.

Kim SK, D'Appolonia BL. 1977. Bread staling studies. III. Effect of pentosans on dough bread, and bread staling rate. Cereal Chem 54: 225.

Knuckles BE, Hudson CA, Chiu MM. 1997. Effect of beta glucan barley fractions in high fibre bread and pasta. Cereal Food World. 42: 94-99.

Malki Y, Myllmaki AK, Suotti T. 1992. Preparation and properties of oat bran concentrates. Cereal Food World. 37: 693.

Pick ME, Hawrysh ZJ, Gee MI, Toth E. 1998. Barley bread products improve glycaemic control of type 2 diabetic subjects. Int J Food Sci Nutr 49: 71-78.

Pomeranz Y, Shogern MD, Finney KF, Bechtel DB. 1977. Fibre in breadmaking-effects on functional properties. Cereal Chem. 54: 25-41.

Rainbird AL, Low AG. 1986. Effect of guar gum on gastric emptying in growing pigs. Brit J Nutr 55: 87-98.

Schwarz PB, Lee YT. 1995. Pages 99-103 in: Progress in Plant Polymeric Carbohydrate Research. Meuser, F., Manners, D.J., & Seibel, W.eds. Behrs'Verlag, Berlin.

Symons LJ, Brennan CS. 2004. The effect of barley β-glucan fibre fractions on starch gelatinisation and pasting characteristics. J Food Sci (in press)

Tudorica CM, Kuri V, Brennan CS. 2002a. Nutritional and physicochemical characteristics of dietary fibre enriched pasta. J Agr Food Chem 50: 347-356

Tudorica CM, Brennan CS, Kuri V, Jones E. 2002b. Yoghurt rheology and microstructure as affected by barley β-glucan inclusion. In: Martinez Boza FJ, Guerro A, Partal P, Franco JM, Munoz J. editors. *Progress in rheology: Theory and applied*. pp. 425-427. Publidisa, Sevilla, Spain,.

Velasquez M, Davies C, Marret R, Slavin JL, Feirtag JM. 2000. Effect of oligosaccharides and fibre substitutes on short-chain fatty acid production by human microflora. Anaerobe 6: 87-92.

Wisker E, Daniel M, Rave G, Feldeim W. 2000. Short chain fatty acids produced by in vitro from fibre residues obtained from mixed diets containing different breads and human faeces during ingestion of diets. Brit J Nutr 84: 31-37.

Wood PJ, Braaten JT, Fraser WS, Riedel D, Poslel M. 1990. Comparisons of viscous properties of oat and guar gum and effects of these and oat bran on glycaemic index. J Agr Food Chem. 38: 753-757.

Wood PJ. 1993. Physicochemical characteristics and physiological properties of oat (1-3) (1-4) β-D-glucan. Pages 83-112 in: Oat bran. Wood PJ. editor. St. Paul, MN: AOAC.

Wood PJ, Beer MU, and Butler G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr 84: 19-23.

Yokoyama WH, Hudson CA, Knuckles BE, Chiu MM, Sayre RN, Turnland JR, Schneeman BO. 1997. Effect of barley β-glucan in durum wheat pasta on human glycaemic response. Cereal Chem 74: 293-296.

Table 1. Wheat flour pasting characteristics peak viscosity (PV), breakdown (BD) and final viscosity (FV) of bread wheat flour substituted with 2.5 and 5% BBG FRACTION

	Control	2.5% BBG FRACTION	5% BBG FRACTION
PV (RVU)	175.28[a]	126.92[b]	131.28[ba]
BD (RVU)	82.95[a]	65.78[b]	73.17[b]
FV (RVU)	233.22[a]	73.17[b]	165.8[b]

[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

PV-peak viscosity; BD-breakdown; FV-final viscosity.

Table 2. Dough and bread physicochemical characteristics

Evaluation parameters	Control bread	2.5% BBG FRACTION bread	5% BBG FRACTION bread
Dough (force g) extensibility (n=9)	19.09[a]	22.62[a]	37.86[b]
Distance (mm)	10.07[a]	10.18[a]	13.84[a]
Loaf volume (cm3)	184[a]	165[b]	133[c]
Loaf height (cm)	5.48[a]	5[a]	4.5[b]

[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

Table 3a and b. Composition of (a) wheat flour mixes and (b) wheat breads substituted with 2.5 and 5% BBG FRACTION

Table 3a Wheat flour mixes

% Component (db)	Control	2.5% BBG FRACTIONF	5% BBG FRACTIONF
Starch	75.61[a]	74.18[a]	73.77[a]
Protein	14.19[a]	13.77[a]	13.81[a]
β-Glucan	0.29[a]	1.68[b]	2.92[c]

Table 3bWheat breads

% Component (db)	Control	2.5% BBG FRACTIONF	5% BBG FRACTIONF
Starch	68.1[a]	67.69[a]	65.62[a]
Protein	14.61[a]	13.94[a]	14.10[a]
β-Glucan	0.29[a]	1.37[b]	2.67[c]

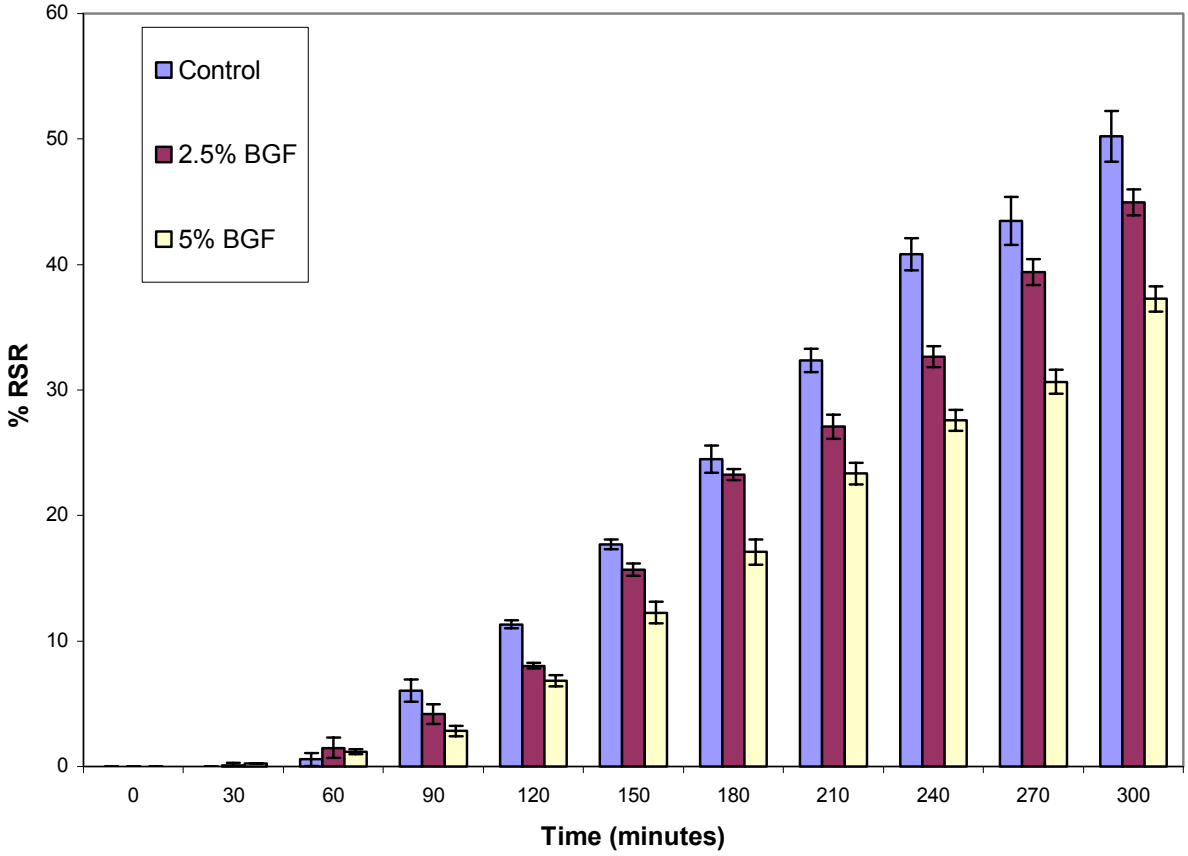
[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

Table 4. Crumb firmness (g) of breads at days 0, 1, 2, and 4.

	Control bread	2.5% BBG	5% BBG
Day 0	5.33[a]	5.47[a]	5.6[a]
Day 1	5.46[a]	5.47[a]	5.6[a]
Day 2	5.55[a]	5.6[a]	6.23[b]
Day 4	5.67[a]	5.71[a]	6.28[b]

[a] means in the same row followed by a different letter are significantly different ($p<0.05$).

Figure 1. Reducing sugar released (RSR), expressed as maltose equivalents, as percentage total available carbohydrates following *in vitro* digestion of control bread and breads with added BBG FRACTION.



[a] points at same time interval with different letters are significantly different ($p<0.05$), data are mean $\pm$ SD. Values at 0, 30, and 60 minutes are not significantly different.

2.4:- The evaluation of the potential nutritional and physicochemical characteristics of wheat breads supplemented with a commercial form of β-glucan Glucagel® (a (1→3) (1→4)-β-D-glucan rich fraction from barley).

Introduction

As mentioned previously, foods high in soluble dietary fibre are considered as low glycaemic index (GI) foods. Low GI foods are differentiated from other foods by the reduced rate at which they are digested and release glucose to the blood (Bjorck *et al* 2000). Soluble dietary fibres are believed slow the release of reducing sugars from the food and hence lower postprandial blood glucose level by several mechanisms, including; reduced amylolysis, but more specifically at the gastrointestinal level through delayed gastric emptying (Rainbird and Low 1986; Cherburt 1995) and reduced nutrient motility (Braaten *et al* 1991).

Epidemiological data strongly correlates low GI diets to reduced insulin resistance (Bjorck *et al* 2000). The potential consequences of a reduced insulin resistance are the control of diabetes and reduction of the possibility of developing risk factors for degenerative diseases, such as obesity (Burley *et al* 1987), hyperlipidaemia (Jenkins *et al* 1985) and hypertension (Anderson, 1983).

The β–glucans from both barley and oat grains have been illustrated to have an important influence on human glycaemic control. When β–glucan was isolated from oats and consumed as oat porridge it was reported to lower postprandial blood glucose (Wood *et al* 1990; Wood 1993). Additionally, barley flour enriched with β–glucans has been shown to have physiological effects comparable to other isolated fibres, such as guar gum, psyllium, and oat β–glucan (Knuckles 1997).

With their low fibre contents, breads are considered as high glycaemic foods. Several workers have examined the glycaemic response of bread as affected by fibre inclusion and reported no or variable effects on sugar release (Malki *et al* 1992; Bhatty 1986, Hudson *et al* 1992; Holm *et al* 1992). However in more recent studies, a strong correlation between the addition of soluble dietary fibre addition to bread and improved glycaemic control has been found. Pick *et al* (1998) and Cavallero *et al* (2002) found that barley β–glucan rich breads, elicit a lower glycaemic response compared to a reference white wheat bread.

In European countries where white bread is commonly consumed it is clear that a demand exists for the supplementation of wheat bread with dietary fibre. However this must be achieved without compromising palatability, a problem often encountered with fibre enrichment, particularly soluble fibre addition (Jenkins *et al* 2002).

The aim of this study was to evaluate the effect of the addition of a commercially available form of β-glucan preparation (Glucagel®, a gelling β–glucan isolate, from barley, *Hordeum vulgare*), on the physicochemical and nutritional properties of white wheat bread, with the aim of obtaining a cereal product with improved nutritional status, that can be successfully manufactured and retains the internal, external and textural eating qualities that make a bread acceptable to the consumer.

This part of the project was aimed at determining if the commercial exploitation of such BBG fractions (as prepared in sections 2.2 and 2.3) could be realistically obtained, and whether these commercial fractions (based on water extraction techniques) have similar potential benefits to the preparations obtained in sections 2.2 and 2.3.

MATERIALS AND METHODS

β–glucan concentrate (Glucagel®)

β–glucan in the form of Glucagel®, a gelling form of β–glucan (supplied by PolyCell Technologies, Crookston, MN) was incorporated into the bread mixes. Glucagel® (83% db β–glucan) was produced from waxy-hulless barley.

Pasting characteristics

Pasting characteristics (peak viscosity (PV), breakdown (BD) and final viscosity (FV) development) of bread wheat flour substituted with 2.5 and 5% Glucagel® were determined using a Rapid Visco Analyser (RVA-4 Newport Scientific PTY, Australia). Bread wheat flour with no fibre addition was used as a control. Test parameters included heating and cooling rates of 12^{o}C per minute, over a temperature range of 50-95^{o}C, and paddle speed of 160rpm. Samples were prepared by mixing 3.5g ($\pm$0.1) in 25ml ($\pm$0.1) distilled water in an aluminium canister. Analysis was performed in triplicate.

Thermal properties of bread wheat flour and Glucagel® mixtures

Differential scanning calorimetry was used to measure thermal parameters (onset of gelatinisation, Tonset; gelatinisation peak temperature, Tp; gelatinisation end point, Tendset; and total enthalpy, ΔH) of bread wheat flour samples substituted with 2.5 and 5% Glucagel®, to ascertain the influence of β–glucan on the starch fraction. Bread wheat flour with no Glucagel® addition was used as a control. Indium was used to calibrate the instrument (Differential Scanning Calorimeter, DSC 12E, Mettler Toledo). Samples were mixed with distilled water prepared (1:4 starch to distilled water ratio) in aluminium crucibles, and left to equilibrate for 1 hour. A blank aluminium pan was used as a blank. Nominal scan rate was 5ºC/min over a 20-100ºC heating rate. Analysis was performed in triplicate.

Bread making

White wheat breads were manufactured using commercial bread wheat flour (Shipton Mill), vegetable fat, dried yeast, salt, sugar and water according to Approved Method 10-09 (AACC 2000), straight dough method. Glucagel® was incorporated into the recipe by replacing bread

wheat flour at 2.5 and 5% proportions (w/w). An additional sample with no Glucagel® was also prepared as a control. Doughs were divided into 70g portions and baked in miniature tins with the following dimensions: top; 85mm(l) by 50mm (w); bottom; 75mm (l) by 40mm (w). Following baking, breads were cooled for 1 hour before subsequent analyses.

Dough Extensibility

Resistance to extension (mean max force g) and extensibility (mean distance at max force mm) of the bread doughs were measured using a texture analyser (TA-XT2) (Stable Micro Systems, Surrey, England), equipped with a Kiefer dough and extensibility rig (A/KIE) using 5kg load cell. 15g dough was placed in an oiled teflon dough form for 20 minutes. After resting dough, strips were removed with the aid of a spatula and subjected to the tensile test. The rig extended the sample by 75mm, at a pre-test, test and post test speed of 2, 3.3 and 10 mm/sec, respectively. The trigger force was 5g. The maximum peak force in compression was recorded as the firmness value in gram units.

Loaf Height

Loaf height was determined using calibrated callipers and reported in centimetres.

Loaf Volume

Loaf volume was measured using Approved Method 10-05 (AACC 2000), guidelines for measurement of volume by rapeseed displacement.

Crumb Firmness

A texture analyser (TA-XT2) (Stable Micro Systems, Surrey, England) was used to measure bread firmness. An AACC 36mm cylinder probe with radius (P/36R) and 5kg load cell was used. The probe compressed the sample by 40% at a pre-test, test and post test speed of 1, 1.7 and 10 mm/sec, respectively. The compression force was 100g. The maximum peak force in compression was recorded as the firmness value in gram units. Measurement was taken from 1cm slices, and samples were discarded after the TA test. For analysis of crumb firmness over

storage (0, 24, and 48 hours) slices was placed in polythene bags, sealed and stored at ambient temperature and on the appropriate day subjected to TA analysis as for samples at day 0.

Chemical composition

Moisture, starch, protein, and β-glucan contents of the breads were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000). Total starch, and β-glucan were determined using the total starch assay kit (Approved Method 76.13 (AACC 2000), and β-glucan enzymatic assay kit (Approved Method 32-23 (AACC 2000), respectively. All assay kits were supplied by Megazyme^{TM} International Ireland Ltd. Nitrogen was determined using a nitrogen analyser (Model FP-2000, Leco Instruments Ltd, St Joseph, MI) and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis.

Reducing sugars (In vitro digestion)

Bread samples were subjected to an *in vitro* digestion, as used by Tudorica *et al* (2002). Samples of bread (equivalent to 2g available carbohydrate) were crumbed (using a food processor) to a size of approx 1cm^3, diluted with sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (HCL acid), and digested with pepsin (from porcine stomach mucosa) (115U per g starch) (Sigma-Aldrich, UK) for 30 minutes at 37^{o}C. The pH of the mixture was re-adjusted to pH 6.9 (NaOH), diluted to 50ml (sodium phosphate buffer), to which porcine pancreatic alpha amylase (110 units/g starch) (Sigma-Aldrich, UK) was added (Sample), the same amount of alpha amylase, but inactivated, by boiling was added to the second sub sample (Blank). The mixtures were transferred to prepared dialysis tubing (Medicell International Ltd, UK), and placed in 450ml of sodium phosphate buffer for 5 hours at 37^{o}C. Tubes were agitated every 10 minutes to simulate gut movements. Aliquots (1ml) duplicate were taken every 30 minutes, replacing the volume each time with 1ml fresh buffer. Dialysate was analysed for total dialyzable sugars by the 3,5-dinitrosalicylic acid method (James 1999).

Reducing Sugars Released (RSR) consisting of the dialysed fragments of digested starch plus the native reducing sugars was expressed, in maltose equivalents as percentage of total available carbohydrates present in the sample using the following calculations of Brighenti et al (1995):

RSR= (A_{sample} x 500 x 0.95/$A_{maltose}$ x SS) x 100

where: A_{sample} is value of absorbance at 540nm, $A_{maltose}$ is value of absorbance of a solution containing 1mg of pure maltose per ml/phosphate buffer, SS is the amount of starch plus sugars (in milligrams) contained within the sample, 500 is the total volume, and 0.95 is the conversion from maltose to starch.

Statistical Analysis

Unless otherwise stated the results of analyses were reported as mean obtained from triplicate samplings of triplicate production runs and triplicate analysis determination. Results were reported as mean + SD. Analysis of variance of the results (ANOVA) was performed using the Minitab 13 statistical software package. Significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

The pasting characteristics and thermal properties of bread wheat flour supplemented with Glucagel®

Rapid Visco Analysis was used to evaluate the influence the pasting characteristics of bread wheat flour substituted with 2.5 and 5% Glucagel®, illustrated in Table 1. Results reveal that the addition of both 2.5 and 5% Glucagel® decreases the PV, BD, and FV development in comparison to the control ($p<0.05$). There was no significant difference between the PV, BD and FV values for 2.5 and 5% substitutions ($p>0.05$).

Table 1. Starch pasting characteristics (peak viscosity (PV), breakdown (BD) and final viscosity (FV)) of bread wheat flour substituted with 2.5 and 5% Glucagel®, expressed as RVU

	Control	**2.5% Glucagel®**	**5% Glucagel®**
PV	1856 ± 41.5^{a}	1509.7 ± 19.7^{b}	1412.7 ± 62.4^{b}
BD	740.3 ± 36.7^{a}	573.7 ± 16.7^{b}	506.3 ± 35^{b}
FV	2167 ± 51.5^{a}	1839.3 ± 38^{b}	1724.7 ± 7.52^{b}

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$)

Pasting properties of wheat flours are generally correlated to the starch characteristics of the flours such as swelling potential, degree of gelatinisation and the subsequent re-association of amylose and amylopectin after granule disruption. Although the reduction in pasting characteristics observed between Glucagel® supplemented flours and control pastes may be as a result of the replacing available starch with Glucagel®, it is also likely that the increased water retention and gel forming capacity of the Glucagel® may affect starch gelatinisation. Preferential hydration of the Glucagel® would limit the available water within the paste mixture and hence limit the swelling characteristics of the starch granules within the pastes, in turn

leading to greater retention of starch granule integrity and a reduction in gelatinisation. The observations of sections 2 and 3 also showed a lowering of PV, BD and FV in starch systems with 5% β-glucan fraction addition. However, Billaderis *et al* (1997) found that when β-glucan was incorporated in maize starch gelation was retarded possibly as a result of interference in the intermolecular associations amongst amylopectin molecules by the β-glucan. It is therefore plausible that the addition of Glucagel® may affect pasting and gelatinisation temperatures.

Table 2. illustrates the thermal parameters of bread wheat flour supplemented with 2.5 and 5% Glucagel®. Tonset of bread wheat flour substituted with 5% Glucagel® was significantly increased compared against the control value. There was a non-significant increase in the Tonset of the control and bread wheat flour substituted with 2.5% Glucagel®. Tendset of both bread wheat flour substituted with 2.5 and 5% Glucagel® was decreased compared to the control, with reduction corresponding with the quantity of fibre added. There was no difference in the Tpeak values amongst the samples. The most pronounced difference was in the reduction of enthalpy (j/g) with the addition of Glucagel®, again corresponding to the level of inclusion

Table 2. Thermal properties (Tonset, Tendset, Tpeak and J/g) of bread wheat flour substituted with 2.5 and 5% Glucagel®

	Control	2.5 Glucagel®	5% Glucagel®
Tonset (^{o}C)	52.97 ± 0.21^{a}	53.4 ± 0.35^{a}	54.43 ± 0.49^{b}
Tendset (^{o}C)	69.9 ± 0.35^{a}	68.4 ± 0.27^{b}	67.7 ± 0.44^{b}
Tpeak (^{o}C)	60.87 ± 0.32^{a}	60.50 ± 0.27^{a}	60.63 ± 0.12^{a}
J/g	5.39 ± 0.19^{a}	4.73 ± 0.10^{b}	3.87 ± 0.19^{c}

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$).

Previous research on the effects of soluble fibres on starch gelatinisation events have yielded similar findings. Eerlingen et al (1996) and Ferrero et al (1996) found the inclusion of soluble fibres results in a starch system resulted in an increase in gelatinisation temperature. This can be accounted for in part, as a result of soluble fibre competing for available water and thereby limiting starch swelling and associated gelatinisation events. Enthalpy of a flour mix can used as an indicator of the amount of starch gelatinisation within the system. One possible

explanation for the reduction in enthalpy on Glucagel® addition is that the fibre creates a matrix around the starch granules thereby impeding gelatinisation events. Tudorica et al (2002) found that the addition of pea, guar and inulin in raw pasta resulted in a decrease in enthalpy.

Again these results appear to correlate with those obtained by us in sections 2-3 (Symons and Brennan, 2004). Here we found a reduction in enthalpy when β-glucan fractions were incorporated into wheat starch systems.

Effect of Glucagel® inclusion on the rheological properties of doughs

Table 3 illustrates the effect of Glucagel® on the rheological properties of doughs. The resistance to extension of bread dough containing 5% Glucagel® was significantly higher ($p<0.05$) than the control bread dough and dough containing 2.5% Glucagel®. The resistance to extension of the control and 2.5% Glucagel® dough were similar ($p>0.05$). The extensibility of the doughs containing both 2.5% and 5% Glucagel® was significantly reduced, and could be correlated to fibre concentration. The production of a stiffer and less extensible dough, may be attributed to the high water absorption capability imparted into the doughs by the β-glucans.

The effects of fibre on dough rheology have been reported. Jankiewicz and Michniewicz (1986) found that pentosans in breads resulted in a substantial increase in water absorption, thus resulting in stiffer doughs with a reduced elasticity. The stiffness of the doughs may be overcome by the addition of water. However the loss of the elasticity of the doughs is not so easily rectified. It is possible that addition of fibre to doughs affects glutenin polymer elasticity. Gomez *et al* (2003) found that in wheat dough supplemented with various fibres, water absorption increased, and extensibility decreased, both effects increasing with fibre concentration

Again these results are similar to those obtained for the BBG fraction used in bread-making in section 3 of this report.

Table 3. Evaluation of dough and baking performance of breads substituted with 2.5 and 5% Glucagel®

Evaluation parameters	Control bread	2.5% Glucagel®	5% Glucagel®
Dough (Force g) extensibility	27.98±3.18[a]	29.19±0.60[a]	51.29±3.9[b]
Distance (mm)	21.32±1.22[a]	13.47±0.58[b]	9.49±0.29[c]
Loaf volume (cm3)	202±16.41[a]	180±12.25[b]	109±7.26[c]
Loaf height (cm)	5.97±0.29[a]	4.82±0.12[b]	3.85±0.18[c]

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$).

The effect of Glucagel® inclusion on loaf volume, height and firmness

Inclusion of Glucagel® in breads resulted in a significant decrease in loaf volume and height ($p<0.05$) (Table 3). The reduction in volume and height increased with a higher addition of Glucagel®.

Reduced loaf volumes as a consequence of barley β-glucan addition have been experienced by Knuckles *et al* (1997) Cavallero *et al* (2002) and Gill *et al* 2002. A reduced loaf volume and a firmer crumb texture may be attributed to gluten dilution as proposed by Pomeranz et al (1977) and Dubois (1978). Physicochemical properties of β-glucan can also affect bread volume and texture indirectly. It is possible that when added to wheat during bread making could tightly bind appreciable amounts of water making it less available for the development of the gluten network, resulting in an underdeveloped gluten network and hence reduced loaf volume and increased firmness. Additionally or alternatively the decreased volume and increased firmness may be attributed to a reduction in steam production as a result of water binding by the β-glucans. Gill *et al* (2002) proposed that β-glucan, due to its high water affinity (Gaosong and Vasanthan 2000) may bind water in the dough, that would otherwise be used in steam generation. Suppression of steam generation, could therefore lead to a reduced volume and firmer crumb.

Table 4. TA loaf Firmness at fresh, 24, and 48 hours.

Hours	Control bread	2.5% Glucagel®	5% Glucagel®
Fresh	5.24±0.17[a]	5.45±0.29[a]	5.64±0.44[a]
24	5.53±0.29[a]	5.59±0.24[a]	5.96±0.43[a]
48	5.64±0.47[a]	5.84±0.27[a]	6.46±0.48[a]

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$).

Table 4 reports changes in crumb firmness over storage time, a phenomenon which, may be related to bread staling. In comparison to the control sample, breads containing Glucagel® appeared to give higher values in compression force measurements, however the differences were only significant for bread containing 5% Glucagel® at days 48 hours. Incorporation of 2.5% Glucagel® did not affect bread firming during the 48 hour storage period ($p>0.05$).

Starch retrogradation is the principal factor involved in the firming of bread, via the time dependent recrystallisation of amylopectin from the completely amorphous state of freshly heated product to the partially crystalline state of a stale product, with the effect of other flour and bread components imparting minimal effects (Kim and D'Appolonia 1977). However as these results illustrate β–glucan gels may retrograde with ageing as observed by Schwarz and Lee (1995).

Table 5. Composition of control bread and bread supplemented with 2.5 and 5% Glucagel®

Component (%)	Control	2.5% Glucagel®	5% Glucagel®
Total Starch	69.99+1.10[a]	66.83+0.71[b]	67.14+0.45[b]
β-glucan	0.31+0.44[a]	2.14+0.05[b]	4.56+0.16[c]
Protein	12.8+0.35[a]	12.66+0.12[a]	12.36+0.14[a]

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$).

Effect of Glucagel® inclusion on the composition of white wheat breads

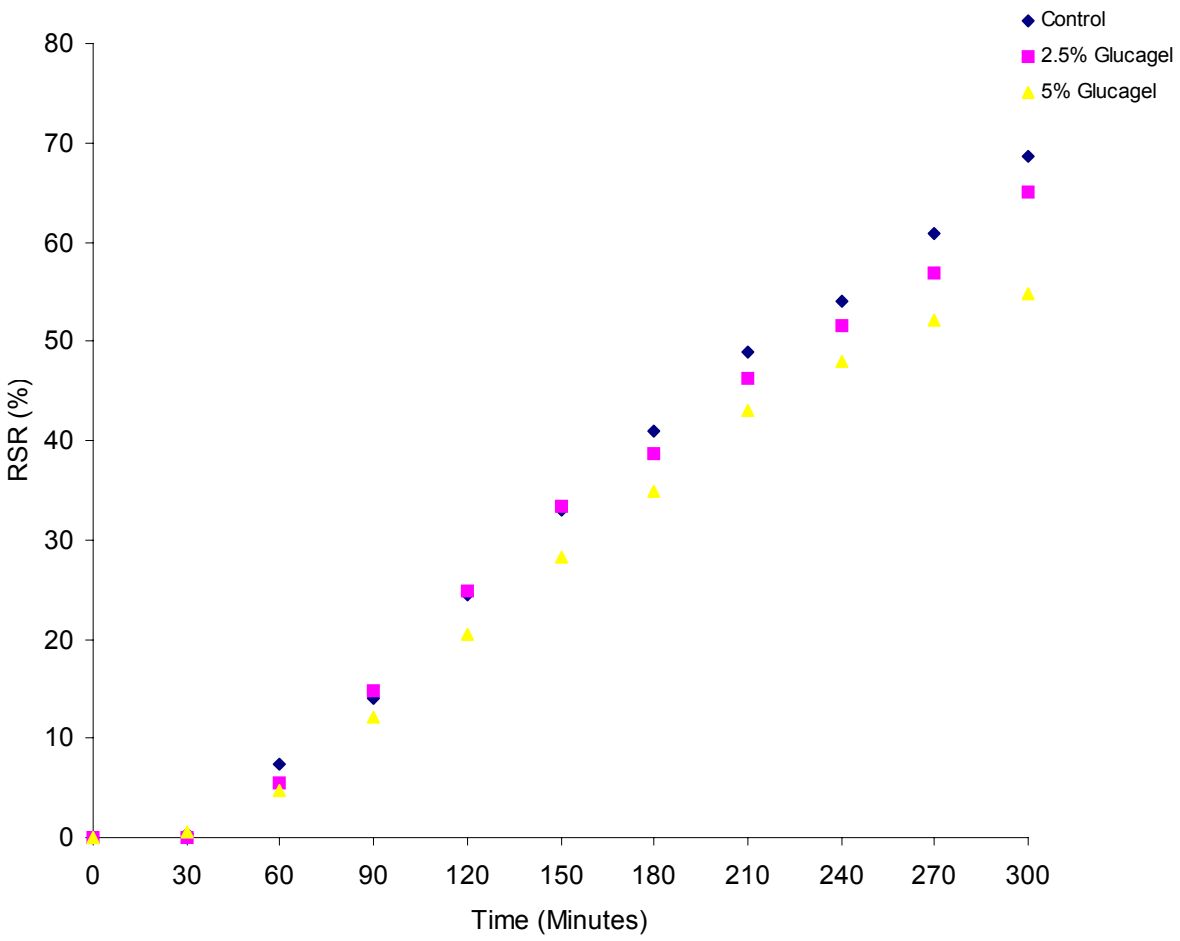
Table 5 illustrates the starch, protein and β-glucan composition of the baked breads with 2.5% and 5% Glucagel® substitution. The results illustrated an expected decline in starch content associated with Glucagel® addition. Protein levels of the flour mixes and the breads were not significantly different between the treatments.

The effect of Glucagel® inclusion on the Reducing Sugar Release of Breads

Figure 1 illustrates the effect of Glucagel® inclusion on the release of reducing sugars from the bread matrix during an *in vitro* digestion process. The results show a significant decrease in reducing sugar release from the 5% Glucagel® bread sample. No significant difference could be found between 2.5% Glucagel® sample and the control white bread. This difference between the 5% Glucagel® bread and control is not only related to the total sugar release of the system but also the rate of sugar release. For instance the evolution of reducing sugars from 60 to 210 minutes is significantly slower.

Reductions in sugar release could arise as a consequence of flour and starch replacement by the inclusion of Glucagel® into the breads. However previous research conducted on the effect of non-starch polysaccharides on the sugar release of pastas (Yokoyama *et al* 1997; Tudorica *et al* 2002a) illustrates that such a reduction in sugar release and starch digestion may occur due to alterations to the structural arrangement of the food matrix and therefore the decrease in sugar release maybe linked to a reduction in starch availability for degradation. This in turn may be related to the ability of starch within the flour matrix to gelatinise during baking, and is consistent with the reduction in pasting characteristics observed in Table 1. It remains unclear as to whether this reduction is a consequence of altered rheological properties of pastes and doughs resulting in a limitation of available water for starch hydration due to β-glucan hydration and gelation, or whether the formation of a glucan gel matrix inhibits enzyme accessibility to partially gelatinised starch granules. It is anticipated that in addition to a reduction in starch available for degradation, attenuation of reducing sugar release, may also be attributed to the increased digesta viscosity and reduced motility as a result of the presence of the β-glucan, (Braaten *et al* 1991; Wood *et al* 2000).

Figure 1. Reducing sugar released (RSR) (expressed as maltose equivalents, as percentage total available carbohydrates



CONCLUSIONS

This part of the study illustrates that the incorporation of 5% Glucagel®, in white wheat breads significantly reduces the rate at which reducing sugars are released, in an *in vitro* digestion model. This in turn may have a potential to regulate *in vivo* sugar release from white wheat bread, a traditionally high glycaemic foods. However in order to deem these fibre enriched breads acceptable to consumers negative changes in the physiochemical properties of the doughs and baked breads must be overcome. It is anticipated that other ingredients (i.e. dough conditioners) maybe incorporated into the breads to counteract the negative effects encountered on baking quality (volume and height loss), but without compromising nutritional benefits.

It was encouraging to note that the results obtained in this part of the study reflected those obtained in sections 2.2 and 2.3. As such the results sever to verify the physicochemical and nutritional effects observed by our own BBG fractions, and also illustrate that there is a commercial potential to both isolate, and subsequently utilise, BBG fractions from barley into bread based systems.

REFERENCES

AACC. 2000. 10th Edition. Approved Methods. St. Paul. Minnesota. The American Association of Cereal Chemists.

Anderson, J.W. 1983. Plant fibre and blood pressure. Ann. Intern. Med. 98: 842-846.

Anon. 2003. Perception and understanding of health components of bread. Federation of Bakers, London. UK.

Bhatty, R.S. 1986. Physicochemical and functional (bread making) properties of hull-less barley fractions. Cereal Chem. 63:31.

Biliaderis, C.G., Arvanitoyannis, I., Izydorczyk, M.S., and Prokopowich, D.J. 1997. Effect of hydrocolloids on gelatinisation and structure formation in concentrated waxy maize and wheat starch gels. Starch-Starke. 49: 278-283.

Bjorck, I., Liljeberg, H., and Ostman, E. 2000. Low glycaemic-index foods. Brit. J. Nut. 83: S149-S155.

Braaten, J.T., Wood, P., Scott, F.W., Riedel, K.D., Poste, L., and Collins, W. 1991. Oat gum lowers glucose and insulin responses after an oral glucose load. Am. J. Clin. Nutr. 53: 1425-1430.

Brighneti F., Pellengrini N., Casiraghi M.C., and Testolin, G. 1995. *In vitro* studies to predict physiological effects of dietary fibre. Eur. J. Clin. Nutr. 49: S81-88.

Burley, V.J., Leeds, A.R., and Bundell, J.E. 1987. The effect of high and low fibre breakfast in hunger satiety, & food intake in a subsequent meal. Int. J. Obesity. 1: 187-93.

Cavallero, A., Empilli, S., Brighenti, F., and Stanca, A.M. 2002. High (1 →3, 1→4)- β-glucan barley fractions in bread making and their effects on human glycaemic response. J.Cereal Sci. 36: 59-66.

Cherburt, C. 1995. Role of gastrointestinal motility in the delay of absorption by dietary fibre. E. J. Clin. Nutr. 49: S74-S80.

Dubois, D.K. 1978. The practical applications of fibre materials in bread production. Bakers Dig. 52: 30-33

Eerlingen, R.C., Jacobs, H., Van Win, H., and Delcour, J.A. 1996. Effect of hydrothermal treatment on the gelatinisation properties of potato starch as measured by differential scanning calorimetry. Journal of Therm. Anal. 47: 1229-1246.

Faivre J. and Bonithon-Kopp, C. 1999. Diets, fibres and colon cancer. Adv. Exp. Med. Biol. 472: 199-206.

Feldheim., W, and Wisker, E. 2000. Studies on the improvement of dietary fibre intake. Dtsch Lebensm-Rundsch. 96: 327-330

Ferrero, C., Martino, M.N., Zartizky, N.E. 1996. Effect of hydrocolloids on starch thermal transitions, as measured by DSC. J. Therm. Anal. 47: 1247-1266

Fernandes, J., Wolever, T.M.S., and Rao, A.V. 2000. Interrelationships between age, total dietary fibre intake and breath methane in humans. Nutr. Res. 20: 929-940.

Gaosong, J., and Vasanthan, T. 2000. Effect of extrusion cooking on the primary structure and water solubility of β-glucan from regular and waxy barley. Cereal Chem. 77: 396-400.

Gill, S., Vasanthan, T., Ooraikul, B., and Rossnagel, B. 2002. Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. J. Cereal Sci. 36: 219-237.

Gomez, M., Randa, F., Blanco, C.A., Caballero, P.A., and Apestegula, A. 2003. Effect of dietary fibre on dough rheology and bread quality. Eur. J. Food Res. Technol. 216: 51-56.

Holm, J., Koellreutter, B., and Wursch, P. 1992. Influence of sterilisation, drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on the rate and extent of in vitro starch digestion. Euro J. Clin. Nutr. 46: 629.

Hudson, C.A., Chiu, M.M., and Knuckles, B.E. 1992. Development and characteristics of high fibre muffins with oat bran, rice bran or barley fibre fractions. Cereal Food World. 37: 373.

James, C.S. 1999. Analytical Chemistry of Foods. Aspen Publishers, USA, 123-5.

Jankiewicz, M., and Michniewicz, J. 1987. The effect of soluble pentosans isolated from rye grain on staling of bread. Food Chem. 25: 241-249.

Jenkins, D.J.A, Wolever, T.M.S., Kalmusky, J., Giudici, S., Giordano, C., Wong, G.S., Bird, J.N., Patten, R., Hall, M., Buckley, G., and Little, J.A. 1985. Low glycaemic index carbohydrate foods in the management of hyperlipidaemia. Am J. Clin. Nutr. 42: 604-617.

Karppinen, S., Liukkonen, K, Aura, A.M., Forsell, P., and Poutanen, K. 2000. *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. J. Food. Sci. 80:1469-1476.

Kim, S.K., and D'Appolonia, B.L. 1977. Cereal Chem. 54: 225.

Knuckles, B.E., Hudson, C.A., and Chiu, M.M. 1997. Effect of Beta glucan barley fractions in high fibre bread and pasta. Cereal Food World. 42: 94-99.

Malki, Y., Myllmaki, A.K., and Suotti, T. 1992. Preparation and properties of oat bran concentrates. Cereal Food World. 37: 693.

Pick, M.E., Hawrysh, Z.J., Gee, M.I., and Toth, E. 1998. Barley bread products improve glycaemic control of type 2 diabetic subjects. Int. J. Food Sci. Nutr. 49: 71-78.

Pomeranz, Y., Shogern, M.D., Finney, K.F. and Bechtel, D.B. 1977. Fibre in breadmaking-effects on functional properties. Cereal Chem. 54: 25-41.

Rainbird, A.L., and Low, A.G. 1986. Effect of guar gum on gastric emptying in growing pigs. Brit. J. Nutr. 55: 87-98.

Schwarz, P.B, and Lee, Y.T. 1995. Pages 99-103 in: Progress in Plant Polymeric Carbohydrate Research. Meuser, F., Manners, D.J., & Seibel, W.eds. Behrs'Verlag, Berlin.

Symons, L.J., and Brennan, C.S. 2004. The effect of barley β-glucan fibre fractions on starch gelatinisation and pasting characteristics. J. Food Sci. (in press)

Tudorica, C.M., Kuri, V., and Brennan, C.S. 2002a. Nutritional and physicochemical characteristics of dietary fibre enriched pasta. J. Agr. Food Chem. 50: 347-356

Tudorica, C.M., Brennan, C.S., Kuri, V., Jones, E. 2002b. Yoghurt rehology and microstructure as affected by barley β-glucan inclusion. In: Martinez Boza, F.J., Guerro, A., Partal, P., Franco, J.M., Munoz, J. eds. *Progress in rheology: theory and applied*. Publidisa, Sevilla, Spain, 425-427.

Velasquez, M., Davies C., Marret, R., Slavin, J.L., and Feirtag, J.M. 2000. Effect of oligosaccharides and fibre substitutes on short chain fatty acid production by human microflora. Anaerobe. 6: 87-92.

Wisker, E., Daniel, M., Rave, G., and Feldeim, W. 2000. Short chain fatty acids produced by in vitro from fibre residues obtained from mixed diets containing different breads and human faeces during ingestion of diets. Brit. J. Nutr. 84: 31-37.

Wood, P.J., Braaten, J.T., Fraser, W.S., Riedel, D., Poslel, M. 1990. Comparisons of viscous properties of oat and guar gum and effects of these and oat bran on glycaemic index. J. Agr. Food Chem. 38: 753-757.

Wood, P.J. 1993. Physicochemical characteristics and physiological properties of oat (1-3) (1-4) β-D-Glucan. Pages 83-112 in Oat Bran. Wood P.J. ed. AOAC. St Paul. MN.

Wood, P.J., Beer, M.U., and Butler, G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit. J. Nutr. 84: 19-23.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnland, J.R., and Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycaemic response. Cereal Chem. 74: 293-296.

2.5:- The potential role of barley flour as a source of β-glucan

Introduction

The main focus of this small area of research was to investigate whether the use of barley flour in food systems could provide similar advantages in physicochemical and nutritional characteristics to BBG fractions, when used in cereal food systems.

In this particular section de-husked and milled barley flour was used in both bread and pasta products.

For the pasta production, the BBG fraction (obtained in section 2) was used to directly compare the effectiveness of barley flour or BBG in pasta quality.

Materials and Methods.

Barley grain

Sunrise, a hulled waxy feed variety barley (5.77% β-glucan, db), was provided by Pertwood organic farms, Salisbury, UK. Whole kernels were finely ground in a laboratory mill (Glen Creston, Stanmore, UK) to pass through a 500-μm-mesh screen.

Bread making

Breads, containing 0, 2.5, 5, 10 and 20% barley flour were manufactured according to Approved Method 10-09 (AACC 2000) as detailed in sections 2.3 and 2.4. Doughs were divided into 70 g portions and baked in test bakery tins (length 85 mm, width 50 mm). Baked breads were cooled for 1 h prior to analysis.

Dough extensibility

Extensibility of the bread doughs was measured using a texture analyzer (model TA-XT2, Stable Micro Systems, Reading, UK) equipped with a Kiefer dough and extensibility rig (A/KIE) using a 5 kg load cell. Dough (15 g) was placed in an oiled teflon dough former for 20 min, removed with the aid of a spatula, and subjected to the tensile test. The rig extended the sample by 75 mm, at a pre-test, test, and post-test speed of 2, 3.3, and 10 mm/s, respectively.

The maximum peak force in tension was regarded as the degree of elasticity of the dough, whereas the distance travelled for dough break was regarded as the extensibility of the dough.

Loaf height and volume

Loaf height was determined using calibrated callipers and reported in centimetres. Measurements were taken from the centre of each loaf. Loaf volume was measured using Approved Method 10-05, volume by rapeseed displacement (AACC 2000).

Crumb firmness

A TA-XT2 texture analyzer (Stable Micro Systems, Reading, UK) was used to measure bread firmness. An AACC 36-mm-cylinder probe with radius (model P/36R) and a 5 kg load cell was used. The probe compressed the sample by 40% at a pre-test, test, and post-test speed of 1, 1.7, and 10 mm/s, respectively. The maximum peak force in compression was recorded as the firmness value in gram units. Measurements were taken on 1-cm slices, Only 1 measurement was taken from the slices; samples were discarded after the test.

Pasta making.

Durum wheat pasta was made using as raw materials: commercial durum semolina (Allied Mills Ltd., UK), water and different types of ingredients (barley flour at 10, 20 and 30% inclusion levels; and BBG fraction at 2.5, 5 and 10% levels). An additional sample with no fibre was also prepared as a control. The mixture was extruded as spaghetti (1.5 mm diameter) using a Fresco M-P15 domestic pasta maker and following a standard formula (500g semolina flour and 160 g water) according to manufacturers recommendations. Samples were wrapped in cling film and stored in air-tight containers and frozen at -40°C until needed.

Cooking procedure.

Optimum cooking time (the time necessary to obtain complete gelatinisation of starch showed by the disappearance of the white central core of the spaghetti strand) was determined as 7 minutes. 50 grams of each pasta sample were than cooked for 7 minutes in 500 ml of boiling distilled water.

After cooking and draining, pasta samples were analysed for dry matter, water absorption and textural properties.

Swelling index

The swelling index of cooked pasta (SI, g water/g dry pasta) was evaluated by drying pasta samples to constant weight at 105^0C, and expressed as: (weight of cooked product, W_1 - weight after drying, W_2)/(weight after drying, W_2). Three measurements were conducted for each pasta type.

Dry matter of raw pasta

Dry matter of raw pasta was determined according to standard methods (AACC, 1996-926.07B) (AACC, 1995).

Texture analysis of Pasta

Textural characteristics of cooked pasta were determined using a Texture Analyser TA.XT2 (Stable Micro Systems, UK), calibrated for a load cell of 25kg.

Elasticity (or *'tensile strength'*) was determined by tension test, using the A/SPR-Spaghetti/Noodle Rig (settings: pre-test speed: 3mm/sec., test speed: 3mm/sec., post test speed: 5mm/sec., distance: 120mm at a rate for data acquisition of: 200pps). Maximum force recorded when the elastic limit is exceeded and the pasta strand snaps gives an indication of pasta elasticity; the test was performed on 15 replicates per sample.

Reducing sugars (In vitro digestion)

Pasta samples were subjected to an in vitro digestion according to the methodology of Tudorica *et al* described in sections 2.3 and 2.4. Samples of bread, equivalent to 3 g available carbohydrate, were crumbed (using a food processor) to a size of approx 1 cm^3, placed in sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (with HCl), and digested with pepsin (Sigma-Aldrich, Pool, UK) (575 units/g starch) for 30 min at 37 ^oC. The pH of the mixture was subsequently re-adjusted to pH 6.9 (with NaOH), and the volume of the liquid made up to 50 mL with a solution of sodium phosphate buffer to which porcine pancreatic alpha amylase (Sigma-Aldrich, Pool, UK) (110 units/g starch) had been added. The mixture was transferred to dialysis tubing (Medicell International Ltd, Reading, UK), and placed in 450 ml of sodium phosphate buffer for 5 h at 37 ^oC. Aliquots (1 ml) duplicate were taken every 30 min.

Dialysate was analyzed for total sugars by the 3,5-dinitrosalicylic acid method. Reducing sugars released (RSR) was expressed in maltose equivalents as percentage of total available carbohydrates present in the sample using the following calculation:

RSR= A_{sample} x 500 x 0.95/$A_{maltose}$ x SS x 100

where: A_{sample} is value of absorbance at 540 nm, $A_{maltose}$ is value of absorbance of a solution containing 1 mg of pure maltose per ml/phosphate buffer, SS is the amount of starch plus sugars (in milligrams) contained within the sample, 500 is the total volume, and 0.95 is the conversion from maltose to starch. .

Statistical analysis

Unless otherwise stated the results of analyses were reported as mean obtained from triplicate samplings of duplicate production runs and duplicate analysis determination. Analysis of variance of the results (ANOVA) was performed using the Minitab 13 statistical software package. Significance was defined as $p<0.05$.

Results

Breads

Table 1 illustrates the effect of barley flour substitution has on the physicochemical properties of breads. The inclusion of barley flour into the bread mixtures had a sever effect on dough extendibility and extensibility. Increasing levels of barley flour also resulted in a decline in loaf structure (loaf volume and height). The lack of dough elasticity is most likely due to the decline in wheat proteins and hence a general reduction in the elastic nature of the doughs.

Table 1. Evaluation of dough and baking performance of breads substituted with barley flour

Evaluation parameters	**Control bread**	**2.5%**	**5%**	**10%**	**20%**
Dough (Force g) extensibility	27.98±3.18[a]	23.12±0.60[b]	20.19±1.7[b]	15.23±6.0[b]	10.11±4.4[c]
Distance (mm)	21.32±1.22[a]	11.11±0.51[b]	8.36±0.92[c]	7.63±0.87[c]	7.41±0.99[c]
Loaf volume (cm3)	202±16.41[a]	167±11.51[b]	162±9.19[c]	156±11.15[c]	144±6.76[c]
Loaf height (cm)	5.97±0.29[a]	4.65±0.21[b]	4.35±0.34[c]	4.12±0.22[c]	3.86±0.29[c]

[a] means values in the same row, followed by a different letter are significantly different ($p<0.05$).

From these results it is clear that the use of barley flour additions (at higher enough levels to yield a significant amount of β-glucan within the food system) would be deleterious to processing and result in poor quality loaves with low consumer appeal. As such no further work was conducted on the nutritional quality of such breads. However research focussed on the use of BBG fractions and barley flour in pasta products.

Pastas

The quality characteristics of pasta depend on the ability of the pasta to soak moisture up during cooking, yet still retain its structure. As such water absorption of pasta and also the overall dry matter of pasta are crucial elements in determining optimum pasta quality. Table 2 illustrates that when barley flour was added to pasta, the water absorption levels of the pasta increased significantly, and the dry matter reduced (compared to a control pasta sample). This behaviour

is probably due to the differences in starch and protein composition of barley flour when compared with that of durum wheat semolina. It may also be due to the particle size of the barley flour, in that durum wheat semolina is produced from hard wheat endosperm whereas the barley flour used in this study was obtained from relatively soft barley endosperms.

Table 2:- Pasta qualities of barley flour and BBG fractions.

Sample	**Addition Level**	**Water Absorbtion**	**Dry Matter content**	**Elasticity (cm)**	**Firmness (g)**
Control	0	49.26	35.0	33.63	20.31
Barley Flour	10%	54.6	29.2	31.15	13.20
	20%	55.49	29.70	21.34	10.92
	30%	58.21	26.70	10.11	3.21
BBG fraction	2.5%		28.7	32.45	20.10
	5.0%		29.4	32.10	19.74
	10.0%		29.4	31.24	19.71

Interestingly, little significant difference was observed between BBG fraction enriched pastas and the control with regards to water absorption and dry matter content (Table 2). There was a slight decline in dry matter content observed, however this was not significant. Such results again illustrate the suitability of BBG fractions in cereal food systems compared to enriched barley flour products.

The elasticity and the extendibility of the pasta samples was also affected by the inclusion of both barley flour and BBG fractions (Table 2). Barley flour inclusion drastically reduced the elastic nature of the pasta by reducing both the force requires to tear the pasta and the distance moved before the pasta tore. The main factor which controls the elastic nature of pasta is the strength and continuity of the gluten matrix within the pasta. Results from table 2 indicate that

barley flour disrupts this matrix formation and hence the pastas are brittle, rather than elastic, in nature.

The inclusion of BBG fractions into the pasta also showed a decrease in pasta elasticity and extendibility, compared to the control pasta sample. However in this case these differences were not significant. The weakening of the pasta matrix (i.e. reduction in pasta elasticity) may be partly explained by the lower Dm of the BBG rich pastas. This in turn may be due to the ability of the BBG fractions to absorb and retain higher levels of water during cooking.

In vitro analysis of the pastas obtained previously indicate that although the addition of barley flour generally reduces the rate of starch digestion, and hence the potential GI of pasta, when compared against the control pasta, this difference was relatively insignificant (Table 3).

However similar analysis for BBG enriched pastas revealed that the BBG fraction had a significant effect in reducing the amount of starch degradation during digestion, and hence had a significant reduction in the potential GI of the pasta (Table 3).

This more substantial reduction in starch degradation and GI values observed in the BBG enriched pasta is probably due to a greater gelling capacity of the extracted BBG fraction compared to that of the beta-glucan within a barley flour. It is distinctly possible that the beta-glucan in the BBG extract reduces the degree of starch granule swelling during cooking. This in turn would reduce the amount of starch gelatinisation, and hence delay the onset of starch degradation. Thus the reduction of the potential GI of BBG enriched pastas would result from the interactions between the fibre, starch and water during pasta cooking.

From these results it is clear that not only does barley flour addition to cereal food products negatively alter the processing and sensorial characteristics of such foods, but that the only process acceptable way of developing beta-glucan rich foods is by using beta-glucan extracts.

Table 3: Comparison of amount of starch digested, and potential GI, from pastas with barley flour and BBG enriched fractions.

Sample	Addition Level	Starch digested after 150min (%)	Starch digested after 180min (%)	Starch digested after 300min (%)	Predicted glycaemic index
Control	0	12.91	19.19	35.77	44.8
Barley Flour	10%	13.21^{b}	$18.55^{a,b}$	34.23^{b}	44.7^{b}
	20%	12.81^{a}	17.37^{a}	34.02^{a}	44.7^{a}
	30%	$12.40^{c,e}$	$17.18^{c,d}$	$33.03^{c,d}$	43.2^{d}
BBG fraction	2.5%	$11.11^{b,c}$	$13.29^{c,d}$	$22.89^{d,e}$	$30.4^{c,d}$
	5.0%	$10.73^{d,e}$	13.22^{d}	$21.08.66^{e}$	$29.8^{c,d}$
	10.0%	$9.76^{b,c,d}$	$12.48^{c,d}$	$20.16^{d,e}$	28.7^{c}

- within the same column, the values with the same letter are not significantly different;

3 Discussion

3.1:- Original project title and objectives

The HGCA project was awarded to the application:-

**"The use of β-glucan fractions (derived from barley) in processed cereal foods:
Effects on the structure, texture, sensory characteristics, and nutritional value of foods."**

The initial project summary and the actual objectives stated in the project are detailed below:-

1. Project summary: *(Max 200 words)*

UK agriculture has shown a rapid decline in the ex-farm price of non-food grade cereals (namely feed wheat and barley). Currently high β-glucan barley grains are regarded as feed value crops only. However, potential exists to use these cereals as food grade materials.

The pilot project had the aims to characterise β-glucan rich components of barley grains; generate generic and process specific information on the inclusion of barley β-glucan fractions, into model cereal food systems (biscuits and pasta, bread); and to determine the effects on the structure, texture, rheology, biochemical and nutritional properties of such food products.

The output should include predictive modelling of β-glucan material in potential foods, scientific credibility to health-promoting effects of barley fibre in functional foods, and optimisation of a new added value product (barley flour with high β-glucan levels) from crop products otherwise be regarded as low-grade animal product.

Outcomes of the project were expected to be the generation of a new market for undervalued cereal grains, thus adding value to the farming / agri-food sector.

The general aims of this project, therefore, are to:-

- investigate the quality characteristics needed from UK high β-glucan barley grain to be used in the production of cereal products
- determine processing parameters required to produce acceptable cereal food products
- evaluate the potential nutritional benefits from β-glucan enriched foods
- analyse market potential for such products and determine the economic feasibility of large scale processing

The objectives of the reasearch are to:

1. Isolate and characterise barley beta glucan fractions from high β-glucan barley grains
2. Determine the rheological behaviour of barley β-glucan inclusion into model starch systems
3. Investigate the effects of barley β-glucan inclusion into model cereal foods
4. Appraise the effect of processing on the rheological and structural behaviour of barley β-glucans in functional food systems
5. Determine the nutritional benefit of barley β-glucan inclusions in food material
6. Explore the feasibility of possible follow -on research investigating the *in vivo* nutritional benefit of barley β-glucan to human health.

3.2 Discussion from the outcome of Objective 1

The research project successfully demonstrated that the possibility of recovering beta-glucan dietary fibre rich fractions from UK barley grain. UK barley provides an excellent source of beta-glucan material. Although different extraction processes were used, extraction rates from relatively simple (and inexpensive) water extractions yielded relatively high beta-glucan contents. This would indicate that such water extraction techniques may be of beneficial use when considering the cost effectiveness of extraction techniques. However, it should be noted that additional purification of the BBG fraction with amylase enzymes has the potential to reduce both protein and starch contents of such fractions. This may be a desirable feature when considering the purity and homogeneousness of extracts.

3.3 Discussion from the outcome of Objective 2

BBG fractions were successfully incorporated into purified wheat starch filler systems. This was later applied also to wheat flour systems. In both cases it was observed that increasing the inclusion of BBG fractions led to a reduction in pasting characteristics, this in turn could lead to a reduction in gelling capacity of such food gels. Although this in itself could be a negative factor when considering the possible inclusion of these materials into freeze-thaw stable foods, the effect that the BBG fractions had on reducing the degree of starch gelatinisation (both in terms of wheat starch and wheat flour) shows a potential to regulate starch degradation. This reduction in pasting characteristics and also gelatinisation of starch appears to be linked to the water retention capacity of the beta-glucans in the BBG fractions. As such the retention of water by the BBG material could protect the starch granules in cereal foods from absorbing moisture during hydration, and thus reduce granule deformation. It is this reduction in granule deformity which could in turn be related to any reduction in starch degradation.

The results from this study clearly illustrate that it is possible to manipulate the pasting profile and starch gelatinisation characteristics of BBG / flour pastes, to obtain a functional gel system in foods.

3.4 Discussion from the outcome of Objectives 3 and 4

Results from eth study show that the inclusion of BBG fractions into doughs, breads and pastas can significantly affect the rheological characteristics and also the structure of such foods. Although the results in the bread analysis do illustrate that high levels of BBG fractions can negatively affect the textural and structural characteristics of both bread and dough, generally these effects were insignificant at levels below 5%. This would therefore be the predicted optimum level of BBG inclusion into cereal food products. It may be possible to manipulate processing conditions further in order to optimise food structure and rheology performance, and this is something a further study may wish to explore.

However, when comparing the effects additions of barley flour (as opposed to BBG fractions) have on food structure, rheology and texture, it is clear that the addition of BBG fractions is the most appropriate method of obtaining BBG rich cereal food products with acceptable sensorial characteristic.

3.5 Discussion from the outcome of Objective 5

The use of *in vitro* degradation studies for both bread and pasta products clearly illustrate the immense potential beta-glucans have in reducing the potential carbohydrate loading from what are normally classified as carbohydrate rich food products. The incorporation of BBG fractions into both breads and pastas significantly reduce the amount of starch degradation observed during the in vitro analysis. This in turn reduces the rate of reducing sugar release from the food and hence potentially restricts the amount of glucose available for the body to absorb. Such a reduction in absorbable glucose would have a dramatic effect in reducing the potential glycaemic index of foods. The inclusion of BBG fractions into cereal foods appear to make the carbohydrates more slowly digestible, and from a nutritional basis, more acceptable in terms of slow and regulated release of sugars into the body.

As such the research project has served to clearly illustrate the potential nutritional benefits of BBG inclusion in food systems, and significantly adds to the weight of evidence suggesting that beta-glucans from barley grains could have a potential health promoting effect. This in turn would enable the promotion of beta-glucans from barley as functional food ingredients, hence leading to a potential exploitation of beta-glucans in the added food ingredient sector.

3.6 Discussion from the outcome of objective 6

The research has successfully achieved all its objectives and has advanced our understanding of the principles behind beta-glucan use in the food system. The success and importance of the findings of this research project are evidenced in the scientific publication output from the project, in internationally recognised journals. More publications, exploring different parts of the project, are planned in the near future.

However the project was only for a small pilot study of this area. As such a more in depth study is required to investigate areas of specific interest. Relatively little work was conducted in this project on the influence of food processing on the solubilities of beta-glucan fractions. Additionally the research directed at investigating the molecular weight of the beta-glucans from different extractions proved unsuccessful as the methods used yielded inconsistent results (results not shown). This are could be developed further.

Also, the study examined the influence of BBG fractions on dough and pasta quality. Both these products were produced from a small variation of formulations and processes. It would be interesting to determine how altering process conditions further, or ingredient provision, would affect the physical and chemical characteristics of foods. Indeed the study of how BBG fractions react in fat –rich or –free foods would be of interest. Further work could also be conducted on eth role of BBG additions to freeze-thaw stable products, and the shelf-life of cereal and no-cereal food products.

The nutritional analysis of the BBG fractions was very successful in illustrating potential health benefits. However, research would need to be conducted on *in vivo* studies to substantiate the preliminary *in vitro* results obtained in this report. Such dietetic information needs to be done to evaluate the potential use of BBG fractions in diabetic and non-diabetic food systems. This would provide valuable information for the potential use of BBG fractions as functional food ingredients.

3.7 Final recommendations

Further research needs to be conducted on the following items:

- Potential exploitation of extraction techniques in industry, and the manipulation of extraction techniques to develop commercial scale extraction procedures
- Comparison of rheological and molecular weight profiles of BBG fractions from a wider range of barley varieties (to include beta-glucan rich barley varieties)
- Determination of process and product characteristics obtained from a wider range of cereal and non-cereal food items enriched with BBG fractions (to include fat free and fat rich food items)
- Substantiation of potential health benefits of BBG rich foods, through clinical and dietetic trials designed to elucidate the role of BBG in manipulating the glyceamic loading of high carbohydrate foods.

Such research should provide the growers and the food industry with supplementary knowledge of how to utilise the findings from this report to commercially exploit the potential function food market for UK grown barley beta-glucan sources.

Commercial exploitation of such sources is currently underway in both New Zealand and America / Canada. It would be beneficial to collaborate with such centres of innovation in order to ensure that the UK market fully capitalises on such potentials.