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THE POTENTIAL USE OF (13, 14)--D-GLUCAN FROM BARLEY AS A FUNCTIONAL FOOD INGREDIENT FOR CEREAL FOODS

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THE POTENTIAL USE OF (1→3, 1→4)-β-D-GLUCAN FROM BARLEY AS A FUNCTIONAL FOOD INGREDIENT FOR CEREAL FOODS

by

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ABSTRACT

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The potential use of (1→3, 1→4)-β-D-glucan from barley as a functional food ingredient for cereal foods

The health related importance of dietary fibre as part of a balanced diet is well known. More recently, soluble fibres, such as (1→3, 1→4)-β-D-glucan (β-glucan), have been shown to influence glycaemic, insulin and cholesterol responses to foods. Barley is a rich source of β-glucan; however, consumption of products containing barley grain or flour is often limited by their negative organoleptic quality. A potential solution lies in the use of barley as an extraction source for β-glucan fractions. One problem with regards to this is the lack of clarity on the use of barley β-glucan fractions in food systems, particularly their physiological and physico-chemical properties.

The aim of this study was to determine the potential of barley β-glucan fractions as functional ingredients in cereal foods. The effects of extraction treatment on fraction composition and physico-chemical properties were investigated. Subsequently, barley β-glucan fractions (from a bench-top and commercial extraction procedure and of differing molecular weight) were incorporated into white wheat bread and durum wheat semolina pasta. The effects on product quality and *in vitro* starch digestibility were investigated. Simultaneously, the effect of processing on the degradation of β-glucan molecular weight was evaluated.

Different extraction treatments may influence the composition and physico-chemical properties of barley β-glucan fractions. The inclusion of barley β-glucan fractions in bread and pasta resulted in a slight reduction of product quality but generally reduced the rate and extent of *in vitro* starch digestibility. Factors such as composition, water retention capacity, integration within the cereal food matrix and molecular weight may influence the behaviour of the fractions. Bread manufacture resulted in degradation of β-glucan molecular weight, although only high molecular weight β-glucans were susceptible to degradation.

The results of the study have both scientific and commercial value and provide foundations for further development of barley β-glucan enriched cereal products.

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LIST OF ABBREVIATIONS

AACC American Association of Cereal Chemists
AAD American Association of Dieticians
ADA American Diabetes Association
ANOVA Analysis of Variance
ARA Arabinose
BBG Barley β-Glucan
BD Breakdown
BMRB British Market Research Bureau
CCK Cholecystokinin
CDA Canadian Diabetes Association
CHD Coronary Heart Disease
cP Centipoises
CV Coefficient of Variation
CVD Cardiovascular Disease
DAA Dieticians Association Australia
DEFRA Department for the Environment, Food and Rural Affairs
DNSG Diabetes Nutrition Study Group
DSC[1] Differential Scanning Calorimeter
DSC[2] Differential Scanning Calorimetry
DWB Dry Weight Basis
EU European Union
FAO Food and Agricultural Organisation of the United Nations
FDA Food and Drug Administration
FV Final Viscosity
GAL Galactose
GALT Gut Associated Lymphoid Tissue
GI Glycaemic Index
GLU Glucagel™
GLX Glucoxylose
HGCA Home Grown Cereals Authority
HMW High Molecular Weight
HPSEC-FD High Performance Liquid Size Exclusion Chromatography-Fluorescence Detection
IoB Institute of Brewing
LMW Low Molecular Weight
MAN Mannose
M_{cf} Calcofluor Average Molecular Weight
MMT Million Metric Tonnes
MW Molecular Weight
NSP Non-Starch Polysaccharide
PV Peak Viscosity
RSR Reducing Sugars Released
RVA[1] Rapid Visco Analyser
RVA[2] Rapid Visco Analysis
SCFA Short Chain Fatty Acid
SD Standard Deviation
SEC Size Exclusion Chromatography
SEM[1] Scanning Electron Micrograph
SEM[2] Scanning Electron Microscope

SEM[3] Scanning Electron Microscopy
TA Texture Analyser
TDF Total Dietary Fibre
T_{endset} Onset of Gelatinisation
TGI Target Group Index
T_{onset} Gelatinisation End Point
T_{peak} Gelatinisation Peak Temperature
UK United Kingdom
US United States
WHO World Health Organisation
WRC Water Retention Capacity
XYL Xylose

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AUTHORS DECLARATION

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iii. Brennan, C.S. and Cleary, L.J., 2005. The potential use of cereal (1→3, 1→4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science*, **42**, 1-13.

iv. Symons, L.J. and Brennan, C.S., 2004. The influence of (1→3, 1→4)-β-D-glucan-rich fractions from barley on the physico-chemical properties and *in vitro* reducing sugar release of white wheat breads. *Journal of Food Science*, **69**, 463-467.

v. Symons, L.J. and Brennan, C.S., 2004. The effect of barley β–glucan fiber fractions on starch gelatinization and pasting characteristics. *Journal of Food Science*, **69**, 257-261.

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Manuscripts Prepared for Submission:

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iii. Brennan, C.S., Symons, L.J. and Tudorica, C.M., 2005. Low GI cereal foods: The role of dietary fibre and food structure. In: Cauvain, S.P., Salmon, S.S. and Young, L.S. *Using Cereal Science and Technology for the Benefit of Consumers: Proceedings of 12th International ICC Cereal and Bread Congress.* Woodhead Publishing Limited, UK, 95-101.

iv. Symons, L.J. and Brennan, C.S., 2003. Pasting behaviour of β-glucan starch gels: effect of barley β-glucan on starch gelatinisation and degradability. In: *Proceedings of Barley Technology / Cereals Chemistry 2003.* Australian Barley Technology Symposium Inc, Australia, 254-256.

v. Symons, L.J. and Brennan, C.S., 2003. Pasting behaviour of β-glucan/starch gels: the effect of barley β-glucan extracts on starch gelatinisation and degradation. In: *Bioactive Carbohydrate for Food and Feed: Proceedings of Dietary Fibre 2003.* AACC / ICC / TNO, the Netherlands, 79.

Conferences Attended:

i. The 12th ICC Cereal and Bread Congress, 23-26 May 2004, Harrogate, UK.

ii. The 11th Barley Technical Symposium and 53rd Cereal Chemistry Conference 2003, 7-10 September 2003, Adelaide, South Australia.

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CHAPTER 1

LITERATURE REVIEW: THE POTENTIAL USE OF CEREAL (1→3, 1→4)-β-D-GLUCAN FROM BARLEY AS A FUNCTIONAL FOOD INGREDIENT FOR CEREAL FOODS

1.1 INTRODUCTION

Cereals are an important economic commodity worldwide. In the United Kingdom (UK), the cereal harvest is dominated by wheat (15.5 million metric tonnes (MMT)), with barley (6 MMT) representing the second most important cereal crop, and oats (0.6 MMT) being a relatively minor crop (Home Grown Cereals Authority (HGCA) 1999). The (1→3, 1→4)-β-D-glucan (hereafter referred to as β-glucan) content of cereals ranges from 1% in wheat grains, 3-7% in oats and 5-11% in barley (Skendi *et al.* 2003). Thus, barley grains are a rich source of β-glucan.

β-glucans from oat and barley have received considerable attention with regard to their hypoglycaemic (Wood *et al.* 1990, 1994a) and hypocholesterolemic capacity (Braaten *et al.* 1994; Beer *et al.* 1995) in humans and also in relation to a reduction in the incidence of diabetes (Li *et al.* 2003a) and cardiovascular disease (CVD) (Keogh *et al.* 2003). In the UK, barley is largely used for animal feed and malt, with use for human consumption very limited; however, because barley is a rich source of β-glucan, an opportunity exists to utilise the grain as a functional food ingredient.

The aim of this chapter is to explore some of the applications and potential nutritional advantages of using β-glucan from barley as a functional food ingredient and to provide a context for the research study. The chapter is divided into five main sections. The first section provides an introduction to the barley crop including, its current economic

importance, grain morphology and composition. In the second section, barley β-glucan, its extraction and rheological properties are discussed. The third section evaluates the role of barley β-glucan as a functional food ingredient and its physiological effects, and the fourth section examines the impact of barley β-glucan on the quality of cereal food products. The fifth and final section evaluates the effect of food processing on the molecular, structural and functional properties of barley β-glucan. The chapter closes with a conclusion summarising the gaps in current knowledge regarding the use of barley β-glucan as a functional ingredient in cereal foods, thus, providing a rationale for the study. Specific aims and anticipated outcomes of the study are also identified.

1.2 BARLEY

Barley is a grass belonging to the family Poaceae, the tribe Triticeae and the genus *Hordeum* (Nilan and Ullrich 1993). Barley can be considered as one of the most ancient crop plants, with its cultivation being mentioned in the Bible. The original area of barley cultivation is reported to be in the Fertile Crescent of the Middle East. Archaeological evidence suggests that the most recent and immediate ancestor of cultivated barley is the two-rowed wild *H. vulgare spontaneum*, which is found growing wild in many areas of South-west Asia and Northern Africa today. Other ancestors have been proposed, but there appears to be no evidence that six-row domesticated barley was derived from any ancestral form other than *H. spontaneum*. Cultivated barley is adapted and produced over a wide range of environmental conditions; it has been or is currently grown from inside the Artic Circle, where soil thaws to only a few inches during summer, to the tropics.

Annual world production of barley is approximately 138 MMT. Leading barley producers are the European Union (EU) (52.96 MMT), the former Soviet Union (32.16 MMT) and Canada (12.13 MMT) (Food and Agricultural Organisation of the United Nations (FAO) 2005). In the UK, barley is the second most cultivated crop; approximately 6 MMT was

produced in 2005 (Department for the Environment, Food and Rural Affairs (DEFRA) 2005a).

1.2.1 Barley Uses

Barley is principally used as feed for animals, particularly pigs, in the form of barley meal for malting and brewing in the manufacture of beer and distilling in whisky manufacture, and to a small extent, human consumption.

1.2.1.1 Feed

Barley, one of the four major feed grains of the world (corn, barley, oats and wheat), is widely used as a livestock feed. In the UK, typically 40-50% of the barley crop is used for animal feed (HGCA 1999). The grain may be used as a major source of energy and protein for pigs (Newman *et al.* 1991) and to support egg production of laying hens. Digestible energy or (metabolisable energy) remains the single most important criterion in feed barley, particularly for monogastric animals. Although the amino acid balance and total protein content in barley is superior to that of corn, its feed value is less because it contains 50-60% starch. In hulled barley, the fibre content is too high and its use is limited to only a small percentage of poultry rations. Work conducted on poultry has clearly illustrated the effect these fibre components have on reducing feed digestibility, metabolisable energy (Annison 1991; Jeroch and Danicke 1995; Classen 1996; Bergh *et al.* 1999) and the occurrence of other negative consequences (i.e. sticky droppings) (Choct *et al.* 1999). As such, barley cannot be used for chicks without treatment with β-glucan degrading enzymes (Almirall *et al.* 1995; Fuente *et al.* 1998; Von Wettstein *et al.* 2003). Similar observations have been made with pigs, which will not make maximum weight gain with barley as the feed grain (Baidoo and Liu 1998; Knudsen and Canibe 2000; Leterme *et al.* 2000). The consequence of these negative nutritional effects is the low economic value of feed barley.

In the last decade, the UK price of feed barley has decreased by approximately 50% (DEFRA 2005b).

1.2.1.2 Malting, brewing and distillation

In the UK, by far the highest domestic use of barley is for malting and brewing in the manufacture of beer and for distilling in whisky manufacture. Both two and six-row barleys are suitable for malting, although the former is generally used in Europe (Kent and Evers 1994). In the UK, approximately 15 varieties are accepted by the Institute of Brewing (IoB) as malting varieties (HGCA 2005). Much research has focused on the role of endosperm components in determining the malting potential of barley (Bathgate *et al.* 1974; Bamforth *et al.* 1979; Henry and Blakeney 1986; Palmer 1987; Brennan *et al.* 1996b, 1997; Molina-Cano *et al.* 2002; Edney and Mather 2004). Levels of β-glucan in the grain have long been regarded as one of the most influential characteristics in relation to the malting potential and brewing yield in barley, regulation of the rate of endosperm modification (Bacic and Stone 1980, 1981; Bourne *et al.* 1982; Bamforth and Martin 1983; Brennan *et al.* 1998; Edney and Mather 2004) and ultimately the viscosity of wort during brewing (Bourne and Pierce 1970).

1.2.1.3 Human consumption

The use of barley for human consumption (other than in malting and distilling) is relatively small in developed countries, and as a result barley is largely considered as a forgotten food, which is rejected as coarse grain for livestock feed. In the UK, only a small proportion of the total crop goes to the food trade. Consumption of barley is greater in the Far and Middle East where much of the barley is consumed as pearled grains for soup, as flours for flat bread and as a ground grain to be cooked and eaten as porridge (Kent and Evers 1994). An interesting feature of barley, which will lead to an increase its utilisation, is the level and range (2-11%) of β-glucan, a major soluble fibre component in barley.

Cereal β-glucans (predominantly from oats and barley) are documented to have hypocholesterolemic and hypoglycaemic capacities, as reported in many human and animal studies (Jenkins *et al.* 1976; German *et al.* 1996; Liljeberg *et al.* 1996).

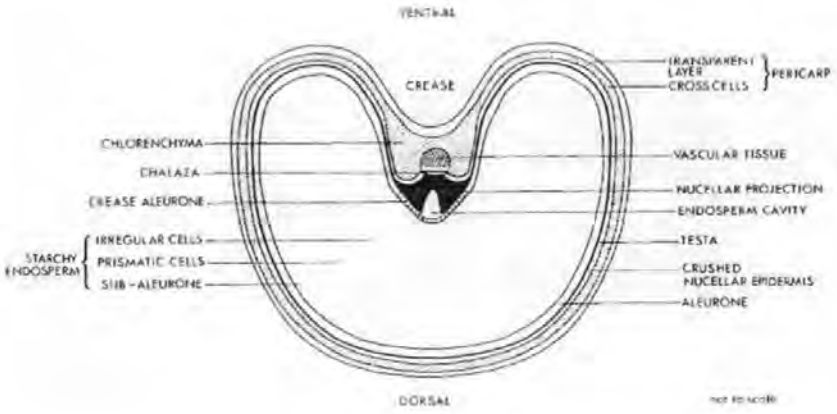
1.2.2 Plant and Grain Morphology

In the mature barley plant, kernels consist of the caryopsis only in naked barleys but which include the lemma, palea and the rachilla, which adheres to the caryopsis in hulled barley. The hull is removable only with difficulty and amounts to about 13% of the grain (by weight); on average the proportion ranges from 7-25% (according to type, variety, grain size and the latitude where barley is grown). Winter barleys have more hull than spring types, six-row (12.5%) more than two-row (10.4%) (Kent and Evers 1994). The proportion of hull increases as the latitude of cultivation approaches. Hull-less or naked barley has been developed in western North America for livestock feed and human food. In hull-less barley, unlike hulled barley, the hull is removed during harvesting. Waxy hull-less types have also been developed especially for human use. Registered hull-less barley cultivars include: Bear, Shonkin, Nubet and Robust in Canada; Azhul, Waxbar, Merlin and an isoline of Compana, Prowashonupana in the United States (US); Waxiro in Australia; and Taiga in Germany (Jadhav *et al.* 1998). Two-rowed hull-less cultivars predominate because of their plump kernel, white aleurone and soft endosperm, which are all desirable in food and industrial applications (Bhatty 1999).

Barley kernels are generally larger and more pointed than wheat, but they are not less broad (1.0-4.4 mm); they have a ventral crease, which is shallower than those of wheat and rye, its presence obscured by the adherent palea. Two to four aleurone layers are present, and cells are approximately 30 μm in each direction. As with most cereals the endosperm forms the largest tissue of the grain; the majority being the starchy endosperm in which (except in some mutants) two populations of starch granules, A and B, exist. Figures 1.1 a

and b represent a transverse and longitudal cross-sectional view of the barley caryopsis respectively.

a)



b)

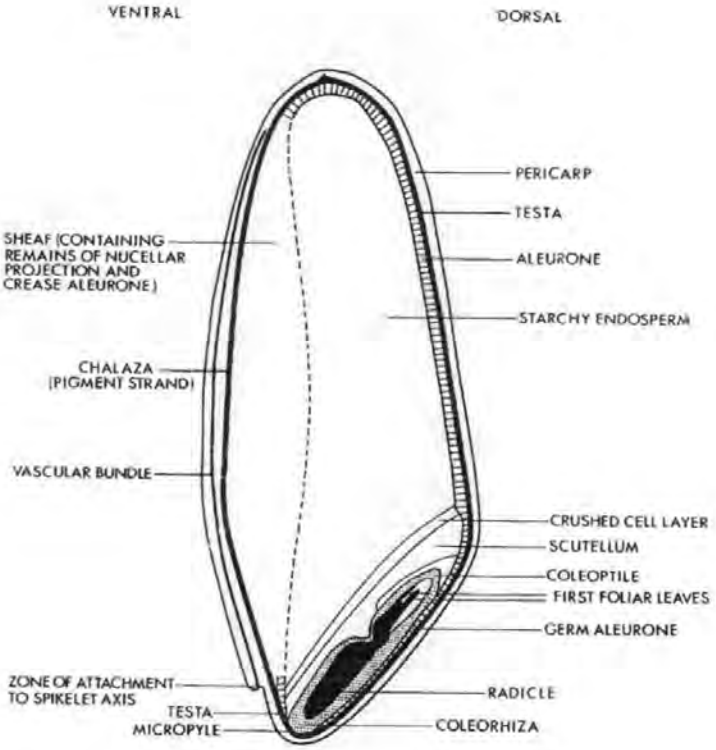


Figure 1.1 Diagram representing: (a) a transverse section cut at mid-grain through a barley caryopsis at the end of the grain filling period; and (b) a longitudal section cut to bisect the crease of a mature barley caryopsis (from MacGregor and Fincher 1993).

At harvest ripeness the moisture content of the grain is about 15%. The dry matter is made up of approximately 80% carbohydrate, 10% protein, 3% lipid and 2% minerals. Table 1.1 presents a full chemical composition of typical mature barley grain.

Table 1.1 Chemical composition of mature barley grain (from Harris (1962) cited by MacGregor and Fincher (1993))

Component	**Dry weight (%)**
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water soluble polysaccharides	1-1.5
Alkali soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2.3
Protein	10-12
Albumins and globulins	3.5
Hordeins	3-4
Glutelins	3-4
Nucleic acids	0.2-0.3
Minerals	2
Others	5-6

Comparisons between the composition of hulled and hull-less barley (data taken from two studies in the US and Sweden) revealed that hull-less barley generally contains more protein and starch, its two major components, and increased β-glucan; this is due to

removal of the fibrous hull, which has a dilution effect on these components (Bhatty and Rossnagel 1998). Hull-less barley also contains more pentosans and total dietary fibre (TDF) components (Klason lignin, cellulose, enzyme resistant starch and uronic acid). Table 1.2 illustrates the effect of the hull-less gene on the composition of barley genotypes.

Table 1.2 Effect of the hull-less gene on the composition of barley genotypes (from Bhatty 1999)

Component (% dry weight)	**Hulled[a] (n=10)**	**Hull-less[a] (n=6)**	**Hulled[b] (n=12)**	**Hull-less[b] (n=24)**
Protein	12.2	15.1	15.9	16.5
Esther extract	2.5	2.7	2.2	2.3
Ash	2.1	1.6	2.8	2.1
Starch	57.7	60.7	53.7	59.7
Total β-glucan	4.8	5.7	5.2	5.6
Soluble β-glucan	2.3	2.9	3	3.1
Pentosans	7.9	5.7	6.5	4.5
Cellulose	4.8	2.9	4.1	2.0
Klason lignin	1.3	0.7	2	0.9
Uronic acid	0.8	0.6	...	...
TDF	20.6	16.6	18.6	13.8

[a]Waxy, normal and high amylose starch barleys (Oscarsson *et al.* 1997). [b]Isotypes of Betzes (CI6598) and Compana (CI5438) barleys (Xue *et al.* 1997).

1.2.3 Non-Starch Polysaccharides of the Barley Grain

The non-starch polysaccharides (NSPs) found in mature grains include, fructans, β-(1→4)-D-glucans (cellulose), β-glucans, arabinoxylans and glucomannans. The β-glucans are

linear molecules with approximately 30% β-(1→3) and 70% β-(1→4) linkages randomly dispersed and associated with firmly linked peptide sequences in the barley endosperm cell wall (Fleming and Kawakami 1977; Forrest and Wainwright 1977).

Differences have been 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 contain 26% β-glucan and 67% arabinoxylan. Both contain similar amounts of glucomannan and cellulose (i.e. 2-4% of each polymer) (MacGregor and Fincher 1993). Table 1.3 illustrates the typical composition of cell walls from barley.

Table 1.3 Composition of cell walls from barley (from MacGregor and Fincher (1993))

Tissue	**Neutral monosaccharide composition of total polysaccharides (%)**					**Protein (%)**	**Phenolic acids (%)**	**Major polysaccharide components (%)**
	Ara	**Xyl**	**Glx**	**Man**	**Gal**			
Aleurone (mature grain)	24	47	26	2	2	16	1.2	71% Arabinoxylan 26% β-glucan 2% Cellulose 2% Glucomannan
Starchy endosperm (mature grain)	11	11	75	3	0	5	0.05	75% β-glucan 20% Arabinoxylan 2% Cellulose 2% Glucomannan

Using fluorescence microscopy, Miller and Fulcher (1994) reported that β-glucan was more uniformly distributed throughout the barley grain endosperm than in oats. In oats, β-glucan was concentrated in the outer or bran layers of the grain, particularly in low β-glucan varieties. Oscarsson *et al.* (1997) illustrated considerable variation in β-glucan distribution among barley genotypes. Cell walls on the ventral side of the kernel and central endosperm in the vicinity of the crease of high β-glucan barley were more heavily stained with Calcofluor than those from low β-glucan barley. Zheng *et al.* (2000) observed that in low β-glucan hull-less barley the β-glucan content was in greatest abundance in the sub-aleurone layer and declined towards the inner layers. In high β-glucan hull-less barley, more than 80% of the β-glucan was distributed evenly through the endosperm.

1.2.4 Occurrence of β-Glucan in the Barley Grain

A number of methods have been developed to estimate β-glucan contents of the barley grain including, enzymatic techniques (McCleary and Glennie-Holmes 1985), use of Calcofluor (Wood and Weisz 1984) and Near Infrared Reflectance (Szczodrak *et al.* 1992). Total levels of β-glucan in barley can vary dramatically between varieties, ranging from 2-11%, but typically fall between 4-7%. A waxy hull-less barley from the US, Prowashonupana, has been reported to have a β-glucan content of approximately 17% (Bhatty 1993). 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, utilisation and nutritional value of barley 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). There is a general agreement that the genetic background of barley is more important than environmental conditions as a determinant of the final β-glucan content of the grain (Gill *et al.* 1982; Henry and Blakeney 1986; Stuart *et al.* 1988). For instance, research conducted by Lehtonen and Ailasalo (1987) reported that two-row barley genotypes had a higher β-glucan content than six-row barley. Studies have also

indicated that waxy (up to 100% amylopectin) barley cultivars have higher levels of β-glucan in the endosperm than non-waxy varieties (Ullrich *et al.* 1986; Yoon *et al.* 1995).

Bhatty *et al.* (1991) observed that cell wall thickness in low (3.9%), medium (4.9%) and high (5.4%) β-glucan genotypes of hulled and hull-less barley was related to β-glucan concentration. In this study, cell walls appeared thicker in the sub-aleurone layer, which suggested a higher concentration of β-glucan. Conversely, Miller and Fulcher (1994) did not observe variations in the thickness of sub-aleurone cell walls when examining different barley cultivars using microspectrofluorometry.

A major environmental factor that influences β-glucan levels in the grain appears to be the availability of water during grain maturation. Dry conditions (heat stress) before harvest result in high β-glucan levels (Bendelow 1975), with a positive relationship between β-glucan level and final grain weight (Savin and Molina-Cano 2001); this observation agrees with field studies on the effect of drought conditions on β-glucan content of the grain (Stuart *et al.* 1988; Coles *et al.* 1991), and may either be related to the fact that final grain fill is adversely affected in drought conditions through impairment of starch synthesis and deposition, or because β-glucan synthesis may be enhanced in dry conditions (Munck *et al.* 2004). Moist conditions have been reported to cause a decrease in β-glucan levels (Stuart *et al.* 1988; Aman *et al.* 1989), so that increased levels of irrigation reduce β-glucan content of the grain (Guler 2003).

1.3 β-GLUCAN

β-glucan is an un-branched polysaccharide composed of cellatriosyl and cellatetraosyl units linked by β-(1→3) and β-(1→4) linkages in a ratio of 3:7 (Woodward *et al.* 1983; Harris *et al.* 1984; Wood *et al.* 1991a; Henriksson *et al.* 1995). The linkage arrangement is not irregular but follows statistical rules (Woodward *et al.* 1983; Buglia *et al.* 1986;

Henriksson *et al.* 1995). Cellotriosyl and cellotertaosyl units constitute 90% of the polysaccharide, with the remaining structure containing longer consecutive β-(1→4) linked glucopyranosyl units (Woodward *et al.* 1988; Wood and McCrae 1996; Izydorczyk *et al.* 1998a). Literature on the fine structure of barley β-glucan has been reviewed by MacGregor and Fincher (1993). The application of high performance anion-exchange chromatography to the separation of the oligosaccharide release by specific hydrolysis has aided the analysis of the fine structure of β-glucans from different botanical sources. Differences have been observed between isolated β-glucans from oats, barley and other cereals. In particular, the ratio between cellotriosyl and cellotetraosyl units is higher in barley than oats (Wood *et al.* 1994b; Cui *et al.* 2000; Tosh *et al.* 2004).

The presence of β-(1→3) linkages gives an irregularity to the β-glucan molecule, which prevents intermolecular association and makes β-glucan partially soluble (Buglia *et al.* 1986). The majority of authors agree that the cellulose-like sequences of up to 14 consecutive β-(1→4) linkages have the potential to aggregate through hydrogen bonds and precipitate from solution (Woodward *et al.* 1983; Letters *et al.* 1985; Bamforth 1994). Longer blocks of contiguous cellotriosyl residues are also believed to be responsible for insolubility (Izawa *et al.* 1993). The associative abilities of β-glucan leads to the formation of gelatinous precipitates, which are of great importance in brewing where their presence impedes proper filtration of the beer (Bohm and Kulkie 1999). Figure 1.2 illustrates the structure of the β-glucan molecule.

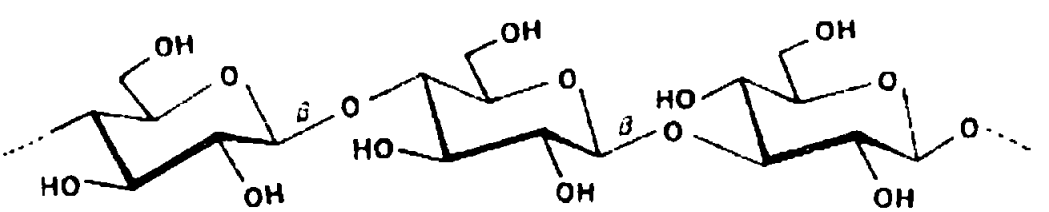


Figure 1.2 The structure of β-glucan (from Tungland and Meyer 2002).

Molecular weight (MW) ranges reported for β-glucans show variability between cereals, with oat β-glucans generally having a higher upper MW (0.065-3 x 10^6 g/mol) than barley (0.15-2.5 x 10^6 g/mol) (Wood *et al.* 1991b; Beer *et al.* 1997a,b; Irakli *et al.* 2004; Lazaridou *et al.* 2004a,b).

1.3.1 Barley β-Glucan Extraction Procedures

Barley and oat β-glucans, together with other NSPs, occur in the walls of the endosperm cells, which enclose starch, matrix proteins and lipids reserves of the grain. Thus, their recovery is not straightforward. The study of the physico-chemical properties of isolated β-glucan fractions requires extraction procedures, which optimise yield, purity and integrity of the β-glucan molecule. To obtain an economically viable functional food ingredient, these considerations have to be balanced and a compromise reached.

Barley flours may be enriched with β-glucan by dry milling and sieving (Knuckles *et al.* 1992) or air classification and sieving (Wu *et al.* 1994; Knuckles and Chiu 1995; Sundberg *et al.* 1995); however, much more research has focused on the impact of wet isolation and purification techniques on the physico-chemical and structural properties of barley β-glucan (Fincher 1975; Klopfenstein and Hoseney 1987; Woodward *et al.* 1988; Bhatty 1993, 1995; Temelli 1997; Burkus and Temelli 1998).

Key extraction methodologies for barley and oat β-glucans were developed by Wood *et al.* (1977, 1978). These researchers assessed the effects of particle size, temperature, pH and ionic strength on β-glucan yield on a laboratory scale. In a further study, the authors prepared an oat gum fraction (from oat bran) on a pilot plant scale by extracting hot 75% ethanol-inactivated oat bran (outer starchy endosperm and overlying aleurone and pericarp-seed coat) with a sodium carbonate solution at pH 10 to give a preparation containing 78% β-glucan (Wood *et al.* 1989). Although this simple extraction process was successful in

generating β-glucan material from cereals, McCleary (1988) illustrated that sequential water extractions at 40, 65 and 95°C increased the extraction rate of barley β-glucan to 90%, thus, enabling an increase in overall yield. Different extractants were investigated by Bhatty (1993) who found that optimum recovery of barley and oat gums (with retained viscosity characteristics) could be obtained using 1 M NaOH; however, there was contamination of this extract with considerable amounts of starch and protein, which resulted in an impure product. To counteract this, Saulnier *et al.* (1994) used a hot water extraction procedure in the presence of thermostable α-amylase to minimise the contamination from starch and yield a more purified barley β-glucan material.

The cost of extraction techniques is one of the major limiting factors in the food industry utilisation of β-glucans. Thus, pure preparations of β-glucans have often been ignored as potential functional food ingredients; this is primarily due to the relatively inexpensive use of barley or oat flour fractions, which in turn has meant that the actual characteristics of these products in food systems are often variable due to fluctuations in protein or starch composition of the flour fractions. Hence, subsequent viscosity, structural and nutritional effects on foods have to be considered in relation to the nature of the β-glucan extract or the composition of the flour material used.

Investigation of different organic solvents as precipitants of barley β-glucan (Beer 1996; Morgan and Ofman 1998) has shown that the extraction solvent affects the structural conformation, MW and solubility of the precipitated β-glucan. To offset these potential negative factors, whilst endeavouring to produce a more cost effective extraction process, Morgan and Ofman (1998) developed a hot water extraction procedure with freezing and thawing for the recovery of β-glucan from barley. The resulting product ('Glucagel™') contained between 89-94% β-glucan (depending on the duration of the initial extraction). Glucagel™ is a β-glucan preparation that is commercially produced for use as a functional

food ingredient. Currently, there is a paucity of studies that have compared the composition, MW and physico-chemical properties of barley β-glucans from solvent precipitation and novel extraction procedures.

The temperature and pH of the extraction process also affects the recovery of barley β-glucan. Temelli (1997) demonstrated that β-glucan extraction increased with temperature. A further evaluation of the influence of extraction conditions on yield, composition and viscosity stability of barley gum was conducted by Burkus and Temelli (1998) using regular barley (Condor) and a waxy cultivar blend. Extraction conditions were evaluated, including, extraction with: no additional treatment; boiling of the extract; prior refluxing of flour with 70% ethanol; and treatment of the extract with thermostable α-amylase. The highest β-glucan purity was achieved with a boiled Condor extract at pH 7 (81.3% yield) and was closely followed by refluxed waxy barley extracted at pH 8 and amylase treated (79.3% yield). Refluxed gums followed by purification at pH 7 exhibited the most stable viscosity.

As previously mentioned, the nature of extraction procedures can have a profound affect on the MW of barley β-glucan and in turn may influence its functional behaviour. Carr *et al.* (1990) observed that the use of NaOH for complete extraction resulted in partial depolymerisation of β-glucan. Although Knuckles *et al.* (1997b) included sodium borohydride in an NaOH extraction at 65°C to prevent alkaline depolymerisation, the MW of the extracted barley β-glucan was lower than that extracted with water at 100°C. Beer *et al.* (1997a) also observed that the MW of β-glucans extracted from oats and barley with NaOH was lower than those extracted with hot water. Knuckles *et al.* (1997b) illustrated that sequential extractions resulted in a decrease in extracted β-glucan MW, and Storsley *et al.* (2003) illustrated that the temperature used for sequential water extractions affects the ratio of (1→4) to (1→3) linkages and the amount of cellulosic regions on the β-glucan

chain. Care must therefore be taken to optimise the yield and rheological characteristics of β-glucan components and avoid depolymerisation during extraction.

1.3.1.1 Influence of endo-β-(1→3, 1→4)-glucanase

Enzymatic hydrolysis may present difficulty in the successful extraction and retention of barley β-glucan with desirable physico-chemical properties. Endo-β-(1→3, 1→4)-glucanase (hereafter referred to as β-glucanase) enzymes originate from micro-organisms or the barley grain itself (Kanauchi and Bamforth 2001). The mechanism by which these enzymes operate is by degradation of the glucan component of the cell walls, acting on both insoluble (hemicellulose) and soluble (gum) glucans (Kanauchi and Bamforth 2001). Alternative arguments propose that the initial digestion of the hemicellulosic fraction involves other enzymes (solubilases), and it is the action of these enzymes that represent the substrate for endo-β-glucanase. Suggested substances include carboxypeptidase (Bamforth *et al.* 1979; Bamforth 1981), phospholipase (Palmer 1987), endo (1→3) glucanase (Bathgate *et al.* 1974), endo (1→4) glucanase (Yin and MacGregor 1988) and ferulic acid esterase (Moore *et al.* 1996). Methods commonly used for inactivating β-glucanase during the preparation of cell wall fractions include, autoclaving, refluxing with hot aqueous ethanol (Carr *et al.* 1990; Beer *et al.* 1996) and treatment with trichloroacetic acid (Forrest and Wainwright 1977).

1.3.2 Flow and Gelling Behaviour of Barley β-Glucan

The rheological characteristics of barley β-glucan has obvious links to its viscosity behaviour (either in native form or as an extract in formulated foods) and potential effects on food structure, texture and nutritional properties. Like other soluble NSPs, β-glucan exists in solution as random coils. The properties of these random coils in solution (viscosity) are largely dependent on concentration and MW (Robinson *et al.* 1982; Bohm

and Kulkie 1999). The flow properties of β-glucans from both oat (Autio *et al.* 1987; Doublier and Wood 1995) and barley (Bhatty 1995; Burkus and Temelli 2005) in solution have been investigated. The pseudoplasticity of high viscosity barley β-glucan gums is an already established fact (Bhatty 1995; Burkus and Temelli 2005). Low viscosity β-glucan gums and high viscosity β-glucan gums at low concentrations have been illustrated to behave like Newtonian fluids (Burkus and Temelli 2005).

In addition to the flow properties of β-glucan, its ability to form a gel is of importance in terms of technological and nutritional functionality. Gelation is the association or cross-linking of long polymer chains to form a 3-dimensional network, which traps and immobilises liquid to form a structure resistant to flow under pressure (Glicksman 1982, cited by Burkus and Temelli 1999). There are relatively limited investigations of the gelation properties of barley β-glucan. Doublier and Wood (1995) reported that hydrolysed oat gums exhibited gel like behaviour, and that high viscosity gums did not gel. In the studies of Burkus and Temelli (1999), low viscosity barley β-glucan was found to form a gel at a concentration of $\geq$5%. Increasing concentration from 5-5.5% resulted in a gel that could withstand 2.5 times higher stress (determined by compression tests). A further increase to 6% resulted in a much lower stress increase. Lazaridou and Biliaderis (2004) demonstrated that the storage modulus (G') of barley β-glucan cryogels increased with decreasing MW, and hence, a reduced gelation time and increased gelation rate were observed. Similarly, Vaikousi *et al.* (2004) illustrated that gelation time was decreased for β-glucan gels from low MW (LMW) sources, and that gels made from high MW (HMW) β-glucan sources exhibited increased yield stress and reduced storage modulus (G'max) values. Similar findings have been reported for water extractable β-glucans from Greek barley cultivars (Irakli *et al.* 2004). Tosh *et al.* (2004) illustrated that differences in the ratio of cellotriosyl/cellotetraosyl units affected the gelation characteristics and elasticity of β-glucan systems. Indeed, reduced solubility of β-glucan systems has been attributed to

higher ratios of cellotriosyl/cellotetraosyl units (Skendi *et al.* 2003). Thus, because the viscoelastic characteristics of β-glucan gels are related to the MW of the isolated fractions, differences in MW observed among β-glucans extracted from different cultivars of barley (Izydorczyk *et al.* 1998b,c) need to be considered in relation to their potential behaviour in food systems.

1.4 ROLE OF BARLEY β-GLUCAN AS A FUNCTIONAL FOOD INGREDIENT

Much of the more recent attention with regards to β-glucans use in food systems has stemmed from its role as a functional dietary fibre. The term dietary fibre is used to collectively describe a group of complex substances in plant material, which resist human digestive enzymes, including non-swellable, more or less hydrophobic components, such as cutins, suberins and lignins, as well as a wide range of hydrophilic compounds, such as soluble and insoluble polysaccharides and non-digestable oligosaccharides. The distinction between soluble and insoluble fibres is based on *in vitro* studies and chemical analyses of plant cell walls. Official definitions of dietary fibre have been made by the Dietary Fibre Technical Committee of the American Association of Cereal Chemists (AACC) (AACC 2000a, 2001, 2003).

Potential health benefits of dietary fibre include: manipulation of bowel transit time (Feldheim and Wisker 2000); prevention of constipation and reduction in risk of colorectal cancer (Bingham 1990; Hill 1997; Faivre and Bonithon-kopp 1999); lowering of blood and serum cholesterol and regulation of blood glucose levels for diabetes management (Bornet *et al.* 1987; Gallaher *et al.* 1993; German *et al.* 1996; Frost *et al.* 1999); and production of short chain fatty acids (SCFAs) (Karppinen *et al.* 2000; Velasquez *et al.* 2000; Wisker *et al.* 2000) for the promotion of colonic health (stimulating the growth of beneficial gut microflora) (Crittenden *et al.* 2002; Tungland 2003).

Research of the physiological behaviour of dietary fibre has broadly examined the effects of soluble and insoluble fractions as purified fibre or in naturally fibre-rich whole foods. High fibre foods have been related to the modulation of glycaemic response on the basis of early studies by Jenkins *et al.* (1976, 1977, 1978, 1980) and more recently, Truswell (2002) and Tudorica *et al.* (2002c) using both purified fibre and naturally fibre-rich foods. In particular, foods high in soluble dietary fibre have been shown to have a positive impact on reducing hyperglycaemia and hyperinsulinaemia in relation to the control of diabetes (Li *et al.* 2003a) and the reduction of risk factors for degenerative diseases including, obesity (Burley *et al.* 1987), hyperlipidaemia (Jenkins *et al.* 1985; Maki *et al.* 2003; Yang *et al.* 2003), CVD (Keogh *et al.* 2003), cancer (Sier *et al.* 2004) and hypertension (Anderson 1983, 1990).

Many attempts have been made to clarify the mechanisms by which soluble dietary fibres behave. With regard to the reduction of glycaemic response, proposed mechanisms include: the concentration and composition 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.* 1987b; Brennan *et al.* 1996a; Tudorica *et al.* 2002c). The cholesterol lowering potential of soluble cereal fibres is primarily considered as a result of increased excretion of bile acids and the increased synthesis of bile acids at the expense of cholesterol (Bengtsson *et al.* 1990).

1.4.1 Functional Foods

Chronic diseases are now the major causes of death and disability worldwide. Non-communicable conditions including, CVD, diabetes, obesity, cancer and respiratory disease account for 59% of 57 million deaths annually and 46% of the global burden of disease (World Health Organisation (WHO) 2003). A relatively few risk factors, which include high cholesterol, high blood pressure, obesity, smoking and alcohol, are

responsible for the majority of the chronic disease burden. Amongst other factors (physical activity and controlling weight, alcohol and tobacco), improving dietary habits to include eating more fruit, vegetables, nuts and wholegrain foods (rich in dietary fibre), moving from saturated animal fat to unsaturated vegetable oil fats and reducing consumption of foods high in fat, salt and sugar can have a significant impact upon reducing rates of chronic disease.

There is widespread recognition that dietary fibre consumption plays an important role in the prevention of CVD, some kinds of cancer and diabetes; however, dietary fibre intakes in many western countries languish far below recommended levels. It is estimated that in some western countries daily dietary fibre intake can be as little as 2-4 g (Mathers and Wolever 2002) against a recommended quantity of >18 g. Greater intake of dietary fibre may be encouraged if a wider choice of palatable and appealing foods that are rich in dietary fibre existed, and this may be achieved by increasing dietary fibre levels in popular food products. The potential of dietary fibre as a food component that can reduce the risk of disease and promote health has positioned it as an ingredient of great interest in functional foods (Sloan 1999).

The history and definition of functional foods has been reviewed extensively by Katan and De Roos (2004) and Arvanitoyannis and van Houwelingen-Koukaliaroglou (2005). There is no universally accepted definition for functional foods or any legal definition of the term in the EU. Currently academic, scientific and regulatory organisations within the EU are working towards a harmonised definition and regulation of the use and marketing of functional foods. The key aim of such regulation is to protect consumers from misleading claims and give the functional foods sector more direction and clarity. Scientifically functional foods have been defined as:

"affecting beneficially one or more target function in the body beyond adequate nutritional effects in a way that is relevant to either improved state of health and well-being and/or reduction in the risk of disease." (Anon 1999)

This definition can be translated into "foods with health benefits beyond nutritional value". The various ways in which a functional food may deliver health benefits include: enhancement or promotion of the intrinsic beneficial properties of a food (or drink); the addition of one or more functional components to a food (or drink); or removal of components of a food (or drink) (Anon 2004).

The functionality of foods is derived from bioactive ingredients. Bioactive ingredients in functional foods may help in the prevention of chronic diseases or the enhancement of performance and well-being. The term 'nutraceutical' is often used interchangeably with functional foods; however, the scope of nutraceuticals is substantially different to that of functional foods. Although the prevention and treatment of disease (medical claims) are related to nutraceuticals, only the reduction of disease is related to functional foods. In contrast to nutraceuticals, including supplements as well as other types of foods, functional foods are expected to be in the form of ordinary foods (Arvanitoyannis and van Houwelingen-Koukaliaroglou 2005).

As will be discussed in the following section of the review, β-glucans from both oat and barley have much potential as functional food ingredients. Such potential, together with the US Food and Drug Administration (FDA) acknowledgment of the relationship between soluble fibre from whole oats, oat bran, whole grain barley and barley containing products and the risk of coronary heart disease (CHD) (Anon 1997a,b, 2005a), has led to a considerable number of companies, both in Europe and the US, creating commercial β-glucan preparations (the majority of which are derived from oat) for inclusion in functional foods (Table 1.4).

Table 1.4 Commercial β-glucan (from oat and barley) preparations

Company (Country)	**Product**
Viljava (Avena) (Finland)	Natureal Oat-bran
Ceba (Sweden)	Swedish Oat Fiber
CreaNutrition–SOF (Switzerland)	Oatwell Oat Bran
Quaker Food/Oats Rhodia (US)	Betatrim (Oat)
Crompton and Knowles (US)	Dri-Flo Healthy Oatbuds
Immunocorp, CA (US)	Norvegian Beta-Glucan (Oat)
Nurture INC, PA (US)	Nurtures OatVantage
	Nurture 1500 (Oat)
GTC Nutrition Golden (US)	Nutureal GI (Oat)
PolyCell (US)	Glucagel (Barley)

1.4.2 Physiological Effects of Barley β-Glucan

The most widely documented nutritional benefit of β-glucan consumption is the flattening of post-prandial blood glucose and insulin rises. Both barley (Hallfrisch *et al.* 2003; Li *et al.* 2003a,b) and oat β-glucans (Wood *et al.* 1990, 1994a; Jenkins *et al.* 2001; Kabir *et al.* 2002; Poyhonen 2004) produce this response. Likewise, both barley (Delaney *et al.* 2003; Li *et al.* 2003a; Yang *et al.* 2003; Smith *et al.* 2004) and oat (Braaten *et al.* 1994; Beer *et al.* 1995; Kang *et al.* 2003; Kerckhoffs *et al.* 2003) β-glucans have been shown to reduce serum and blood cholesterol levels.

Other notable, but less documented, nutritional benefits of β-glucan (from both oat and barley) consumption include: reduced gastrointestinal enzyme activity (Schneeman and Gallaher 1985); diminished absorption of nutrients (Edwards *et al.* 1988; Lund *et al.* 1989); delayed gastric emptying (Johansen *et al.* 1996, 1997); prolonged post-prandial

satiety (Anderson 1990; Bourdon *et al.* 1999); increased stool bulk and relief of constipation (Hojgaard *et al.* 1980; Valle-Jones 1985; Odes *et al.* 1993); prevention of colorectal cancer (Thorburn *et al.* 1983; Reimer *et al.* 2000); and immunostimulatory effects (Causey *et al.* 1998; Fulcher *et al.* 2000).

1.4.2.1 Attenuation of glycaemic response

On the basis of studies by Jenkins *et al.* (1976, 1977, 1978, 1980), high fibre foods have been correlated to the modulation of glycaemic response and as a consequence retarded and diminished insulin secretion. The importance of viscosity for this effect has been observed by Jenkins *et al.* (1978) and Wood *et al.* (1994a) who illustrated that rises in post-prandial blood glucose and insulin concentrations were reduced after meals containing viscous polysaccharides. The hypoglycaemic effect of foods enriched with fibres is commonly expressed in relation to the Glycaemic Index (GI). Jenkins and co-workers introduced the concept of GI, an established physiologically based method, to classify foods according to their blood glucose raising potential (Jenkins *et al.* 1981). GI is defined as the incremental blood glucose area (0-2 hours) following 50 g available carbohydrate in the test product and is expressed as the percentage of the corresponding area following an equivalent amount of carbohydrate from a reference product (typically glucose or white bread).

There is a strong consensus that the reduction of the glucose and insulin peak after consumption of viscous soluble fibres, such as β-glucan, is as a result of an increase of the viscosity of the contents of the stomach and small intestine. This increase in viscosity reduces the absorption rate of digested nutrients from the small intestine by resistance of the convective effects of intestinal contractions (Edwards *et al.* 1988; Adiotomre *et al.* 1990). Glucose transport in the intestinal tract wall is inhibited partly by an increase in the resistance of the mucosal diffusion barrier that is brought about by the greater viscosity of

the intestinal bolus containing the β-glucan. The mobility of fluid layers surrounding and overlying the intestinal villi is also greatly reduced (Edwards *et al.* 1988; Lund *et al.* 1989).

Mathematical correlations of blood glucose level to concentration and MW of β-glucan have been illustrated by Wood *et al.* (1994a, 2000) who demonstrated an inverse linear relationship between log (viscosity) of oat β-glucan in a drink model (varying MW/dose) and the magnitude of 50 g 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. Tappy *et al.* (1996) also found that inclusion of oat β-glucan into breakfast cereals could reduce the post-prandial glycaemic response by up to 50%, which at low levels (below 5%) appeared to be dose responsive. Levels above 5% did not show any further reductions in glycaemic response, possibly indicating a saturation point. Jenkins *et al.* (2001) indicated that 1 g of β-glucan per 50 g of ingested carbohydrates could reduce the GI of food by four units. Information on the dosage necessary for hypoglycaemic effect is an important factor when considering appropriate levels of β-glucan inclusion in food systems.

Although an increase in luminal viscosity is the most likely explanation for the blood glucose lowering properties of soluble fibres, it has been proposed that other physical mechanisms are involved, especially when soluble fibres are part of a solid food matrix such as bread or pasta. In addition to rheological effects, soluble polysaccharides may also inhibit the rate of digestion of solid starch products by forming an enzyme resistant barrier around starch granules (Ellis *et al.* 1991). Brennan *et al.* (1996a) investigated the topological relationship between guar gum and starch in wheat bread and the influence of guar gum on the rate of *in vitro* hydrolysis of wheat starch by pancreatic α-amylase. Micro-structural studies of digesta taken 4 hours post feed from pigs revealed that guar gum was closely associated with the starch granules in the bread. This suggests that in

addition to rheological effects, soluble polysaccharides may act as a physical barrier to enzyme-substrate interactions and the release of nutrients from the food matrix. Results of the *in vitro* starch digestibility studies were consistent with the structural observations in that the hydrolysis of starch in guar gum wheat bread was reduced significantly compared with the control; these effects were independent of the MW of guar gum contained in the wheat bread. Currently, there are limited studies investigating the effect of β-glucan from either oat or barley on the micro-structure of cereal foods and starch digestibility.

The hypothesis on the formation of a soluble fibre barrier around starch granules is also supported by the theory of 'thermodynamic incompatibility' of biopolymers (Tolstoguzov 2003a,b). Thermodynamic incompatibility of bio-polymers implies that macro-molecules show a preference to be surrounded by their own type in mixed solutions. For example, self-association, typical for amylopectin, is intensified in the presence of other macro-molecules, such as guar gum. Thermodynamic incompatibility was utilised to explain why the addition of guar gum increases the quantity of starch that resists digestion (Tolstoguzov 2003b). According to Tolstoguzov (2003b), amylopectin is incompatible with guar gum, and therefore, when added to a starchy mixture, guar gum can lead to a phase separation, encapsulation of the starchy phase by the guar gum enriched phase and possibly prevention of the leaching of amylose. This phase separation is thought to increase the concentration of macro-molecules inside the starch granules, which consequently decreases starch digestion.

A less explored mechanism for the effectiveness of soluble fibres in lowering post-prandial blood glucose after consumption of starchy foods, is the ability of these fibres to limit the rate of digestion of the starch component by inhibiting gelatinisation (i.e. competing for available water), as proposed by Holm *et al.* (1988) and Tester and Sommerville (2003).

After initial basic research, application of oat and barley β-glucan for glycaemic control has been concentrated in studying the effects of cereal fractions or products and prototype products from these. More attention has been paid to oat products due to the higher viscosity given by its β-glucan as compared to barley β-glucan. Studies of the hypoglycaemic effect of native oat and oat β-glucan products are reviewed extensively by Wursch and Pi-Sunyer (1997) and Malkki (2004). Research specifically examining the hypoglycaemic effect of barley β-glucan additions has been conducted with promising results. When flat breads in which part of the conventional wholemeal barley flour was replaced by Prowashonupana barley (20% β-glucan) were fed to healthy humans, the GI was decreased by 30 units, and the insulin response was also significantly lowered compared to wholeflour products (Liljeberg *et al.* 1996). Similarly, Pick *et al.* (1998) observed that barley breads (5 g β-glucan/day) fed to diabetic men significantly lowered glycaemic response compared to a control white bread. Cavallero *et al.* (2002) incorporated barley β-glucan rich flour and fractions into wheat bread. Four breads were produced: 100% bread wheat flour (total β-glucan 0.1%: soluble β-glucan 0.1%); 50% bread wheat flour and 50% barley flour (total β-glucan 2.4%: soluble β-glucan 2.0%); 50% bread wheat flour and 50% sieved barley fraction (total β-glucan 4.2%: soluble β-glucan 2.8%); and 50% bread wheat flour and 50% water-soluble barley fraction (total β-glucan 6.3%: soluble β-glucan 5.7%). Eight adults were fed test meals of each of the four breads, and GIs were calculated from finger prick capillary tests. A linear decrease in GI was associated with increasing β-glucan concentration. The 50% wheat/barley flour bread showed a reduction in GI from the control bread (GIs=85.42 and 89.49 respectively); however, only the bread containing the water-soluble fraction produced a significantly reduced GI (GI=69.67) compared to the control bread (GI=89.49). The authors concluded that it was the β-glucan levels in the bread (notably the increased soluble β-glucan levels) that were responsible for the reduction in GI and that this did not result from impaired food

degradation and amylolysis but through the effect of β-glucan on digesta viscosity and glucose absorption.

Yokoyama *et al.* (1997) compared blood glucose and insulin responses of healthy individuals following the ingestion of control durum wheat pasta (100 g of available carbohydrate and 5 g of TDF (negligible β-glucan)) to that of a pasta sample with added barley β-glucan (100 g of available carbohydrate and 30 g of TDF (of which 12 g was β-glucan)). Post-prandial blood glucose and insulin responses were significantly reduced following ingestion of the pasta enriched with barley flour. The authors attributed this reduction in the glycaemic response to the incorporation of β-glucan and increased TDF content of the experimental pasta samples. Similar findings have been reported by Knuckles *et al.* (1997a) and Bourdon *et al.* (1999). Izydorczyk *et al.* (2005) observed reduced rates of *in vitro* starch digestibility in durum wheat pastas containing hull-less barley flour fractions. The authors reported that changes to product micro-structure and starch granule availability were responsible for the reduced rates of *in vitro* starch digestion. The exact role of β-glucan in modulating the GI of pasta warrants further investigation.

As discussed, barley β-glucan enriched foods have potential in the modulation of the glycaemic response in both diabetic and non-diabetic subjects. Despite the long experience in the use of other pharmaceutical viscous fibre preparations (i.e. guar) for diabetic patients and a number of successful studies with both healthy and diabetic people fed food products containing viscous soluble fibre, the attitude of authorities and professional societies towards the use of viscous fibre containing functional foods as part of a low glycaemic diet remains somewhat conservative. This is reflected in a statement of the American Diabetes Association (ADA) (2002) which concludes the following:

"with regard to the glycemic effects of carbohydrate, the total amount of carbohydrate in meals and snacks is more important than the source or type----Although the use of low glycemic food may reduce postprandial hyperglycemia, there is not sufficient evidence of long term benefit to recommend use of low glycemic index diets as a primary strategy in food/meal planning for individuals with type 1 diabetes.

Carbohydrate and type 2 diabetes

It thus appears that ingestion of large amounts of fiber is necessary to confer metabolic benefit. It is not clear whether the palatability and gastrointestinal side effects of fiber in this amount would be acceptable to most people." (ADA 2002)

In contrast, the Dieticians Association of Australia (DAA) (Perlstein *et al.* 1997), the Canadian Diabetes Association (CDA) (CDA 2002) and the Diabetes Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (DNSG 2004) recommend high fibre, low GI foods for individuals with diabetes as a means of improving post-prandial glycaemia. The Nutrition Subcommittee of the Diabetes Advisory Committee of Diabetes UK (Connor *et al.* 2003) view the GI as a broad guide to good carbohydrate food choices, which stratifies foods into low, medium and high GI choices; moreover, the latest studies that have investigated the hypoglycaemic effect of barley β-glucan have paid attention to the known critical factors of amount, viscosity, palatability and compliance and are convincing enough to suffice for health claim purposes.

1.4.2.2 Hypocholesterolemic properties

One of the greatest scientific and public health interests on soluble dietary fibre has been its role in reducing blood cholesterol and the reduction of CVD. Following a review of 37 original studies (Anon 1996) in 1997, the FDA of the US published a final ruling on the relationship between soluble fibre from whole oats or oat bran and the risk of CHD (Anon 1997a,b). It concluded that β-glucan soluble fibre is the component responsible for the hypocholesterolemic properties of oats when part of a diet low in saturated fat and cholesterol. More recently, the agency has announced that whole grain barley and barley containing products are allowed to claim that they reduce the risk of CHD (Anon 2005a).

The principle mechanism by which β-glucan lowers blood cholesterol is believed to be as a result of increased excretion of bile acids at the cost of cholesterol, which occurs through increased intestinal viscosity (De Schrijver *et al.* 1992; Jensen *et al.* 1993; Jonnalagadda *et al.* 1993). The highly viscous β-glucan solution absorbs bile acids secreted in the duodenum and impedes the re-adsorption of bile acids in the small intestine (Bengtsson *et al.* 1990). Attention has also been placed upon other mechanisms. Since the hypoglycaemic effect of viscous soluble fibre causes a reduction of insulin secretion, this reduces cholesterol synthesis in the liver, insulin being an activator of a key enzyme in cholesterol synthesis, hydroxymethyl-CoA reductase. This enzyme is also inhibited by bile acids, especially deoxycholic acid, the proportion of which increases in the bile acid pool of people consuming oat bran based diets (Marlett *et al.* 1994). More recently, Yang *et al.* (2003) suggested that up-regulation in the activity of cholesterol 7 alpha-hydroxylase (CYP7A1), an enzyme associated with the regulation of the pathway through which cholesterol is converted into bile acids, was responsible for hypocholesterolemic behaviour of β-glucan. The bile acid binding properties of cereal β-glucans have been examined by several authors; Bowles *et al.* (1996) using ^{13}C CP/MAS NMR and by Kahlon and Woodruff (2003) in *in vitro* studies. Both studies failed to show any significant evidence that the cholesterol-lowering properties of β-glucans were due to binding of bile acid salt molecules.

Studies of the hypocholesterolemic role of oat β-glucan rich diets in animals and humans have been discussed by Rispin *et al.* (1992), Kahlon and Chow (1997) and Kerckhoffs *et al.* (2003). Likewise, the hypocholesterolemic effect of barley β-glucan rich diets has also been examined in numerous studies with animals (Fadel *et al.* 1987; Newman *et al.* 1991, 1992; Oda *et al.* 1991, 1993; Ranhotra *et al.* 1991; Wang *et al.* 1992; Kalra and Jood 2000; Yang and Moon 2002; Bird *et al.* 2004) and humans (Newman *et al.* 1989; McIntosh *et al.* 1991; Lupton *et al.* 1994; Smith *et al.* 2004).

Attempts have been made to ascertain if the botanical source of β-glucan influences its cholesterol-lowering capacity. In particular, the study of Delaney *et al.* (2003) compared the cholesterol-lowering capacity of β-glucans from barley and oats using a hamster model system. Although the diets rich in oat or barley β-glucan significantly reduced the cholesterol levels of the hamsters, no significant difference was observed between the two experimental diets, thus, leading to a conclusion that the cholesterol-lowering potency of β-glucan is not dependent on botanical source. A similar observation was recorded by Hallfrisch *et al.* (2003) in a comparison of the effect of barley and oat β-glucan diets on glucose and insulin responses in humans.

There has been considerable interest in the level of β-glucan supplement needed to achieve a significant cholesterol-lowering benefit. Most studies have been on the effectiveness of dietary fibre or oat and barley β-glucans in relation to food labelling claims through the US FDA. The FDA have adopted a recommendation of 3 g per day of β-glucans (0.75 g/serving) as having a nutritional effect; this as a component of the recommended 30-35 g of dietary fibre per day advised by the American Association of Dieticians (AAD) (Anon 1997a).

1.4.2.3 Satiety and weight reduction

Besides restriction of food intake and increased physical activity, obesity is also affected by consumption of dietary fibre. In a large multi-cultural study with middle-aged men, high fibre intake was found on a population level to affect body fat levels more effectively than low fat diets (Kromhout *et al.* 2001). Fibre does not only affect satiety physically by increasing gastric distension and delaying gastric emptying but also by affecting endocrine and intestinal hormone secretions (Malkki 2004). In addition to insulin and glucagon, attention has been paid to the gut hormone cholecystokinin (CCK). Physiological responses to CCK released from the small intestine, include delayed gastric emptying,

blunted glycaemic responses and increased satiety, factors also related to consumption of viscous polysaccharides (Liddle *et al.* 1986, 1988). CCK release is stimulated by amino acids and fats (Holt *et al.* 1992), and it has been discovered that soluble fibres may also increase its secretions (Mossner *et al.* 1992). It is postulated that prolonged contact of lipid and amino acid with intestinal cells, due to slow digestion after a meal containing viscous fibre, may promote a greater release of CCK (French and Read 1994; Burton-Freeman and Schneeman 1996). Investigations of the satiation effects of barley β-glucan are relatively limited. Bourdon *et al.* (1999) found that consumption of barley β-glucan rich pasta resulted in CCK levels being increased for a longer period than after a control meal.

1.4.2.4 Anti-carcinogenic properties

One of the many health-providing roles attributed to cereal dietary fibre is the prevention of colorectal cancer (Bingham 1990; Hill 1997). Mechanisms behind the anti-carcinogenic effect of dietary fibre remain unclear and controversial. Large-scale epidemiological studies do not show any or only weak correlation between fibre intake and a reduction of risk of colorectal cancer (Fuchs *et al.* 1999); however, these studies do not differentiate the effects of TDF and viscous soluble fibre, and to date there is still a paucity of studies that have examined the role of cereal β-glucans in protecting against colorectal cancer.

The role of fibre in protecting against tumour development is believed to stem from the fermentation of fibre by intestinal bacteria. Proposed mechanisms include, the supply of the colonic epithelium with SCFAs (principally butyric, acetic and propionic) and suppression of microbial protein metabolism, bile acid conversion and other toxigenic bacterial reactions (Bingham 1990; Kritchevsky 1998; D'Argenio and Mazzacca 1999; Rieger *et al.* 1999; Dongowski *et al.* 2002). Butyric acid stimulates proliferation of normal cell lines, both *in vitro* and in the normal cell epithelium, but retards the growth of carcinoma cell lines and induces apoptosis in cultured colonic adenoma and carcinoma

cells (Kim *et al.* 1980; Hague *et al.* 1993); however, it appears that with soluble fibres (such as cereal β-glucans), which are rapidly fermented, the protecting effect of butyric acid does not extend to the rectum, since it is for a greater part either used by colonic mucosa or absorbed (Kashtan *et al.* 1992). The anti-carcinogenic effect of barley fibre has been examined by McIntosh *et al.* (1993, 1996) who investigated its role in preventing dimethyl hydrazine induced tumours. In these studies insoluble dietary fibre was found to be the active component.

1.4.2.5 Prebiotic effect and fermentation in the large bowel

Fermentable carbohydrates reaching the colon have been illustrated to have a prebiotic effect, such that on reaching the colon they provide a selective advantage for the proliferation of particular bacterial microflora groups, which are of benefit to the host (probiotics) (Fuller 1989). Many of the carbohydrates not absorbed in the small intestine can support the growth of colon bacteria, but for prebiotic action the effect must be selective (Gibson and Roberfroid 1995). Bifidobacteria and Lactobacilli are considered beneficial genera within the human intestinal microflora and are the predominantly used probiotics (Crittenden *et al.* 2002). Whilst a considerable number of studies have examined the prebiotic effect of oat bran and oat β-glucan (reviewed by Malkki and Virtanen (2001)), relatively few studies have examined the prebiotic effect of barley or barley isolates. Dongowski *et al.* (2002) found that barley diets containing between 7-12 g β-glucan/100 g increased *Lactobacillus* numbers in the caecum and colon of rats compared to a control. In the same study, SCFA production was increased compared to a control.

1.4.2.6 Immunological effect

Dietary components and their digestive products are in very intimate contact with the immune system of the gut (gut associated lymphoid tissue (GALT)), and specific nutrients are known to be important in the development and function of the immune system

(Alexander 1995). Although much less is known about the potential of dietary fibres in influencing immune function, several studies have demonstrated a lower incidence of bacterial translocation across the gut barrier on the administration of fermentable dietary fibre (Deitch *et al.* 1993; Frankel *et al.* 1995), thus, suggesting dietary fibre may modulate immunity. Although the mechanisms for the effect of fermentable dietary fibres on immune function in the gut have not been fully established, a number of interesting hypothesis have been proposed including: direct contact of lactic acid bacteria (Schiffrin *et al.* 1995) or bacterial products (cell wall or cytoplasmic components) (Tejada-Simon *et al.* 1999) with immune cells in the intestine; production of SCFAs from fibre fermentation (Bohmig *et al.* 1997; Jenkins *et al.* 1999); and modulation of mucin production (Deitch *et al.* 1993; Frankel *et al.* 1995). Schley and Field (2002) have extensively reviewed the immune enhancing effect of dietary fibre and proposed mechanisms of action.

A vast majority of studies investigating the immunity modulating ability of β-glucan have been made with (1→3)-β-D-glucans from edible mushrooms or from fungal or yeast cell walls (Malkki *et al.* 2004). Cereal β-glucans are long thought to be passive biological polymers and as such their immune enhancing effect has been subject to few studies; however, in recent years both immunostimulatory and immune restrictive effects have been investigated. The majority of these studies have been conducted with oat β-glucan, with a limited number examining the immune enhancing capacity of barley β-glucan. In human monocyte cell cultures, the biological activity of both highly purified oat and barley β-glucan was found to bind macrophage receptors and induce macrophage differentiation, the effect depending on origin and MW (Causey *et al.* 1998; Fulcher *et al.* 2000). A 6-fold increase in macrophages was observed with 100 mg/ml HMW oat β-glucan, and a 7.5-fold increase was noted at an equivalent concentration of LMW oat β-glucan. LMW barley β-glucan was three times stimulatorier than a polymer free basal medium.

1.5 IMPACT OF BARLEY β-GLUCAN INCLUSIONS ON THE QUALITY OF CEREAL FOODS

β-glucan from barley has been incorporated into numerous cereal systems including, bread (Kunckles *et al.* 1997a; Pick *et al.* 1998; Cavallero *et al.* 2002) and pasta (Knuckles *et al.* 1997a; Marconi *et al.* 2000). Although not discussed in this review, there are studies also emerging that have investigated the incorporation of barley β-glucan into non-cereal systems including, extruded meat products (Morin *et al.* 2002, 2004), yoghurt and cheese curds (Tudorica *et al.* 2002a,b) and beverages (Temelli *et al.* 2004).

1.5.1 Starch

Mixtures of starch and NSPs can be used to modify and control food texture, improve moisture retention, control water mobility and improve the sensory quality of foods (Appelquist and Debet 1997). The inclusion of NSPs (i.e. guar gum, locust bean gum, xanthan gum, carrageenans and carboxymethylcelluose) in starch systems is known to influence starch structure, melting, gelatinisation, fragmentation and retrogradation (Donovan 1977, 1979; Lund 1984, Lai and Kokini, 1991; Kokini *et al.* 1992; Bahnassey and Breene 1994; Fanta and Christianson 1996). The literature on the subject of starch and NSP interactions is far too extensive to discuss within the confinements of this thesis but is thoroughly reviewed by Appelqvist and Debet (1997). Studies investigating the influence of β-glucans (from oat and barley) on the functional properties of starch are limited. Biliaderis *et al.* (1997) studied the interactions of oat β-glucan at 1 to 2% (w/w) in concentrated aqueous dispersions (40%, w/w) of maize and wheat starch. The presence of β-glucan did not affect the gelatinisation temperature of the maize or the wheat starch or the rheology of the wheat starch gel. This study cannot be viewed as a complete representation of the behaviour of β-glucan in starch systems as the source, structure and MW of the β-glucan will impact upon its physico-chemical properties and behaviour; these

factors were not addressed. The lack of information on the effects of barley β-glucan in a starch system is a limiting factor in its use as a functional food ingredient.

1.5.2 Bread

In previous studies, bread has been enriched with dietary fibre including wheat bran (Ranhotra *et al.* 1990; Sidhu *et al.* 1999), gums and modified celluloses (Pomeranz *et al.* 1977) and barley β-glucan (Knuckles *et al.* 1997a; Cavallero *et al.* 2002; Gill *et al.* 2002). Enrichment of bread with fibre material often has a negative impact on the quality of dough (high water absorption, increased shortness and decreased fermentation tolerance (Gan *et al.* 1992; Park *et al.* 1997; Laurikainen *et al.* 1998)) and final product quality (reduced volume, height, increased firmness and impaired colour and flavour) (Pomeranz *et al.* 1977; Lai *et al.* 1989; Knuckles *et al.* 1997a)).

There is relatively limited published information available on the impact of barley β-glucan in dough and bread-making. Knuckles *et al.* (1997a) evaluated the effect of incorporating barley flour fractions in bread. Water absorption and mixing time (determined by farinographs) increased with level of fibre, and loaf volume decreased compared to a control. Reduced volumes in barley-enriched breads have also been observed by Kahlon *et al.* (1993), Cavallero *et al.* (2002) and Gill *et al.* (2002). Conversely, Dhingra and Jood (2001) found that wheat breads supplemented with up to 15% barley flour were of an acceptable organoleptic quality. Differences in barley flour composition, physico-chemical properties and inclusion level are likely to explain some of the differences in bread quality observed between different studies. There are few studies investigating the effect of adding concentrated barley β-glucan fractions to breads. As these fractions are a concentrated source of β-glucan, they may be added at lower levels, which will possibly limit negative changes to product quality.

1.5.3 Pasta

Many workers have investigated the effects of adding high fibre material, from sources other than durum wheat, to pasta. Low glycaemic responses have been obtained with pastas formulated with guar (Gatti *et al.* 1984; Giorato *et al.* 1986; Carra *et al.* 1990). Addition of fibre enriched durum bran to semolina flour resulted in a tasteful product but increased cooking loss and reduced firmness in the cooked pasta (Kordonowy and Youngs 1985). Dougherty *et al.* (1988) incorporated oat fibres in pasta, and this resulted in lowered product quality. More recently, Tudorica *et al.* (2002c) evaluated the effects of adding inulin, pea and guar fibre to pasta. Pea and inulin fibre significantly increased the cooking loss from the pastas, and low inclusions of guar resulted in decreased cooking loss.

Few studies have been conducted to assess the impact of barley β-glucan inclusion on the physico-chemical properties and cooking quality of pastas. A small number of studies have examined the incorporation of barley β-glucan enriched flour fractions. Knuckles *et al.* (1997a) evaluated the effect of adding barley flours to pasta and observed that overall acceptability decreased with increasing fibre content. Marconi *et al.* (2000) examined the composition and utilisation of barley pearling by-products for making pasta rich in dietary fibre and β-glucan. Pastas enriched by substituting 50% standard durum wheat semolina with β-glucan enriched barley flour fractions (9.1-10.5% β-glucan) had reduced but acceptable cooking qualities (stickiness, bulkiness, firmness, and total organic matter released into cooking water); however, notable differences were observed in colour (barley pastas being darker than the control). The popularity, versatility and convenience of pasta make it an ideal food for enrichment with barley β-glucan, although like many cereal products, quality specifications are high. Barley β-glucan addition to pasta certainly warrants further study.

1.6 EFFECT OF FOOD PROCESSING ON THE MOLECULAR, STRUCTURAL AND FUNCTIONAL PROPERTIES OF BARLEY β-GLUCAN

Relatively little work has investigated the role of food processing on the rheological or nutritional characteristics of β-glucans, and only recently is this important issue being addressed. The molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding and solubility) of β-glucans are highly unstable and subject to change under selected processing conditions. The degree to which these changes impact upon the sensory, physiological and ultimately the health benefits of β-glucans are subject to continued debate (Yokoyama *et al.* 2002).

The MW of β-glucans is easily reduced by enzymatic or chemical hydrolysis, mechanical shear or heat treatment. These conditions are typically encountered in extraction and subsequent processing of β-glucans. Loss of barley β-glucan MW has been reported in extruded ready to eat barley cereals (Klamczynski *et al.* 2004) and during bread-making (Sundberg *et al.* 1996; Andersson *et al.* 2004; Trogh *et al.* 2004). MW degradation in breads is considered to occur during mixing and fermentation by endogenous enzymes within the flour or yeast. Despite loss in barley β-glucan MW, Andersson *et al.* (2004) found no significant changes to cellotriosyl/cellotetraosyl ratio of barley β-glucan throughout the mixing, fermentation and baking of bread dough. Relatively few studies have investigated whether loss of barley β-glucan MW has an impact on its physico-chemical and nutritional properties. Thus, there is a need to understand and manipulate processing in order to ensure that the possible alterations to the structure of barley β-glucan do not compromise its nutritional properties.

1.7 CONCLUSIONS AND RATIONALE FOR STUDY

There is little doubt that barley β-glucan offers many nutritional and rheological advantages to the food industry. An area of considerable nutritional interest is the ability of barley β-glucan rich foods to attenuate the glycaemic response of both healthy and non-diabetic subjects. The majority of studies investigating the glycaemic properties of barley β-glucan have been conducted with native barley grains or flours produced from them; however, frequent human consumption of such products is limited by a decline in product palatability and acceptability. A potential solution lies in using barley grain as an extraction source for β-glucan fractions, which can then be incorporated into popular and frequently consumed foods. It is reasonable to say that there is still a lack of clarity on the use of barley β-glucan rich fractions, in particular their physiological and physico-chemical properties. Challenges exist in developing extraction procedures for the production of economical and functional barley β-glucan fractions from native grains and flours.

Due to their popularity and frequency of consumption, cereal foods, namely bread and pasta, are ideal candidates for enrichment with barley β-glucan fractions; however, quality specifications for these products are high, and at present there is a paucity of investigations examining the effects of barley β-glucan fraction incorporation on the quality of cereal-based food systems. Research is also required to determine the effect of process parameters on the MW profiles and physico-chemical behaviour of barley β-glucan fractions. Such research would also broaden the understanding of how barley β-glucan can affect the nutritional characteristics of foods by altering their structure, texture and viscosity.

1.7.1 Study Aims

The overall aim of this study was to address the lack of knowledge regarding the use of β-glucan fractions from barley as functional ingredients in cereal food products. The specific aims of the study were to:

1. Investigate different extraction treatments for the isolation of β-glucan fractions from barley and the effects of their inclusion in wheat starch (Chapter 2).

2. Investigate and compare the influence of different barley β-glucan fractions on the physico-chemical properties, micro-structure and *in vitro* starch digestibility of white wheat bread (Chapter 3).

3. Investigate and compare the influence of different barley β-glucan fractions on the physico-chemical properties, micro-structure and *in vitro* starch digestibility of durum wheat semolina pasta (Chapter 4).

4. Investigate and compare the effects of differing MW barley β-glucan fractions (high and low) on the physico-chemical properties, microstructure and *in vitro* starch digestibility of white wheat bread and durum wheat semolina pasta (Chapter 5).

5. Investigate the susceptibility of barley β-glucan fractions to MW degradation during fermentation, baking and *in vitro* digestion (Chapter 3 and 5).

It is anticipated that the results of these investigations will have both scientific and commercial value and provide foundations for further development and optimisation of barley β-glucan enriched cereal products.

CHAPTER 2

THE EFFECT OF BARLEY β-GLUCAN FRACTIONS ON WHEAT STARCH GELATINISATION AND PASTING CHARACTERISTICS

2.1 INTRODUCTION

The recognition of the beneficial physiological properties (hypoglycaemic and hypocholesterolemic capacities) of β-glucans has led to a demand for the development of concentrated sources for incorporation into various food systems. Key extraction methodologies of β-glucans from barley and oat were developed by Wood *et al.* (1977, 1978) and Bhatty (1993, 1995) who investigated the influence of different solvents on the recovery and viscosity of barley and oat β-glucans. More recently, Temelli (1997) and Burkus and Temelli (1998) have investigated the effects of concentration, temperature and pH on the rheological properties of β-glucan rich gums from barley. These authors concluded that extraction conditions have an influence upon the yield, composition and viscosity stability of barley β-glucan gum. At present, there are few studies that have investigated the effects of these β-glucan preparations on the physico-chemical properties of model food systems, thus, their current use as functional ingredients in food is limited.

Starch-NSP interactions have been investigated by many workers for more than two decades, and such studies have examined a wide variety of NSPs, with very diverse chemical structures (guar gum, locust bean gum, xanthan gum, pectin, aliginate, kappa-carrageenan, hydroxypropylmethylcellulose, arabinoxylan, konjac flour and gellan), in a wide spectra of cereal flours and starches (maize starch, wheat flour, waxy maize, wheat starch, corn, waxy corn and tapioca) (Christianson *et al.* 1981; Alloncle *et al.* 1989; Alloncle and Doublier 1991; Cameron *et al.* 1993; Bahnassey and Breene 1994; Biliaderis

et al. 1997; Rojas *et al.* 1999; Shi and BeMiller 2002; Tester and Sommerville 2003). The effects of these NSPs at generally low concentrations (0.1-0.2% (w/w)) on starch during pasting/gelatinisation have been measured by a diverse range of methods (viscometer, amylographic analysis, differential scanning calorimetry (DSC^2), dynamic rheometry, Rapid Visco Analysis (RVA^2), optical microscopy and ultra violet spectrophotometry). These studies have revealed that variations in starch pasting characteristics (increase or decrease greatly, or slightly or no effect) are dependent upon NSP, starch source, concentration and method of measurement.

2.1.1 Rationale and Aim

The food industry has the potential to be an important user of β-glucans; however, there is a lack of information on the behaviour and functionality of β-glucans in food systems, particularly those containing starch. Thus, the aim of this study was to investigate different extraction treatments for the isolation of β-glucan fibre fractions from barley (BBG fibre fractions) and the effect of BBG fibre fraction inclusion in a model food system (wheat starch).

2.1.1.1 Objectives

- Extract BBG fibre fractions from barley flour using four different aqueous-solvent based extraction treatments (water only, refluxed, purified and alkali), and compare fibre fraction yield and β-glucan recovery, composition (TDF, β-glucan, starch and protein) and water retention capacity (WRC).

- Incorporate BBG fibre fractions into wheat starch and examine effects on gelatinisation and thermal characteristics, as determined by RVA^2 and DSC^2.

2.2 MATERIALS AND METHODS

2.2.1 Materials

2.2.1.1 Barley flour, wheat starch and reagents

Whole kernels of Cindy, a waxy barley (5.77% β-glucan, dry weight basis (dwb)) (Pertwood Cereal Partners, Salisbury, UK), were finely ground in a laboratory mill (Glen Creston, Stanmore, UK) to pass through a 500 μm mesh screen. Un-modified wheat starch (14% moisture) (S/8040/60) was used in the thermal processing experiments (Fisher Scientific, UK). Unless otherwise stated, all general laboratory reagents were purchased from Fisher Scientific (UK).

2.2.2 Methods

2.2.2.1 Extraction of BBG fibre fractions

BBG fibre fractions were prepared from barley flour using the original procedure of Wood *et al.* (1978) and some of the modifications used by Temelli (1997). Four extraction treatments were used: (1) flour and water extraction only (water fraction); (2) flour refluxed once with ethanol (75% v/v) for 4 hours at 85°C (refluxed fraction); (3) extract of refluxed flour was treated with thermostable α-amylase (Termamyl, National Centre for Biotechnology Education, Reading, UK) at an inclusion level of 1 ml enzyme per 100 ml extraction buffer for 1 hour at 98°C to eliminate starch impurities (purified fraction); and (4) flour and water extraction at pH 10 achieved by the addition of a few ml of 1 M NaOH (alkali fraction). All treatments were performed at 55°C and pH 7, except for the alkali treatment, which was conducted at pH 10. Each extraction treatment was repeated three times. Figure 2.1 illustrates the basic extraction process.

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

↓

Centrifuge 15 minutes (15,000 x *g*, 4°C)

↓

Supernatant to pH 4.5 (centrifuge, 20 minutes, 21,000 x *g*, 4°C)

↓

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

↓

Recover precipitate (centrifuge, 10 minutes, 3000 x *g*)

↓

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

↓

Dry under constant flow (1.5 hours)

Figure 2.1 Generalised BBG fibre fraction extraction procedure (Wood *et al.* 1978; Temelli 1997).

2.2.2.2 Chemical composition

Moisture, starch, protein, TDF and β-glucan contents of BBG fibre fractions were determined. Moisture was determined according to Approved Method 44-15A (AACC 2000b). Total starch, TDF and β-glucan were determined using the total starch assay kit (Approved Method 76.13, AACC 2000b), dietary fibre assay kit (Approved Method 32-07, AACC 2000b) and β-glucan enzymatic assay kit (Approved Method 32-23, AACC 2000b) respectively. All assay kits were supplied by Megazyme™ International Ireland Ltd (Wicklow, Ireland). Nitrogen was determined using a nitrogen analyser (Model FP-2000, Leco Instruments Ltd, St Joseph, Michigan, US), and protein content was estimated by using a conversion factor of 6.25. Results are from duplicate analysis of a composite

sample (hereafter a composite sample is defined as a homogenous mix of three individual sample units produced from the same treatment) reported on a dwb.

2.2.2.3 WRC

The ability of a fibre to bind water has importance with regard to technological and physiological functionality. Binding can be determined by filtration (water holding capacity), centrifugation (water-binding capacity) or freeze drying (Chaplin 2003). WRC is defined as the amount of water retained by a known weight of fibre under the conditions used (Robertson *et al.* 2000). This definition arose from Pro-fibre, a European concerted action group, and is preferred to either water holding capacity or water binding capacity. The WRC of a fibre is related to structural and chemical composition, more specifically the amount of soluble and insoluble fibre within the matrix (Robertson and Eastwood 1981; Robertson *et al.* 2000).

The WRC of the BBG fibre fractions was determined by the procedure of Robertson *et al.* (2000) with some modifications. BBG fibre fractions (1 g of each) were hydrated in pre-weighed tubes with 30 ml of distilled water for 18 hours at room temperature. Following hydration samples were centrifuged (3000 x *g* for 20 minutes). The supernatant was carefully decanted, and the sample was left to drain. Sample fresh weight was recorded before drying (120°C for 2 hours). WRC was calculated as the amount of water retained by the pellet (g/g dry weight) after draining.

2.2.2.4 Pasting characteristics of wheat starch

Native starch granules are generally insoluble in cold water (below 50°C) due to strong hydrogen bonds holding starch polymers together (Glicksman 1969). When an aqueous suspension of starch is heated in water, granules begin to swell when sufficient energy is

present to overcome the bonding forces of starch molecules. With continued heat, a temperature will be reached at which the hydrogen bonding forces are sufficiently weakened and allow water to be absorbed by the starch granules. Subsequently the starch granules begin to swell tangentially and simultaneously lose their characteristic maltese crosses. This process is known as initial gelatinisation (Crossland and Favor 1948). Continued heating of the starch granules results in rupturing, disintegration and a dispersion of amylose, amylopectin and granule fragments and is referred to as gelatinisation (Moore 1984).

The Brabender Amylograph has traditionally been used to determine the gelatinisation and pasting properties of starch and starch/gum dispersions during heating/stirring and cooling/stirring. The Rapid Visco Analyser (RVA[1]) is a less widely used instrument but can provide similar information in a shorter time and with a smaller sample (Ross *et al.* 1987a). Thus, the RVA[1] can be used as a quick and convenient tool for demonstrating the effect of NSPs on starch gelatinisation during a classic heat-hold-cool cooking cycle.

Within this study, measurements were made (using an RVA[1]) of three key points during the gelatinisation of wheat starch and BBG fibre fraction mixtures: peak viscosity (PV), breakdown (BD) and final viscosity (FV). PV occurs at an equilibrium point between swelling and polymer leaching, which causes an increase in viscosity, and rupture and polymer alignment, which causes a decrease. During the hold period of the test, the sample is subjected to a period of constant high temperature (usually 95°C) and mechanical shear stress. This will further disrupt the granules, and amylose molecules will generally leach out into solution and undergo alignment. This period is accompanied by a reduction in viscosity, which is called BD or shear-thinning. The rate of reduction depends on the temperature and degree of mixing or shear applied to the mixture and the nature of the

material itself. As the mixture is subsequently cooled, re-association between starch molecules, especially amylose, occurs to a greater or lesser degree. In sufficient concentration this usually causes the formation of a gel and a viscosity increase to FV. FV is the most commonly used parameter to define a particular samples quality as it indicates the ability of the material to form a viscous paste or gel after cooking or cooling.

PV, BD and FV development of wheat starch substituted with 1 and 5% BBG fibre fractions were determined using an RVA[1] (RVA-4) (Newport Scientific PTY, Warriewood, Australia). An RVA[1] Standard One Profile was used with heating and cooling rates of 12°C per minute, a temperature range of 50-95°C and a paddle speed of 160 rpm. Samples were prepared by mixing 3.5 g in 25 ml of distilled water in an aluminium canister. An example RVA[1] pasting curve is illustrated in Appendix I.

2.2.2.5 Gelatinisation characteristics of wheat starch

The progression of starch from a semi crystalline to amorphous material when heated in excess water (gelatinisation) can be characterised using DSC[2]. During this process that encompasses an onset of gelatinisation (T_{onset}), gelatinisation peak temperature (T_{peak}), gelatinisation end point (T_{endset}) and an associated enthalpy (J/g) of gelatinisation, double helices registered in the crystalline regions progressively unravel as hydrogen bonds break, which leads to dissociation of the crystalline regions, with associated hydration and swelling of the granules. Heating beyond T_{endset} leads to a loss of granule form and gelation/solubilisation (Tester *et al.* 1998; Tester and Debon 2000; Tester *et al.* 2000; Tester and Sommerville 2003).

A differential scanning calorimeter (DSC[1]) (DSC 12E, Mettler Toledo, Leicester, U.K) was used to measure the gelatinisation characteristics (T_{onset}, T_{peak}, T_{endset} and enthalpy) of

wheat starch substituted with 1 and 5% BBG fibre fractions. Wheat starch with no fraction addition was used as a control, and indium was used to calibrate the instrument. The starch to distilled water ratio was 1:4. Nominal scan rate was 10°C/minute over a 30-100°C heating rate. An example DSC[1] trace is illustrated in Appendix II.

2.2.2.6 Statistical analysis

Unless otherwise stated, all results are from triplicate determinations of a composite sample, and mean ± standard deviation (SD) values are presented. Analysis of variance (ANOVA) of BBG fibre fraction yield and β–glucan recovery values only was performed using the Minitab 13 statistical software package (Minitab Inc., State College, Pennsylvania, US) followed by Tukey's test. Significance was defined as $P<0.05$.

2.3 RESULTS AND DISCUSSION

2.3.1 Effect of Extraction Treatment on the Yield of BBG Fibre Fractions and β–Glucan Recovery

Figure 2.2 illustrates the effect of extraction treatment on the yield of BBG fibre fractions. Yield of each BBG fibre fraction (weight of fraction/50 g flour) from different extraction treatments ranged from 2.25-5.50%. The water extraction had the highest BBG fibre fraction yield (5.50%), followed by the alkali extraction, which had a significantly lower yield of 4.16% ($P<0.05$). The refluxing and purification extractions resulted in a further lowering of yield (2.25 and 2.39% respectively) ($P<0.05$) (the difference between the two extractions was not significant ($P>0.05$)). Temelli (1997) reported barley gum yields from 3.28-5.54% when investigating effects of temperature (40, 45, 55 and 50°C) and pH (7, 8 and 10) on the extraction of β-glucan from Condor barley. Highest yield (5.54%) was achieved at pH 8 and 55°C. Burkus and Temelli (1998) evaluated the effect of heat treatment (no heat, boiled, refluxed and purified) on the yield and composition of barley β-glucan gum; lowest yield was achieved from a purified treatment (3.2%).

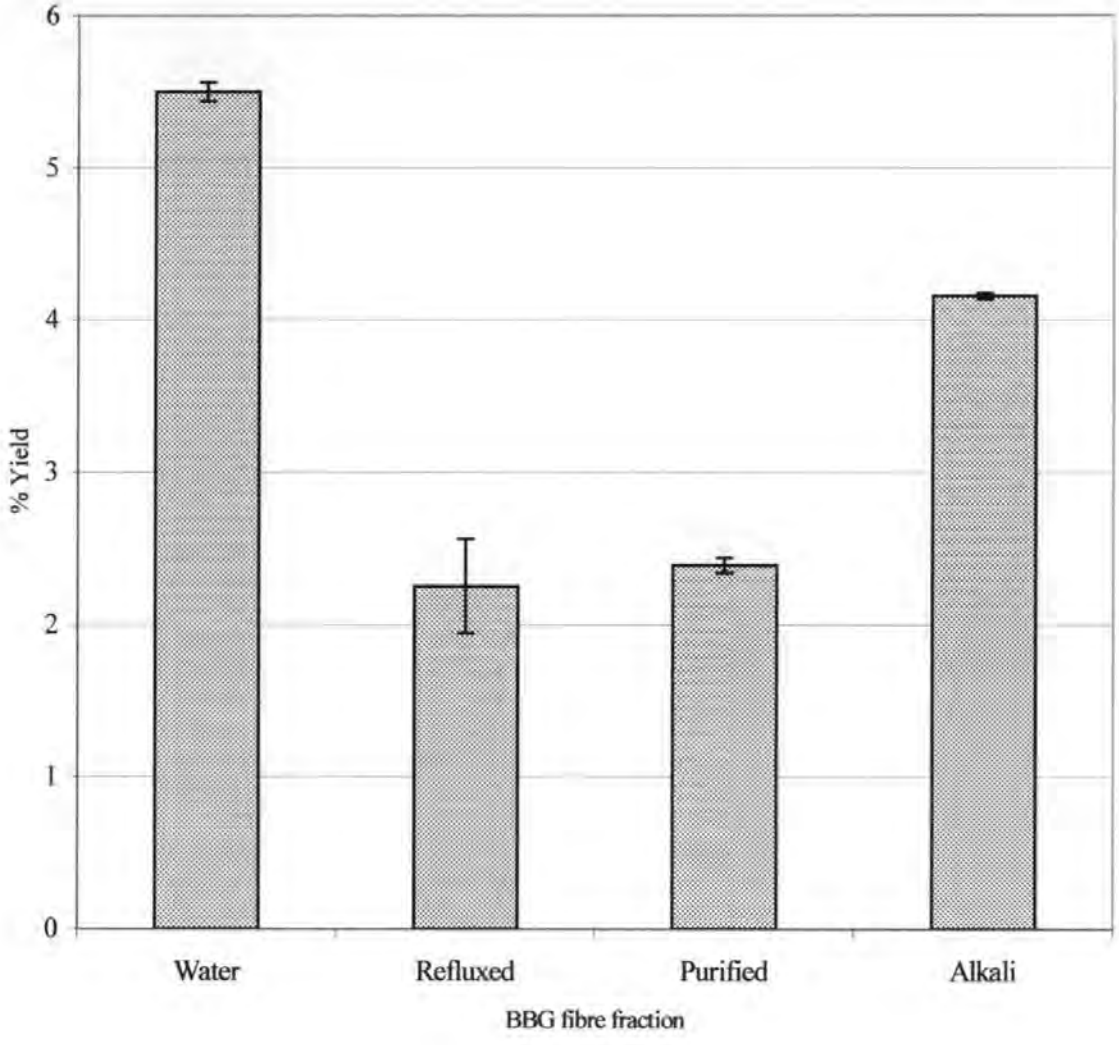


Figure 2.2 Percentage BBG fibre fraction yield from water, refluxed, purified and alkali extraction treatments. SD of three independent extractions is included on the figure as error bars.

Efficiency of β–glucan extraction was determined by dividing weight (g) of β–glucan in each BBG fibre fraction recovered by weight (g) of β–glucan in 50 g flour. The extraction efficiency of the different treatments is illustrated in Figure 2.3. The water extraction gave the greatest β–glucan recovery (65.93%) and was followed by the alkali extraction, which significantly lowered β–glucan recovery efficiency to 51.04% ($P<0.05$). Beer *et al.* (1996), Temelli (1997) and Burkus and Temelli (1998) have also reported loss of β–glucan recovery under alkali conditions. The lowest extraction efficiencies were encountered with

the refluxing (25.97%) and purified (30.19%) treatments (the difference between the two extractions was not significant (P>0.05)). These lower recovery efficiencies may be attributed to thermal degradation and in the case of the refluxed fraction to starch contamination during refluxing. Beer *et al.* (1996) observed lower β-glucan yields from oat bran treated with 75% ethanol for 4 hour at 80°C compared with non-treated oat bran.

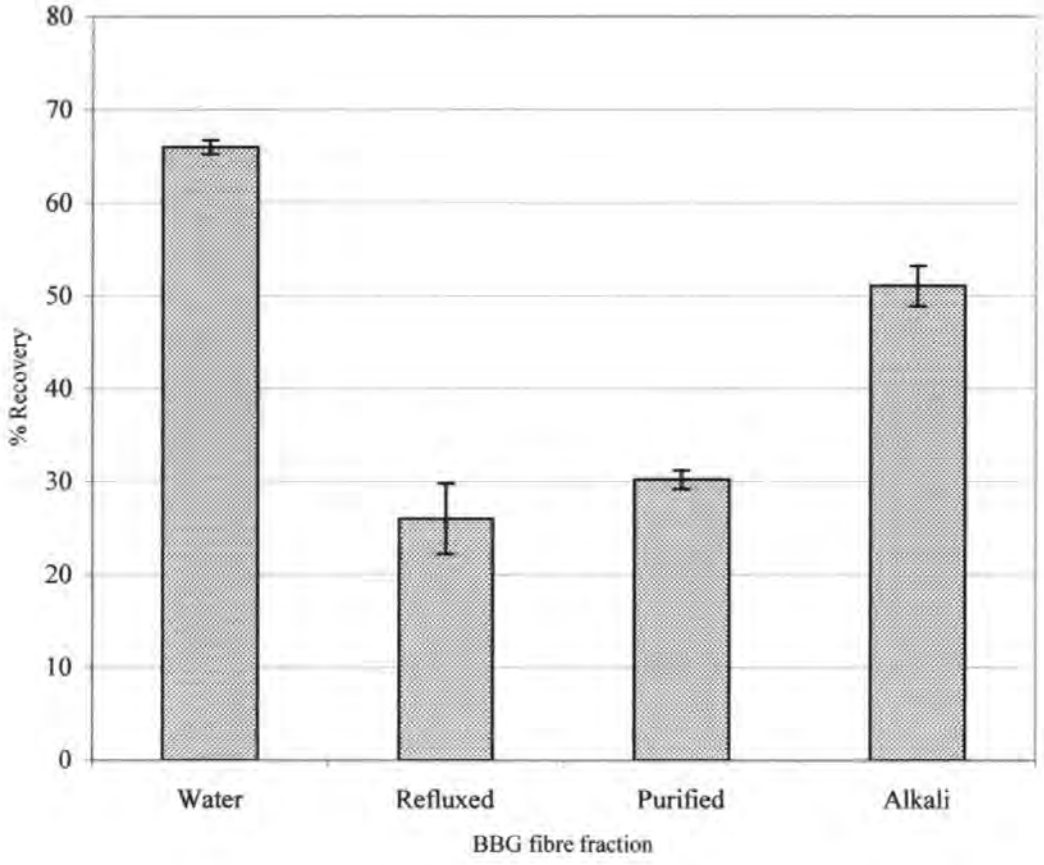


Figure 2.3 Percentage β-glucan recovery from water, refluxed, purified and alkali extraction treatments. SD of three independent extractions is included on the figure as error bars.

2.3.2 Effect of Extraction Treatment on the Composition of BBG Fibre Fractions

β–glucan, TDF, protein and starch contents of BBG fibre fractions from different extraction treatments are compared in Table 2.1. The results indicate that extraction

treatment may affect the composition of the BBG fibre fractions. The β–glucan content of the fractions ranged between 65.03-73.39%. The purified fraction had the highest β–glucan contents (73.39%). A lower purity was achieved with the alkali (69.60%) and water fractions (68.62%). The refluxed fraction had the lowest β–glucan content (65.03%).

TDF contents of the BBG fibre fractions ranged between 78.70-91.82%. The purified fraction had the greatest TDF content (91.82%) and was followed by the alkali fraction, which had a lower TDF content of 81.97%. The lowest TDF contents were observed in the water and refluxed fractions (79.01 and 78.70% respectively). The results indicate that in addition to β-glucan, the treatments may result in co-extraction of other fibres. Both Temelli (1997) and Burkus and Temelli (1998) have reported contamination of β-glucan preparations with co-extracted fibres, predominantly pentosans, when using similar extraction procedures.

Table 2.1 Composition of BBG fibre fractions extracted at 55°C with differing treatments[1]

BBG	Extraction pH	β-glucan (%)	TDF (%)	Protein (%)	Starch (%)
Water	7	68.62 ± 0.40	79.01 ± 0.52	5.77 ± 0.06	12.50 ± 0.35
Refluxed	7	65.03 ± 0.65	78.70 ± 0.45	3.16 ± 0.04	14.14 ± 0.17
Purified	7	73.38 ± 0.77	91.82 ± 0.60	2.95 ± 0.01	1.23 ± 0.03
Alkali	10	69.60 ± 0.44	81.97 ± 0.44	5.26 ± 0.01	9.77 ± 0.26

[1]Results are mean ± SD of duplicate determinations of a composite sample reported on a dwb.

The starch content of the BBG fibre fractions was between 1.23-14.14%. The refluxed fraction had the highest level of starch contamination (14.14%) and was followed by the

water (12.50%) and alkali (9.77%) fractions. A similar effect of starch contamination of oat gums at high concentrations has been reported by Dawkins and Nnanna (1993). Burkus and Temelli (1998) observed that refluxing waxy barley with ethanol resulted in gums with a lower β-glucan contents and higher levels of starch contamination (25%) compared to non-refluxed samples (59.5 vs 72.0% β-glucan respectively). Wood *et al.* (1978) concluded that starch gelatinisation commences above 63°C, and extraction treatments of 45°C were the optimum to avoid contamination. Purification of β–glucan preparations by starch hydrolysis with thermostable α-amylase has been used by several authors (Bhatty 1995; Burkus and Temelli 1998). Burkus and Temelli (1998) reported that the starch content of refluxed samples decreased from 25-2% upon purification treatment. In this study, the employment of α-amylase in the purified extraction resulted in a terminal starch content of 1.23%.

The protein content of the BBG fibre fractions ranged between 2.95-5.77%. The water fraction had the highest protein content (5.77%) and was followed by the alkali fraction. Protein contamination was lowest in refluxed and purified fractions (3.16 and 2.95% respectively), which indicates prior refluxing of the flour may reduce protein contamination.

2.3.3 Effect of Extraction Treatment on the WRC of BBG Fibre Fractions

Figure 2.4 illustrates that extraction treatment may affect the WRC of the BBG fibre fractions. The purified fraction exhibited the highest WRC (12.31 g/g dwb) and was followed by the alkali and refluxed fractions. The lowest WRC was exhibited by the water fraction (7.80 g/g dwb). In this particular study, WRC of the gums may be a reflection of the TDF content of the fraction, a higher fibre content being related to an increased WRC value. From a technological perspective, the ability of the BBG fibre fractions to retain

water may have important functional implications, possibly modifying food texture, moisture retention and water mobility, which will bear an influence on the physico-chemical properties and sensory quality of food products (Appelqvist and Debet 1997).

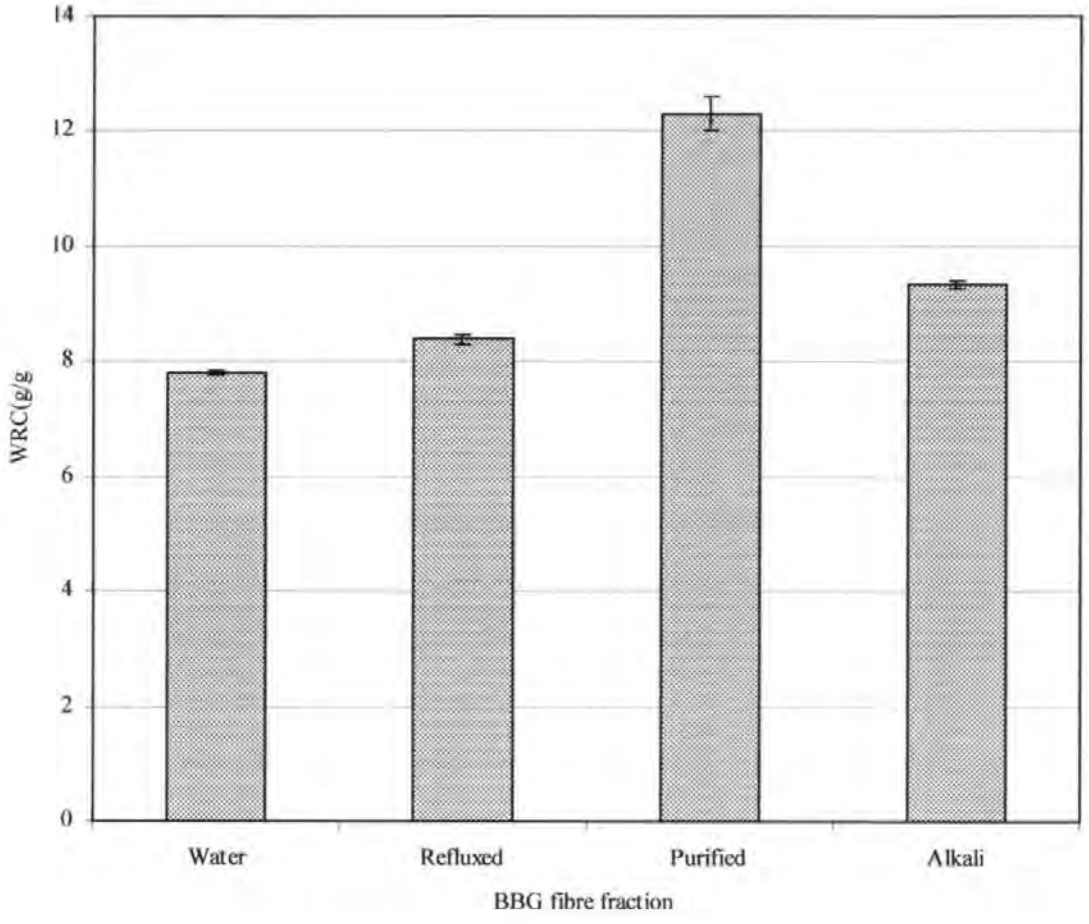


Figure 2.4 WRC of BBG fibre fractions from water, refluxed, purified and alkali extraction treatments. SD of triplicate determinations of a composite sample is included on the figure as error bars.

2.3.4 Pasting Characteristics of Wheat Starch Substituted with 1 and 5% BBG Fibre Fractions

The pasting characteristics (PV, BD and FV) of wheat starch pastes with 1 and 5% BBG fibre fraction substitutions are illustrated in Table 2.2. Values are expressed in centipoises (cP), and comparisons are made against the control.

The results indicate that substitution of wheat starch with 1% BBG fibre fractions may result in an increase in the PV of pastes compared to the control. Conversely, the results indicate that substitution of wheat starch with 5% BBG fibre fractions may decrease the PV of pastes compared to the control.

The results indicate that substitution of wheat starch with 1% BBG fibre fractions may have a minimal effect on BD values of wheat starch; however, substitution of wheat starch with 5% BBG fibre fractions may result in a decrease in BD compared to the control.

Substitution of wheat starch with 1% BBG fibre fractions may result in a increase in FV values compared to the control, whilst substitution of wheat starch with 5% BBG fibre fractions may result in a decrease in FV values compared to the control.

Table 2.2 Pasting characteristics (PV, BD and FV) of wheat starch substituted with 1 and 5% water, refluxed, purified and alkali BBG fibre fractions (expressed as cP[1])

BBG fibre fraction	PV	BD	FV
Control	4046 ± 9.90	1163 ± 8.50	4623 ± 82.70
1% Water	4458 ± 34.60	1046 ± 44.50	5054 ± 18.40
5% Water	3753 ± 117.40	651 ± 39.60	4123 ± 170.40
1% Refluxed	4950 ± 0.00	1146 ± 0.00	5391 ± 0.00
5% Refluxed	3552 ± 38.90	773 ± 2.10	3883 ± 14.80
1% Purified	4756 ± 142.10	1359 ± 128.70	4980 ± 79.90
5% Purified	3417 ± 50.20	767 ± 32.50	3628 ± 67.20
1% Alkali	4397 ± 33.20	1064 ± 91.20	4947 ± 21.20
5% Alkali	3818 ± 115.30	695 ± 44.50	4198 ± 152.70

[1]Results are mean ± SD of triplicate determinations of a composite sample.

The apparent increase in the PV and FV of wheat starch with 1% BBG fibre fractions may be a result of the viscous effect of BBG fibre fractions within the starch system.

The effect of NSPs on the cooking and cooling of starch systems has been reported by many authors (Christianson *et al.* 1981; Alloncle and Doublier 1991; Bahnassey and Breene 1994). Several theories, sometimes conflicting, have been postulated to explain the increase in viscosity brought about by NSP inclusion:

- Christianson *et al.* (1981) reported that gum media in a starch hydrocolloid magnifies viscosity increases due to changes in granule size or shape during swelling.

- Alloncle and Doublier (1991) described starch dispersions as composites where viscoelastic properties in the pasted and gelled states are governed primarily by the volume occupied by swollen particles. The dispersed phase consists primarily of amylopectin and accounts for two thirds of the total volume. The continuous phase is predominantly amylose, which makes an additional contribution due to exclusive viscoleastic properties. Increasing the concentration of NSP within the continuous phase has a role in increasing the viscosity of the composite. Starch gelation takes place upon cooling and is influenced by the gelation of amylose, which is modified by the added NSP.

- Bahnassey and Breene (1994) proposed that the increase in PV of starch/NSP systems is associated with the release of amylose and low MW amylopectin and the subsequent formation of polymer complexes, which significantly alters the

viscosity of the system. A similar explanation was proposed by Shi and BeMiller (2002).

The apparent reduction in wheat starch PV, BD and FV brought about by the inclusion of 5% BBG fractions could be a result of removing starch from the system; however, it is also possible that any reduction may be related to the WRC of the fractions, which leads to water being withheld from the starch granules by the β-glucan. The reduction of available water in the system would reduce initial starch granule swelling and explain the lower PV and FV of the pastes.

It is also plausible that the apparent reduction in the PV and FV of 5% BBG fibre fraction-starch gel systems is a result of the interference of intermolecular associations among amylopectin molecules by the polysaccharide, this in accordance with the theory proposed by Biliaderis *et al.* (1997).

2.3.5 Gelatinisation Characteristics of Wheat Starch Substituted with 1 and 5% BBG Fibre Fractions

The gelatinisation characteristics (T_{onset}, T_{endset}, T_{peak} and enthalpy) of wheat starch substituted with 1 and 5% BBG fibre fractions are illustrated in Table 2.3. The results suggest that incorporation of BBG fibre fractions (1 or 5%) into wheat starch may not affect T_{onset}, T_{endset} or T_{peak} compared to the control.

Addition of 5% BBG fibre fractions may result in a decrease in the total enthalpy value of wheat starch compared to the value of the control. Lowest enthalpies were observed in wheat starch substituted with 5% water fraction (7.19 J/g) and refluxed fraction (7.36 J/g).

Addition of 1% BBG fibre fraction may not have any affect on the enthalpy value of wheat starch compared to the control.

Several authors have examined the gelatinisation of starch in the presence of β-glucan and have observed variable results on gelatinisation parameters (Kim *et al.* 1986; Kim and Setser 1992; Biliaderis *et al.* 1997). It is difficult to make direct comparisons between the data sets due to the variations in materials, conditions and moreover, varying outcomes of starch gelatinisation.

The apparent decrease in enthalpy of wheat starch substituted with 5% BBG fibre fractions within this study may be a direct result of replacement of starch with BBG fibre fraction; however, the ability of soluble polysaccharides to immobilise water and restrict starch gelatinisation may also be a mechanism. The inclusion of NSP to starch water systems has been reported to decrease the free volume of water and hinder molecular mobility (Scandola *et al.* 1991), and this in turn effects the plasticisation of amorphous regions and the dissociation of double helices during the gelatinisation process. Both Ferrero *et al.* (1996) and Tester and Sommerville (2003) have reported the 'anti-plasticising' effect of NSP in starch-water systems.

Table 2.3 Gelatinisation characteristics (T_{onset}, T_{peak}, T_{endset} and enthalpy) of wheat starch substituted with 1 and 5% water, refluxed, purified and alkali BBG fibre fractions[1]

BBG fibre fraction	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)	Enthalpy (J/g)
Control	52.55 ± 0.07	68.55 ± 0.92	59.05 ± 0.07	7.80 ± 0.02
1% Water	52.40 ± 0.14	67.80 ± 0.42	58.70 ± 0.00	7.89 ± 0.03
5% Water	53.30 ± 0.28	66.95 ± 0.21	59.10 ± 0.28	7.19 ± 0.06
1% Refluxed	52.20 ± 0.14	68.20 ± 0.00	59.20 ± 0.00	8.01 ± 0.01
5% Refluxed	52.50 ± 0.42	67.10 ± 0.14	58.95 ± 0.21	7.36 ± 0.00
1% Purified	52.90 ± 0.71	68.10 ± 0.41	58.65 ± 0.07	7.79 ± 0.19
5% Purified	52.60 ± 0.42	67.55 ± 0.64	59.20 ± 0.14	7.37 ± 0.08
1% Alkali	51.50 ± 0.00	67.20 ± 0.35	58.75 ± 0.07	7.82 ± 0.10
5% Alkali	53.21 ± 0.28	67.65 ± 0.78	59.05 ± 0.21	7.45 ± 0.04

[1]Results are mean ± SD of triplicate determinations of a composite sample.

2.4 CONCLUSIONS

This study illustrates that the composition and functional behaviour of barley β-glucan fractions may be influenced by the choice of extraction treatment. A purification treatment may yield a fraction with the highest β-glucan purity; however, the yield and recovery of β-glucan from this treatment is low and also considerably costly and hazardous when taking into consideration the volume of organic solvent consumed in production.

The observations from this study may have importance in the application of BBG fibre fractions in foods and in human nutrition, particularly where starch is a primary ingredient (i.e. bread and pasta). The possible ability of BBG fibre fractions at a low level of inclusion to increase the viscosity of wheat starch pastes may have implications on the textural and eating quality of foods. The WRC exhibited by BBG fibre fractions and

possible reduction in gelatinisation of starch at a high level of inclusion has relevance to human nutrition where the degree of starch gelatinisation can affect the post-prandial sugar availability from food and regulation of the *vitro* and *in vivo* glyceamic response to carbohydrate rich diets.

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 and pasta where water absorption can significantly influence processing and final product quality. Further studies are warranted to investigate these effects.

CHAPTER 3

THE EFFECT OF β-GLUCAN RICH FRACTIONS FROM BARLEY ON THE PHYSICO-CHEMICAL PROPERTIES AND *IN VITRO* STARCH DIGESTIBILITY OF WHITE WHEAT BREAD

3.1 INTRODUCTION

Bread, in its various forms, is considered to be one of the oldest and most popular processed cereal products consumed globally; however, in recent years the role of bread in the daily diet of UK adults has been scrutinised as a result of the increasing emphasis being placed upon the relationship between diet and health. The popularity of diet regimes such as Atkins (and other low-carbohydrate diets) and low-GI is believed to be partly responsible for the decline in household consumption of bread (Anon 2005b). In a UK study conducted by the Target Group Index (TGI) and British Market Research Bureau (BMRB) in 2004, which surveyed 25,000 UK adults, 99% of consumers were found to consume bread; however, a decline of approximately 5% (2002-2004) was reported in the number of consumers claiming to be heavy users (eating pre-packed bread more than once a day). Whilst bread can be considered low in fat and a source of complex carbohydrates, white bread, the most popular variety in the UK, is a poor source of dietary fibre (Anon 2003).

White wheat bread (hereafter referred to as bread) is generally considered to be a high GI food (Foster Powell *et al.* 2002); however, in some studies a strong correlation between the addition of soluble dietary fibre 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 elicited lower glycaemic responses compared to a reference white bread.

The hypoglycaemic efficacy of native and extracted β-glucans is in part related to dose, MW, fine structure and rheological characteristics (Wood *et al.* 1990, 1994a). There is also increasing evidence to suggest that the hypoglycaemic capacity of β-glucans when in a cereal food matrix is partly a result of their ability to decrease the digestibility of the starch fraction by reducing susceptibility to amylolytic attack (Hudson *et al.* 1992; Izydorczyk *et al.* 2005).

The addition of barley β-glucan to wheat flours (typically in the form of barley flour addition) has been reported to have a negative impact on dough and baked bread quality (Knuckles *et al.* 1997a; Cavallero *et al.* 2002; Gill *et al.* 2002). This negative impact may be partly a result of the replacement of a significant proportion of conventional wheat flour with that of barley flour, which due to a difference in composition has reduced baking performance (Knuckles *et al.* 1997a). Incorporating smaller quantities of barley β-glucan fractions may reduce the negative effect.

The production of barley β-glucan rich fractions requires extraction procedures that may result in β-glucan with different physico-chemical properties. These extraction procedures may also cause different degrees of β-glucan MW degradation (Beer *et al.* 1997a,b; Knuckles *et al.* 1997b), which in turn may reduce hypoglycaemic efficacy. Food processing conditions, particularly bread fermentation and baking (Aman *et al.* 2004) and also the conditions of the gastrointestinal tract (Johansen *et al.* 1997) have been reported to result in the MW degradation of β-glucan.

3.1.1 Rationale and Aim

Whilst a number of studies have examined the effect of barley β-glucan rich flour inclusion on the physico-chemical and nutritional properties of breads, there are few studies that

have examined the effects of incorporating barley β-glucan fractions. As a concentrated source of β-glucan, these fractions can be incorporated to breads in smaller quantities than flours but still yield breads with high levels of β-glucan. It is acknowledged that the composition and physico-chemical properties of barley β-glucan fractions will vary depending upon the extraction procedure used in their production, thus these different barley β-glucan fractions are likely to have varying effects when included into a cereal food system, such as bread. In addition, these fractions may also differ in their susceptibility to MW degradation during the conditions of fermentation and baking and those of digestion, which may further change their physico-chemical and hypoglycaemic properties.

The overall aim of this study was to investigate and compare the influence of two barley β-glucan fractions, BBG fibre fraction (as prepared in Chapter 2) and a commercial barley β-glucan preparation (Glucagel™), on the physico-chemical properties and *in vitro* starch digestibility of bread and the susceptibility of these preparations to MW degradation during fermentation, baking and *in vitro* digestion.

3.1.1.1 Objectives

- Incorporate an aqueous-solvent extracted BBG fibre fraction (as prepared in Chapter 2) and a commercial barley β-glucan fraction (Glucagel™) into bread at different inclusion levels and investigate and compare effects on the rheological properties of dough (resistance to extension and extensibility) and quality of baked bread (height, volume, colour, appearance and firmness).

- Examine and compare the influence of barley β-glucan fraction inclusions on the micro-structure of baked and *in vitro* digested breads.

- Determine and compare the influence of barley β-glucan fractions on the digestibility of starch in bread using a multi-enzymic *in vitro* digestion method.

- Profile and compare the MW of barley β-glucan fractions during fermentation, baking and *in vitro* digestion.

Such information will provide clarity on the suitability of different barley β-glucan fractions for potential inclusion as functional ingredients in bread and also highlight formulation and process modifications, which may need to be employed in order to make such breads of an acceptable quality to consumers.

3.2 MATERIALS AND METHODS

3.2.1 Materials

3.2.1.1 BBG fibre fraction

BBG fibre fraction (approximately 69% β-glucan dwb) was prepared from Cindy barley flour using extraction method (a) detailed in Chapter 2 (*2.2.2.1*).

3.2.1.2 Glucagel™

Glucagel™, a gelling form of β–glucan (approximately 75% β–glucan dwb), was supplied by Polycell Technologies (Crookston, Minnesota, US).

Table 3.1 illustrates BBG fibre fraction and Glucagel™ composition (as determined using methods detailed in Chapter 2 (*2.2.2.2*)). Due to their importance in bread-making, the arabinose and xylose content of BBG fibre fraction and Glucagel™ were determined using the 'Uppsala Method' (Approved Method 32-25, AACC 2000b). Particle size (determined

using meshed screens), colour (visually determined) and WRC (as determined in Chapter 2 (*2.2.2.3*)) of the two fractions are also illustrated (Table 3.1).

Table 3.1 BBG fibre fraction and Glucagel™ composition and physical properties

	BBG fibre fraction	**Glucagel™**
% Component (dwb)[1]		
β-glucan	68.62 ± 0.40	75.03 ± 0.20
Arabinose	1.80 ± 0.04	0.90 ± 0.08
Xylose	2.60 ± 0.05	0.90 ± 0.02
TDF	79.01 ± 0.52	77.10 ± 0.43
Total starch	12.50 ± 0.35	16.90 ± 0.21
Protein	5.77 ± 0.06	4.95 ± 0.03
Physical properties		
Particle size (μm)	<500	<500
Colour	Beige	Light tan
WRC (g/g)	7.80	6.25

[1]Results are mean ± SD of duplicate determinations of a composite sample reported on a dwb.

3.2.1.3 Bread making ingredients

Bread wheat flour was supplied by Shipton Mill (Stroud, UK). Sugar, salt, yeast and vegetable fat were purchased from a local supermarket.

3.2.1.4 Reagents

Unless otherwise stated, all general laboratory reagents were purchased from Fisher Scientific (UK) or Sigma Aldrich (UK/Sweden).

3.2.2 Methods

3.2.2.1 Bread making

Breads were manufactured using a straight dough, long fermentation basic bread process. Bread wheat flour was substituted with BBG fibre fraction or Glucagel™ at three different levels (variations in the amount of BBG fibre fraction and Glucagel™ used at each level are a reflection of the difference in the composition of the two preparations and were necessary to achieve breads with similar levels of β-glucan):

Level 1: BBG (2.5%), Glucagel™ (2.3%).

Level 2: BBG (5%), Glucagel™ (4.6%).

Level 3: BBG (7.5%), Glucagel™ (6.9%).

An additional sample with no β-glucan was also prepared as a control. Table 3.2 illustrates the control, BBG fibre fraction and Glucagel™ bread formulations. The moisture level of the mixtures was adjusted on manufacture to produce visually optimum doughs that could be used to form pup loaves.

Table 3.2 Control, BBG fibre fraction and Glucagel™ bread formulations

Bread	Bread wheat flour (g)*	β-glucan (g)	Water (ml)
Control	125	0	70
BBG level 1	121.875	3.125	70
BBG level 2	118.750	6.250	73
BBG level 3	115.625	9.375	75
Glucagel™ level 1	122.125	2.875	70
Glucagel™ level 2	119.250	5.750	73
Glucagel™ level 3	116.375	8.625	75

*yeast (6g), salt (6g), sugar (1g) and vegetable fat (6.25g) were constant for all breads.

All dry ingredients were mixed in a food processor (RoboCoupe R4, Robot Coupe Ltd, UK) for 10 seconds to ensure complete homogeneity; water was then added, and the mixture was processed for 45 seconds to allow formation of dough. Doughs were fermented at 40°C for 2 hours. The doughs were then kneaded and divided into 70 g portions and placed in miniature pup loaf tins with the following dimensions: top 85 mm (length) by 50 mm (width); and bottom 75 mm (length) by 40 mm (length). The doughs were then fermented for a further 60 minutes. Following fermentation doughs were baked in a fan assisted oven (Zanussi Combiwave FCVM/E62, UK) at 220°C for 25 minutes. After baking, breads were cooled for 1 hour before subsequent analyses. Proximate composition of the breads was determined as detailed in Chapter 2 (*2.2.2.2*), with the exception of available starch, which was determined as the sum of total starch minus resistant starch using the resistant starch assay kit (Approved Method 32.40, AACC 2000b) as supplied by Megazyme™ International Ireland Ltd (Wicklow, Ireland). Table 3.3 presents the proximate composition of the breads.

Table 3.3 Proximate composition of control, BBG fibre fraction and Glucagel™ breads[1]

Bread	Available starch (%)	Protein (%)	TDF (%)	β-glucan (%)
Control	69.52 ± 0.25	16.09 ± 0.02	5.88 ± 0.78	0.14 ± 0.00
BBG level 1	67.15 ± 0.15	15.57 ± 0.01	8.02 ± 0.34	1.69 ± 0.03
BBG level 2	64.05 ± 0.01	15.46 ± 0.04	9.99 ± 0.41	3.38 ± 0.10
BBG level 3	62.51 ± 0.32	15.25 ± 0.01	11.80 ± 0.35	4.90 ± 0.10
Glucagel™ level 1	67.87 ± 0.17	15.76 ± 0.16	7.65 ± 0.68	1.61 ± 0.01
Glucagel™ level 2	65.97 ± 0.21	15.56 ± 0.26	9.51 ± 0.59	3.27 ± 0.05
Glucagel™ level 3	64.07 ± 0.82	15.43 ± 0.82	11.19 ± 0.21	4.94 ± 0.15

[1]Results are mean ± SD of duplicate determinations of a composite sample (dwb).

3.2.2.2 Dough rheology

The final product of dough mixing is a visco-elastic mass that after appropriate proofing and baking produces an aerated solid called bread. Bread has a sponge like structure (the voids are interconnected), the structural elements being primarily gelatinised starch and denatured protein. The rheological characteristics of dough are primarily responsible for achieving the desired result (Stauffer 1998). Dough rheology is traceable to the nature of the matrix elements, which are in wheat dough, the gluten proteins (hydrated glutelins (glutenins) and prolamines (gliadins)). A great deal research of dough has measured rheological characteristics and correlated them with bread characteristics (Bloksma and Bushuk 1988; Hoseney and Rogers 1990).

Extensibility and extensibility resistance of wheat dough are readily accessible physical measurable qualities, which allow good assessment of baking behaviour. Such methods have been established globally as AACC Standard Methods (Extensigraph (Approved Method 54-10) and Alveograph (Approved Method 54-30)); however, the disadvantage of these methods is the substantial quantities of material required to perform the analysis, for example up to 300 g of flour can be required for duplicate analysis. It is this large amount of sample required that is the limiting factor in research applications where only a small quantity of test material is available. This problem has been partially remedied by other authors who have used a Stable Microsystems Kiefer Dough and Gluten Extensibility Rig in which as little as 10 g dough can be used for six replications. It has been demonstrated that the results obtained in micro-extension trials can provide the same information as the macro-methods (Kieffer *et al.* 1998).

In this study, the rheological properties (resistance to extension (expressed as mean maximum force g) and extensibility (when elastic limit is exceeded and sample breaks)

(expressed as the mean distance at maximum force mm)) of bread doughs were measured using a texture analyser (TA) (TA-XT2) (Stable Micro Systems, Surrey, UK) equipped with a Kiefer dough and extensibility rig (A/KIE) (calibrated for a load cell of 5 kg). Dough (15 g) 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 75 mm at a pre-test, test and post-test speed of 2, 3.3 and 10 mm/sec respectively. The trigger force was 5 g. An example of the TA trace obtained can be found in Appendix III.

3.2.2.3 External, internal and texture quality evaluation of bread

The quality assessment of bread typically fits into three broad categories: external; internal; and texture/eating quality. Quality assessments may be measured by descriptive means; however, less subjective measurements can be made using instrumental techniques. In this study, external (height, volume and crust colour), internal (loaf appearance and crumb colour) and texture (firmness) qualities of the breads were quantified.

3.2.2.3.1 Loaf height

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

3.2.2.3.2 Loaf volume

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

3.2.2.3.3 Crumb firmness

A TA (TA-XT2) (Stable Micro Systems, Surrey, UK) was used to measure bread firmness. An AACC 36 mm radius cylinder probe (P/36R) was used (calibrated for a load cell of 5

kg). 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 100 g, and maximum peak force in compression was recorded as the firmness value in gram units. Measurements were taken from 1 cm slices, and samples were discarded after the TA test. An example of the TA trace obtained can be found within Appendix IV.

3.2.2.3.4 Crust and crumb colour

Crust and crumb colour were determined using L*a*b* colour space (also refereed to as CIELAB). L* indicates lightness (L: lightness, 100 = white, 0 = black), and a* and b* are the chromaticity co-ordinates (a: + red, - green), (b: + yellow, - blue). Values were obtained on a Model CR-200 Chroma Meter (Minolta, Ramsey, New Jersey, US). For crust colour, measurements were taken from three loaves at three different positions. For crumb colour, measurements were taken from three slices at three different positions.

3.2.2.3.5 Loaf appearance

Cross-sectional images of baked breads were taken using a digital camera (Canon Power Shot A400 Digital Camera, Canon INC, Japan).

3.2.2.4 In vitro digestion

The relationship between the rate of starch digestion and GI has been established by investigators of *in vitro* amylolytic hydrolysis (O'Dea *et al.* 1981; Jenkins *et al.* 1982, 1987; Ross *et al.* 1987b; Heaton *et al.* 1988; Bornet *et al.* 1989; Englyst *et al.* 1992, 1999, 2003; Grandfeldt *et al.* 1992; Brighenti *et al.* 1995; Araya *et al.* 2002; Seal *et al.* 2003). Although at present *in vitro* methods are certainly no substitute for *in vivo* evaluation, particularly for clinical or epidemiological purposes, *in vitro* methods are ideal as a screening tool of foods before exposure to expensive and laborious *in vivo* studies.

In this study bread samples were subjected to an *in vitro* digestion based upon the method of Brighenti *et al.* (1995), slightly modified. This method is based on a multi-enzymic digestion confined within a dialysis tube, followed by analysis of the reducing sugars released into the dialysate. The use of dialysis offers certain advantages over unrestricted digestion. Primarily the method determines susceptibility of starch to amylolytic attack, but effects not directly related to starch digestion are also reflected, for example the appearance of digest products in the dialysate will be affected by internal viscosity of the digestion tubing. Although the method is less sophisticated than more recently published techniques aimed at predicting the GI of different foods, for example the rapidly available glucose measurements developed by Englyst *et al.* (1999, 2003), it still provides a good estimate of the rate of sugar release from starchy foods. When restricted *in vitro* digestions were employed by Brighenti *et al.* (1995) to predict the physiological effects of dietary fibre, significant correlations between starch hydrolysis and *in vivo* GI were obtained. *In vitro* digestions have also been employed by Brennan *et al.* (1996a, 2004), Hudson *et al.* (1992), Tudorica *et al.* (2002c) and Izydorczyk *et al.* (2005) to investigate the effect of soluble dietary fibres on the starch digestibility of cereal foods.

In this study, samples of bread (equivalent to 2 g available starch) were reduced to a size of approx 1 cm^3, diluted with sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (HCL acid) and digested with pepsin (from porcine stomach mucosa) (115 U/g starch) (Sigma-Aldrich, UK) for 30 minutes at 37°C. The pH of the mixtures was re-adjusted to pH 6.9 (NaOH), diluted to 50 ml (sodium phosphate buffer) and porcine pancreatic α-amylase (110 U/g starch) (Sigma-Aldrich, UK) was added. A sample blank (with deactivated enzyme) was also prepared. The mixtures were transferred to prepared dialysis tubing (Medicell International Ltd, UK) and placed in 450 ml of sodium phosphate buffer for 5 hours at 37°C. Tubes were agitated every 15 minutes to simulate gut movements.

Duplicate aliquots (1 ml) were taken every 30 minutes, replacing the volume each time with 1 ml fresh buffer. Dialysate was analysed for total dialysable sugars by the 3,5-dinitrosalicylic acid method (James 1999).

Reducing sugars released (RSR), consisting of the dialysed fragments of digested starch, was expressed in maltose equivalents as a percentage of available carbohydrate present in the sample using the following calculation of Brighenti *et al.* (1995):

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

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

3.2.2.5 Micro-structure

The ultra-structure of cereal foods, in particular the accessibility of starch to digestive enzymes, has a strong influence on its digestibility (Brighenti *et al.* 1995). Several authors have used microscopic techniques, such as scanning electron microscopy (SEM^3), to examine the micro-structure of foods in relation to their digestibility and have observed correlations between starch granule availability and post-prandial blood glucose response/rate of *in vitro* starch digestibility (Brennan *et al.* 1996a; Giacco *et al.* 2001; Tudorica *et al.* 2002c).

In this study samples of baked and *in vitro* digested (samples taken at 300 minutes *in vitro* digestion) breads were frozen in liquid nitrogen and freeze dried. The freeze dried samples

were transversely fractured to expose interior surfaces, and 1 mm sections were mounted onto pre-glued stubs. All prepared specimens were sputtered coated with gold (Emitech K550 Sputter Coater, Ashford, UK) and examined by a scanning electron microscope (SEM^2) (SEM JEOL JSM6100, Oxford, UK).

3.2.2.6 Extraction and analysis of β-glucan Calcofluor average MW (Mcf) and MW distribution

The ability to measure the molecular conformation of β-glucan has enabled the elucidation of mechanisms of physico-chemical and physiological responses. A widely employed technique for determining the MW of β-glucan is high performance size exclusion chromatography with fluorescence detection (HPSEC-FD) (Wood *et al.* 1991a; Suortti 1993). Calcofluor (disodium 4,4-bis{4-anilino-6-[bis(2-hydroxyethyl)-amino]-1,3,5-triazin-2-yl}amino-2,2-stilbenedisulfonate) is specific for (1→3, 1→4)-β-D-glucans in cereal extracts (Wood *et al.* 1983), which cause large increases in the fluorescent intensity of the dye. Extracts of β-glucan are initially separated by size exclusion chromatography (SEC) and then mixed with Calcofluor. The β-glucan-Calcofluor complex results in an increase in intensity that can be detected by a fluorescence detector. The measurement is not affected by the presence of other polysaccharides since Calcofluor is selective for β-glucan, thus, the technique provides a simple procedure to determine MW of β-glucan extracts without the need for prior purification. This particular type of SEC is highly precise as only elution volume and the relative detector signal are measured to determine MW; however, accurate measurement is dependent upon the accuracy of the calibration curve. Wood *et al.* (1991a) used pullulan standards to calibrate the columns, but this has been illustrated to lead to over-estimation of MW (Varum *et al.* 1991; Wood *et al.* 1991a). An alternative approach is the use of purified β-glucan of known average MW for the calibration (Wood *et al.* 1991a; Suortti 1993). Rimsten *et al.* (2003) recently developed an

improved calibration and calculation of the average MW. In this method, a purified β-glucan was fractionated into narrow MW ranges and the average MW was determined before analysis of samples on the HPSEC-FD system. One of the limitations of HPSEC-FD is that the detection method will exclude β-glucans of a lower MW (<10,000) (Jorgensen 1988; Manzanares *et al.* 1993).

In this study bread wheat flour, BBG fibre fraction, Glucagel™ and control, BBG fibre fraction and Glucagel™ (level 3) containing doughs (1 and 3 hours fermentation) and baked and *in vitro* digested breads (samples taken at 30, 150 and 300 minutes *in vitro* digestion) were selected for β-glucan MW characterisation. Samples with a moisture content of >10% were freeze dried prior to analysis. Enzymes in the samples were inactivated by boiling in 50% ethanol for 15 minutes. β-glucans in the products (100 mg) were extracted with hot deionised water (20 ml) with added $CaCl_2$ (0.28 mg/ml of water) and thermostable α-amylase (50 µl, Megazyme International Ireland Ltd, Wicklow, Ireland), following the method of Rimsten *et al.* (2003). The mixtures were immediately placed in a boiling water bath for 90 minutes, with occasional mixing by vortex. After cooling to room temperature, tubes were centrifuged (1500 *x g* for 15 minutes), and supernatants were filtered (0.45 µm) before injecting into the HPSEC-FD system (set up according to Wood *et al.* 1991a,b, with some modifications).

The HPSEC-FD system consisted of two pumps (LC-10AD, Shimadzu, Miniato, Japan) coupled to a degasser (SDU 2006, Prolab, Reinach, Switzerland), one delivering the eluent (0.1M NaNo3 with 0.02% NaN_3) at a flow rate of 0.5 ml/min and the other one delivering Calcofluor solution (0.05% fluorescent brightner 28 (Sigma) in 0.1 M tris(hydroxymethyl)-(aminomethane) (Tris) adjusted to pH 8) at a flow rate of 0.5 ml/min though a pulse reducer. An injector (Midas type 830, Spark, Emmen, Holland) was coupled to the system

before a guard column (OHpak SB-G, Shodex, Showa Denko KK, Kawasaki, Japan) and two columns in series (OHpak SB-806HQ and SB-804HQ, Shodex, Showa Denko KK, Kawasaki, Japan). Calcofluor was delivered postcolumn by a mixing loop placed together with the columns in an oven maintained at 60°C. For detection, a fluorescent detector (1100 series G1321A, Agilent Technologies, Germany) was used with the wave-lengths λ_{ex} = 415 nm and λ_{em} = 445 nm according to Suortti (1993) at a gain setting of 8. An image of the HPSEC-FD system used is contained within Appendix V.

The system was calibrated using β-glucan fractions with narrow MW ranges. By using the regression line of the calibration curve, M_{cf} could be calculated. The M_{cf} over the distribution divided into n slices was defined as:

$$M_{cf} = \frac{\sum_{i=1}^{n}(w_i c_i)}{\sum_{i=1}^{n} c_i}$$

where: w_i is the MW at a slice i given by the calibration and c_i is the corresponding concentration, expressed as Calcofluor response. This average includes only β-glucan molecules large enough to be detected with Calcofluor. Percentiles were also calculated describing the MW at which 10, 50, and 90% of the distribution fall below that value. Results are means of duplicate analyses.

3.2.2.7 Statistical analysis

Unless otherwise stated, all determinations were made in triplicate (samples taken from three independent production runs), and mean ± SD values are presented. Data was statistically evaluated by ANOVA as detailed in Chapter 2 (*2.2.2.6*). Significance was defined as $P<0.05$.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of BBG Fibre Fraction and Glucagel™ on the Rheological Properties of Bread Dough

The first basic step in bread manufacture is combining water with wheat flour and kneading (imparting mechanical energy) to the mixture to form an elastic dough (Bushuk 1985; Hoseney 1985). Two main contributors to bread quality, that is volume and a fine crumb, are dictated by certain optimum properties in the dough matrix. The two characteristics that define a 'good' dough are the ability to retain gas (carbon dioxide) generated during fermentation in the form of numerous small gas cells and a proper balance of viscous flow and elastic strength so that the loaf can expand adequately during proofing and the early stages of baking, yet retain a rounded form (Stauffer 1998).

Table 3.4 illustrates the effect of BBG fibre fraction and Glucagel™ inclusion on the rheological properties of bread doughs. The resistance to extension of all BBG fibre fraction containing doughs was significantly higher than the control dough, the magnitude of resistance increasing with greater addition of BBG fibre fraction ($P<0.05$). Doughs containing Glucagel™ levels 2 and 3 exhibited significantly higher resistance to extension than the control ($P<0.05$); however, the resistance to extension of dough with Glucagel™ level 1 inclusion was not significantly different to the control ($P>0.05$).

The extensibility of all doughs containing BBG fibre fraction was significantly reduced compared to the control, the reduction increasing with BBG fibre fraction concentration ($P<0.05$). Only wheat doughs with Glucagel™ level 2 and 3 inclusions exhibited a significant decrease in extensibility ($P<0.05$) compared to the control; however, this reduction was not as great as that exhibited by the BBG fibre fraction containing doughs.

Table 3.4 Rheological properties of control, BBG fibre fraction and Glucagel™ doughs[1]

Dough	Extension (g)	Distance (mm)
Control	$33.34^{d} \pm 0.65$	$-29.83^{b} \pm 0.47$
BBG level 1	$40.26^{c} \pm 2.70$	$-20.62^{d} \pm 0.47$
BBG level 2	$51.56^{b} \pm 3.30$	$-19.82^{d} \pm 0.28$
BBG level 3	$66.99^{a} \pm 0.44$	$-15.15^{e} \pm 0.44$
Glucagel™ level 1	$38.28^{c,d} \pm 1.63$	$-31.65^{a} \pm 0.95$
Glucagel™ level 2	$42.15^{c} \pm 2.75$	$-24.63^{c} \pm 0.72$
Glucagel™ level 3	$63.41^{a} \pm 2.70$	$-25.98^{c} \pm 0.43$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

These results suggest that barley β-glucan fractions greatly affect the rheological behaviour of bread dough and possibly final product quality. Dough resistance to extension is thought to be an indicator of dough strength and ability to retain gas, and extensibility is considered as a predictor of the processing/handling characteristics of the dough (Wang *et al.* 2002). The results gathered in this investigation indicate that barley β-glucan fractions increase the strength and stiffness of dough and possibly gas retaining capacity. The decrease in extensibility suggests that barley β-glucan fractions simultaneously interfere with gluten structure and development, possibly limiting the free expansion of the dough and its ability to stretch into thin membranes during fermentation and baking. These results are in agreement with Gomez *et al.* (2003) who observed decreased extensibility of wheat dough supplemented with various fibres.

Differences in the TDF composition of BBG fibre fraction and Glucagel™ may help to explain the variation in the resistance to extension and reductions in extensibility observed amongst the doughs. The BBG fibre fraction contains a higher content of arabinose and xylose than Glucagel™ (Table 3.1), co-extracted with β-glucan during the extraction procedure, and these components have been reported to have a strong influence on the rheology of wheat doughs, holding approximately 9-11 times their own weight in water (Jelaca and Hlynka 1972). In addition to increasing water absorption, it has been postulated that arabinoxylans form links with gluten proteins to increase the resistance of doughs to extension and decrease its extensibility (Hoseney and Faubion 1981).

3.3.2 Effect of BBG Fibre Fraction and Glucagel™ on Loaf Volume, Height and Firmness

The inclusion of BBG fibre fraction and Glucagel™ in bread resulted in a significant decrease in loaf height and volume compared to the control ($P<0.05$) (Table 3.5), the magnitude of loss increasing with fibre concentration. Glucagel™ breads exhibited greater losses in height and volume compared to their BBG fibre fraction counterparts. Appendix VI illustrates cross-sectional views of breads with BBG fibre fraction and Glucagel™ inclusions. There was a non-significant rise in the firmness of breads containing BBG fibre fraction and Glucagel™ compared to the control ($P>0.05$).

Reduced loaf height and volumes as a consequence of barley β-glucan addition have been experienced by Knuckles *et al.* (1997a), Cavallero *et al.* (2002) and Gill *et al.* (2002). Deleterious effects of fibre addition on bread structure have been suggested to be due to dilution of the gluten network, which in turn impairs gas retention. Pomeranz *et al.* (1977) detected through microscopic examination a major difference between the crumb structure of control and fibre containing bread. The crumb structure of the control bread was

composed of thin filaments, which were essentially absent in fibre enriched bread. According to Gan *et al.* (1992), fibre materials in expanded dough appeared to disrupt the starch gluten matrix and also restrict and force gas cells to expand in a particular dimension, greatly distorting the gas cell structure.

Table 3.5 Loaf height, volume and firmness of control, BBG fibre fraction and Glucagel™ breads[1]

Bread	Height (cm)	Volume (ml)	Firmness (g)
Control	$6.18^{a} \pm 0.08$	$212^{a} \pm 2.00$	$5.40^{a} \pm 0.02$
BBG level 1	$5.94^{b} \pm 0.04$	$190^{b} \pm 2.00$	$5.48^{a} \pm 0.13$
BBG level 2	$5.21^{c} \pm 0.07$	$165^{c} \pm 1.15$	$5.54^{a} \pm 0.39$
BBG level 3	$4.19^{e} \pm 0.08$	$123^{e} \pm 2.31$	$5.75^{a} \pm 0.61$
Glucagel™ level 1	$5.41^{c} \pm 0.06$	$170^{c} \pm 0.00$	$5.75^{a} \pm 0.17$
Glucagel™ level 2	$4.71^{d} \pm 0.11$	$133^{d} \pm 5.77$	$5.80^{a} \pm 0.23$
Glucagel™ level 3	$4.09^{e} \pm 0.05$	$112^{f} \pm 2.89$	$6.01^{a} \pm 0.18$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

The physico-chemical properties of barley β-glucan fractions can also affect bread volume and texture indirectly. It is possible that when added to wheat flour during bread making, barley β-glucan fractions retain appreciable amounts of water and make it less available for the development of the gluten network, which results in an underdeveloped gluten network, and hence, reduced loaf height and volume. Alternatively, the decreased height and volume may be attributed to a reduction in steam production as a result of the WRC of

the barley β-glucan fractions. Jiang and Vasanthan (2000) and Gill *et al.* (2002) proposed that barley β-glucan, due to its high water affinity, may retain water in the dough, which would otherwise be used in steam generation. Suppression of steam generation could possibly lead to a reduced loaf height and volume.

The difference between the extent of loaf height and volume loss observed between the BBG fibre fraction and Glucagel™ breads may again be explained by the greater content of arabinose and xylose in the BBG fibre fraction. Water-soluble arabinoxylans have been illustrated to have important technological potential in bread making, improving baking quality by exerting a viscous influence on gluten-starch films, which protects gas retention in dough and in turn enhances bread volume (Delcour *et al.* 1991). Therefore, it is plausible that the presence of arabinoxylans in the BBG fibre fraction breads counteracts the negative effects of the barley β-glucan, which results in breads with higher loaf height and volume than Glucagel™ counterparts.

3.3.3 Effect of BBG Fibre Fraction and Glucagel™ on Bread Crust and Crumb Colour

L*a*b* colour space values for control, BBG fibre fraction and Glucagel™ bread crusts and crumbs are presented in Table 3.6. The L* crust colour values of all BBG fibre fraction breads were similar to the control ($P>0.05$), whilst Glucagel™ breads had significantly darker crusts (lower L* crust colour values) than the control and BBG fibre fraction breads ($P<0.05$). The a* crust colour values of all BBG fibre fraction breads were significantly lower (less red) ($P<0.05$) than the values for the control and Glucagel™ breads (which were similar ($P>0.05$)). With the exception of bread with BBG fibre fraction level 2 inclusion (significantly lower ($P<0.05$)), the b* crust colour values (yellowness) of control, BBG fibre fraction and Glucagel™ breads were similar ($P>0.05$).

Table 3.6 Crust and crumb colour of control, BBG fibre fraction and Glucagel™ breads[1]

Bread	Crust			Crumb		
	L*	A*	b*	L*	a*	b*
Control	$69.20^{a} \pm 2.00$	$13.12^{a} \pm 0.43$	$23.08^{a} \pm 1.68$	$73.11^{b} \pm 1.34$	$3.65^{b} \pm 0.15$	$11.56^{b} \pm 0.57$
BBG level 1	$70.01^{a} \pm 0.87$	$10.13^{b} \pm 0.81$	$20.43^{a,b} \pm 1.74$	$72.93^{b} \pm 2.27$	$3.95^{b} \pm 0.21$	$11.61^{b} \pm 1.26$
BBG level 2	$70.30^{a} \pm 1.37$	$8.23^{b} \pm 0.43$	$18.16^{b} \pm 0.34$	$75.87^{a,b} \pm 1.63$	$4.99^{a} \pm 0.24$	$14.58^{a} \pm 0.38$
BBG level 3	$71.06^{a} \pm 2.18$	$10.01^{b} \pm 0.20$	$20.54^{a,b} \pm 2.76$	$75.89^{a,b} \pm 0.21$	$5.08^{a} \pm 0.12$	$14.19^{a} \pm 0.31$
Glucagel™ level 1	$61.18^{b} \pm 1.29$	$14.34^{a} \pm 0.39$	$22.12^{a,b} \pm 0.91$	$76.92^{a} \pm 0.91$	$4.86^{a} \pm 0.14$	$10.81^{b} \pm 0.47$
Glucagel™ level 2	$56.94^{b} \pm 1.72$	$14.25^{a} \pm 0.65$	$20.10^{a,b} \pm 1.59$	$74.35^{a,b} \pm 0.45$	$5.10^{a} \pm 0.15$	$10.60^{b} \pm 0.03$
Glucagel™ level 3	$57.20^{b} \pm 4.51$	$15.02^{a} \pm 1.48$	$21.42^{a,b} \pm 0.88$	$75.07^{a,b} \pm 0.30$	$5.18^{a} \pm 0.05$	$10.43^{b} \pm 0.35$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

The L* crumb colour values of control, BBG fibre fraction and Glucagel™ breads were similar ($P>0.05$), with the exception of bread with Glucagel™ level 1 inclusion, which had a significantly higher value ($P<0.05$). With the exception of bread with BBG fibre fraction level 1 inclusion, BBG fibre fraction and Glucagel™ breads had significantly higher a* crumb colour values (more red) ($P<0.05$) compared to the control. Breads with BBG fibre fraction level 2 and 3 inclusions were significantly more yellow (higher b* crumb colour values) than the control, whilst breads with BBG fibre fraction level 1 and Glucagel™ inclusions had b* crumb colour values similar to that of the control ($P>0.05$).

Both Knuckles *et al.* (1997a) and Gill *et al.* (2002) have evaluated the effect of barley β-glucan rich flour inclusions on the crust and crumb colour of wheat breads. Knuckles *et al.* (1997a) observed that breads substituted with 5% barley flour had crumb lightness similar to the control, whilst increasing substitution to 20% resulted in breads with a darker crumb. Conversely, Gill *et al.* (2002) observed no change in the crumb colour of breads formulated with 5, 10, and 15% native barley flour. Differences observed between the crust and crumb colour of BBG fibre fraction and Glucagel™ breads are most likely to be attributed to differences in the colour of the two fractions (Table 3.1).

3.3.4 Effect of BBG Fibre Fraction and Glucagel™ on the *In Vitro* Starch Digestibility of Bread

Table 3.7 illustrates the effect of BBG fibre fraction and Glucagel™ inclusion on the *in vitro* starch digestibility (as measured by RSR) of bread. A graphic representation of the *in vitro* starch digestibility of control and BBG and Glucagel™ (level 3) breads is given in Appendix VII to allow a visual appreciation of the differences in the rate of RSR. The results reveal a consistent significant decrease in RSR from breads with BBG fibre fraction level 2 and 3 inclusions compared to the control bread after 30 and 60 minutes *in vitro*

digestion respectively ($P<0.05$). No consistent significant decrease in RSR was observed from bread with BBG fibre fraction level 1 inclusion compared to the control ($P>0.05$). The RSR from breads with Glucagel™ levels 2 and 3 inclusions was generally significantly lower than the control after 120 minutes *in vitro* digestion ($P<0.05$). No consistent significant decrease in RSR could be found from bread with Glucagel™ level 1 inclusion compared to the control bread ($P>0.05$). Post 150 minutes *in vitro* digestion there was generally no difference between the RSR from BBG fibre fraction breads and Glucagel™ counterparts ($P>0.05$).

These results indicate that β-glucan fractions from barley have the ability to modify the starch digestibility of breads, which in turn may have implications for the *in vivo* regulation of sugar release from bread, a traditionally high glycaemic food. These results are in agreement with the studies of Pick *et al.* (1998) and Cavallero *et al.* (2002) who both observed significant reductions in the glycaemic responses of healthy individuals fed barley β-glucan rich breads compared to white wheat controls.

Several theories exist to explain the effect of soluble polysaccharides on the starch digestibility of cereal products. It is widely accepted that *in vivo* reductions in glycaemia by soluble fibre are a result of increased intestinal viscosity (Jenkins *et al.* 1978; Wood *et al.* 1990); however, the majority of these studies are with homogenous solutions of soluble fibre and glucose as opposed to foods with a solid matrix where the structure and interaction of fibre with other macro-molecules (starch and protein) is of importance. Some authors have proposed that changes to the micro-structure of cereal products in the presence of soluble fibre are responsible for reductions in starch digestibility (Brennan *et al.* 1996a; Tudorica *et al.* 2002c). Other studies indicate that the limitation of water availability as a consequence of soluble non-starch polysaccharide hydration can restrict

gelatinisation of starch and hence, reduce hydrolysis by α-amylase (Jankiewicz and Michniewicz 1987; Tester and Sommerville 2003).

The attenuated *in vitro* starch digestibility of BBG fibre fraction and Glucagel™ breads may be a consequence of a limitation of available water for starch hydration due to barley β-glucan hydration and gelation (as observed in the starch gelatinisation investigations of Chapter 2), the formation of a glucan gel matrix which inhibits enzyme accessibility to partially gelatinised starch granules, reductions in sugar motility as a result of increased digesta viscosity or a combination of all these mechanisms. Further studies to characterise the viscous influence of the fibres may be warranted, although data from *in vitro* studies examining this effect should be viewed conservatively, since acidity, osmolatity, volume and concentration of sugars all contribute to the viscous effect of fibres in the intestine and *in vitro* techniques are unlikely to detect this (Brand Miller and Holt 2004).

The ability of the BBG fibre fraction to lower RSR from bread earlier in digestion (90 minutes) than Glucagel™ (150 minutes) may be attributed to differences in the degree of gum hydration. It is possible that the BBG fibre fraction was hydrated and incorporated into the bread matrix more rapidly and thoroughly than Glucagel™, resulting in an earlier attenuation of RSR. Differences in rate of soluble fibre (gum) hydration have been reported by Ellis *et al.* (1991) to be of importance in hypoglycaemic efficacy and may partly explain the variable responses (effect and no effect) reported in studies investigating the same soluble fibre but in different forms of preparation (Wursch and Pi-Sunyer 1997).

Table 3.7 *In vitro* starch digestibility (RSR, expressed in maltose equivalents as a percentage of total available carbohydrate) of control, BBG fibre fraction and Glucagel™ (GLU) breads[1]

Bread	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins	210 mins	240 mins	270 mins	300 mins
Control	$0.00^{a}\pm0$	$3.36^{a}\pm0.44$	$11.23^{a,b}\pm0.68$	$18.84^{a}\pm0.93$	$26.85^{a}\pm1.52$	$35.49^{a}\pm1.42$	$40.40^{a}\pm0.98$	$45.98^{a}\pm0.39$	$51.76^{a}\pm0.64$	$53.48^{a,b}\pm0.71$
BBG 1	$0.00^{a}\pm0$	$1.99^{a,b}\pm1.03$	$9.51^{b,c}\pm0.91$	$16.57^{a,b}\pm1.27$	$26.16^{a,b}\pm1.52$	$33.10^{a,b}\pm1.14$	$38.42^{a,b}\pm1.43$	$43.28^{b,c}\pm0.15$	$50.34^{a,b}\pm0.26$	$55.46^{a}\pm0.89$
BBG 2	$0.00^{a}\pm0$	$1.58^{b}\pm0.70$	$8.65^{c}\pm0.98$	$13.13^{b,c}\pm1.99$	$22.00^{c,d}\pm1.42$	$27.63^{c,d}\pm1.54$	$35.22^{c}\pm1.41$	$42.30^{c}\pm1.04$	$48.61^{b,c}\pm0.89$	$50.15^{c}\pm0.71$
BBG 3	$0.00^{a}\pm0$	$2.22^{a,b}\pm0.43$	$5.76^{d}\pm0.94$	$10.99^{c}\pm1.99$	$19.88^{d}\pm1.29$	$27.33^{d}\pm1.36$	$33.95^{c}\pm0.32$	$39.02^{d}\pm0.75$	$46.18^{c}\pm0.39$	$48.32^{c}\pm1.22$
GLU 1	$0.00^{a}\pm0$	$3.29^{a,b}\pm0.76$	$11.75^{a}\pm0.26$	$18.29^{a}\pm0.26$	$24.25^{a,b,c}\pm1.34$	$31.12^{b}\pm0.52$	$36.66^{b,c}\pm1.42$	$45.89^{a,b}\pm0.89$	$48.30^{b,c}\pm0.69$	$53.55^{a,b}\pm2.51$
GLU 2	$0.00^{a}\pm0$	$3.46^{a}\pm0.72$	$11.68^{a}\pm0.65$	$17.50^{a}\pm0.07$	$22.89^{b,c,d}\pm2.11$	$30.92^{b,c}\pm1.53$	$36.74^{b,c}\pm1.28$	$43.82^{b}\pm1.25$	$48.38^{b,c}\pm0.32$	$52.11^{a,b,c}\pm1.14$
GLU 3	$0.00^{a}\pm0$	$3.28^{a,b}\pm0.88$	$8.24^{c}\pm0.16$	$16.29^{a,b}\pm0.88$	$21.47^{c,d}\pm0.65$	$30.78^{b,c}\pm0.08$	$34.39^{c}\pm0.40$	$38.04^{d}\pm1.44$	$46.65^{c}\pm1.92$	$51.47^{b,c}\pm1.61$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

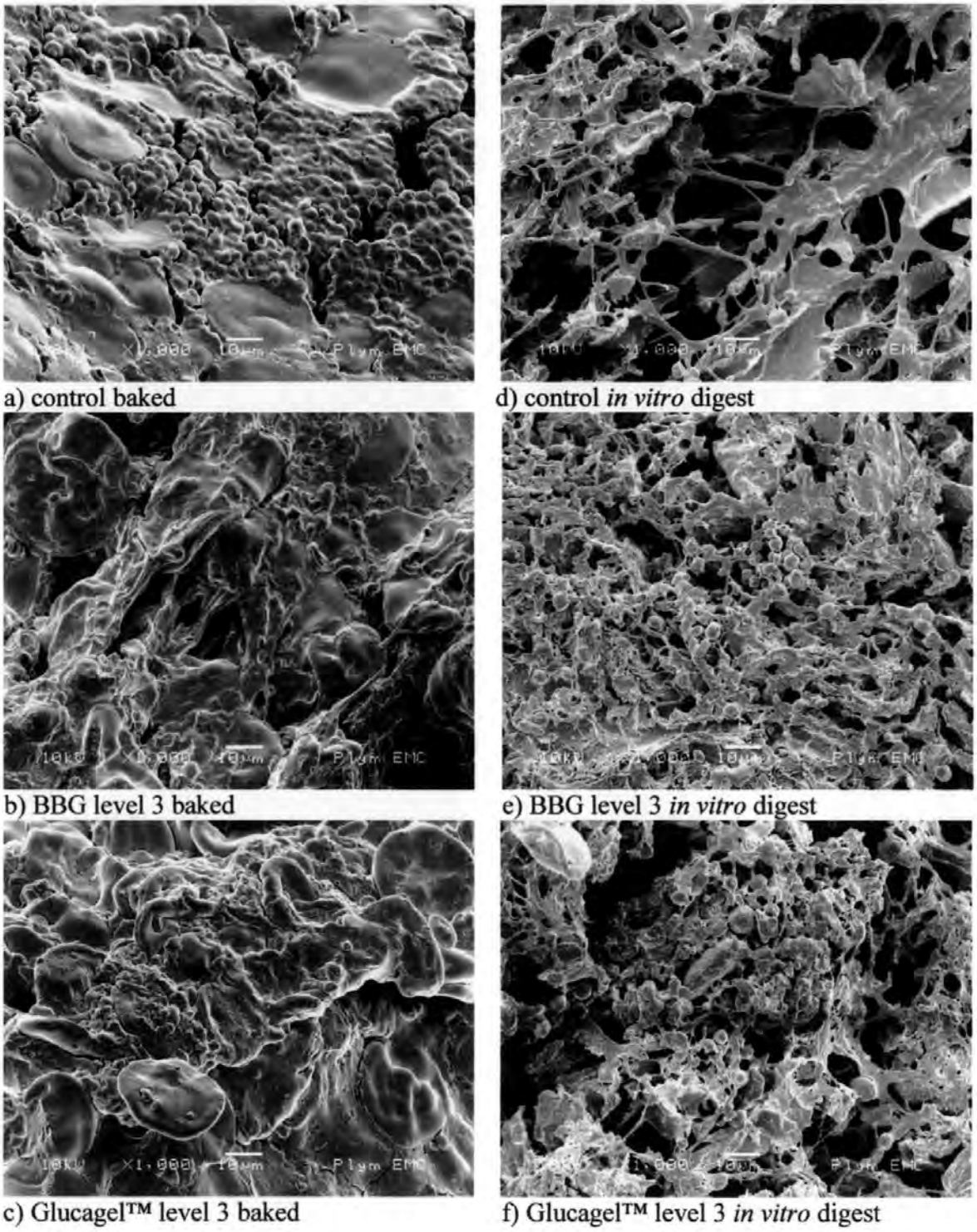
[a]means values in the same column followed by the same letter are not significantly different ($P>0.05$).

3.3.5 Effect of BBG Fibre Fraction and Glucagel™ on the Micro-Structure of Baked and *In Vitro* Digested Bread

Traditional breads have a porous structure with small and highly dispersed starch granules that are exposed and susceptible to enzymic degradation. This susceptibility to amylase degradation of the starch is in part due to the gelatinisation of starch during baking, which in turn results in a high glycaemic response from eating bread (Giacco *et al.* 2001).

SEM[3] was used to evaluate the effect of BBG fibre fraction and Glucagel™ on the micro-structure of baked and *in vitro* digested bread. Figure 3.1 illustrates scanning electron micrographs (SEMs[1]) of baked and *in vitro* digested (300 minutes) control, BBG fibre fraction and Glucagel™ (level 3 inclusion, the level at which maximum attenuation in RSR was observed) breads. Additional SEMs[1] of all baked and *in vitro* digested BBG and Glucagel™ breads are contained in Appendix VIII.

Figure 3.1a illustrates the baked control bread, which exhibits a porous structure with a high dispersion of small starch granules. Addition of both BBG and Glucagel™ (Figures 3.1b and 3.1c respectively) appears to result in a loss of porosity and starch granule accessibility. Particularly noticeable is the formation of a 'gummy looking' matrix (Figure 3.1b) in the baked BBG fibre fraction bread. The *in vitro* digested control bread (Figure 3.1d) has a very porous structure with few undigested starch granules. The presence of BBG fibre fraction and Glucagel™ in the *in vitro* digests (Figures 3.1e and 3.1f respectively) results in a more compact structure and a noticeable retention of small undigested starch granules. The changes observed in the micro-structure of the breads and *in vitro* digests does not appear to vary substantially between BBG fibre fraction and Glucagel™ addition.



a) control baked
d) control *in vitro* digest
b) BBG level 3 baked
e) BBG level 3 *in vitro* digest
c) Glucagel™ level 3 baked
f) Glucagel™ level 3 *in vitro* digest

Figure 3.1 SEMs[1] of baked and *in vitro* digested (300 minutes) breads (x 1000): (a) control baked; (b) BBG level 3 baked; (c) Glucagel™ level 3 baked; (d) control *in vitro* digest; (e) BBG level 3 *in vitro* digest; and (f) Glucagel™ level 3 *in vitro* digest.

Ellis *et al.* (1991) illustrated that in addition to a rheological affect, guar gum inhibited the rate of digestion of the starch components of wheat bread by altering the micro-structure. In the studies of Brennan *et al.* (1996a), microscopic analysis of digestas taken from pigs 4 hours after they had been fed guar gum containing bread revealed that the galactomannan component of the guar was still closely associated to the individual starch granules in the bread, thus, forming and enzyme-resistant barrier around the starch granules. *In vitro* digestibility studies were consistent with the structural observations in that the hydrolysis of starch in guar gum wheat bread was reduced significantly compared with the control. Whilst in this current study it is not possible to detect if the barley β-glucan fractions are in intimate contact with starch granules, the SEM[1] images clearly illustrate the way in which barley β-glucan fractions integrate within a food system has an impact on the physico-chemical properties of breads and the rate of amylolytic activity and starch hydrolysis.

3.3.6 Effect of Fermentation, Baking and *In Vitro* Digestion on the M_{cf} and MW Distribution of β-Glucan from BBG Fibre Fraction and Glucagel™

M_{cf} and MW distribution of β-glucan within bread wheat flour, BBG fibre fraction, Glucagel™ and control, BBG fibre fraction and Glucagel™ (level 3 inclusion) doughs, baked and *in vitro* digested breads were determined after extraction with boiling water and hydrolysis of starch with a thermostable α-amylase from *Bacillus licheniformis*. This method is illustrated to extract between 7-75% of the β-glucan in cereal samples with no apparent depolymerisation (Rimsten *et al.* 2003).

The M_{cf} and MW distribution of β-glucan from BBG fibre fraction and Glucagel™ were similar (P>0.05) (Table 3.8 and Figure 3.2 respectively), both being relatively low. The similarity in M_{cf} of β-glucan from BBG fibre fraction and Glucagel™ clarifies that MW was not responsible for the variance between the physico-chemical properties and *in vitro*

RSR of BBG fibre fraction and Glucagel™ containing breads. The conditions of fermentation and baking did not significantly change the M_{cf} of β-glucan in either preparation. The distributions in the control β-glucan are of less importance because of the very low β-glucan content compared to the breads with added β-glucan.

The degradation of barley β-glucan MW during bread processing has been illustrated by Knuckles *et al.* (1997b), Andersson *et al.* (2004) and Trogh *et al.* (2004). These studies have clearly demonstrated an enzymatic hydrolysis of the β-glucan most likely from enzymes present in the flour or in added yeast. The absence of degradation of β-glucan in either BBG fibre fraction or Glucagel™ is difficult to explain; however, it is plausible that β-glucanases preferentially degrade HMW β-glucan (as illustrated by the degradation of β-glucan contained within the control) and/or there is a terminal MW at which β-glucan is no longer susceptible to molecular degradation.

The distributions in β-glucan from the BBG fibre fraction and Glucagel™ *in vitro* digested breads do not change drastically during treatment (Figure 3.3). The slight increase in M_{cf} (Table 3.9) might be explained by an increase in extractability or a reduction in bread components as reducing sugars are released.

Table 3.8 M_{cf} and MW distribution of β-glucan from bread wheat flour, BBG fibre fraction, Glucagel™ and control, BBG fibre fraction and Glucagel™ (level 3 inclusion) doughs (1 and 3 hours fermentation) and baked breads (percentiles describing MW (x 10^4 g/mol) at which 10, 50 and 90% of the distribution fall below that value[1])

Sample	(M_{cf}) (x 10^4 g/mol)	CV[2]	Distribution		
			10%	50%	90%
Bread wheat flour	70^{a}	1.6	$4.5^{c,d}$	36^{a}	186^{a}
BBG	$12^{c,d}$	1.8	$4.3^{d,e}$	9.5^{d}	21^{c}
Glucagel™	$11^{c,d}$	1.5	$4.0^{e,f}$	8.8^{d}	21^{c}
Dough 1 hour fermentation					
Control	22^{b}	1.7	5.3^{a}	15^{b}	45^{b}
BBG	$11^{c,d}$	1.1	$4.5^{c,d}$	9.3^{d}	19^{c}
Glucagel™	$11^{c,d}$	4.3	$4.2^{d,e,f}$	8.8^{d}	20^{c}
Dough 3 hour fermentation					
Control	21^{b}	1.3	3.9^{f}	12^{c}	46^{b}
BBG	10^{d}	1.9	$4.3^{d,e,f}$	8.5^{d}	17^{c}
Glucagel™	$11^{c,d}$	5.0	$4.0^{e,f}$	8.4^{d}	19^{c}
Baked bread					
Control	20^{b}	8.2	$4.8^{b,c}$	13^{c}	44^{b}
BBG	$13^{c,d}$	5.5	4.9^{b}	$10^{c,d}$	23^{c}
Glucagel™	$11^{c,d}$	8.5	$4.1^{e,f}$	8.8^{d}	19^{c}

[1]Results are mean ± SD of duplicate determinations (samples taken from independent production runs).

[2]Coefficient of variation (CV) (%) for M_{cf}.

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

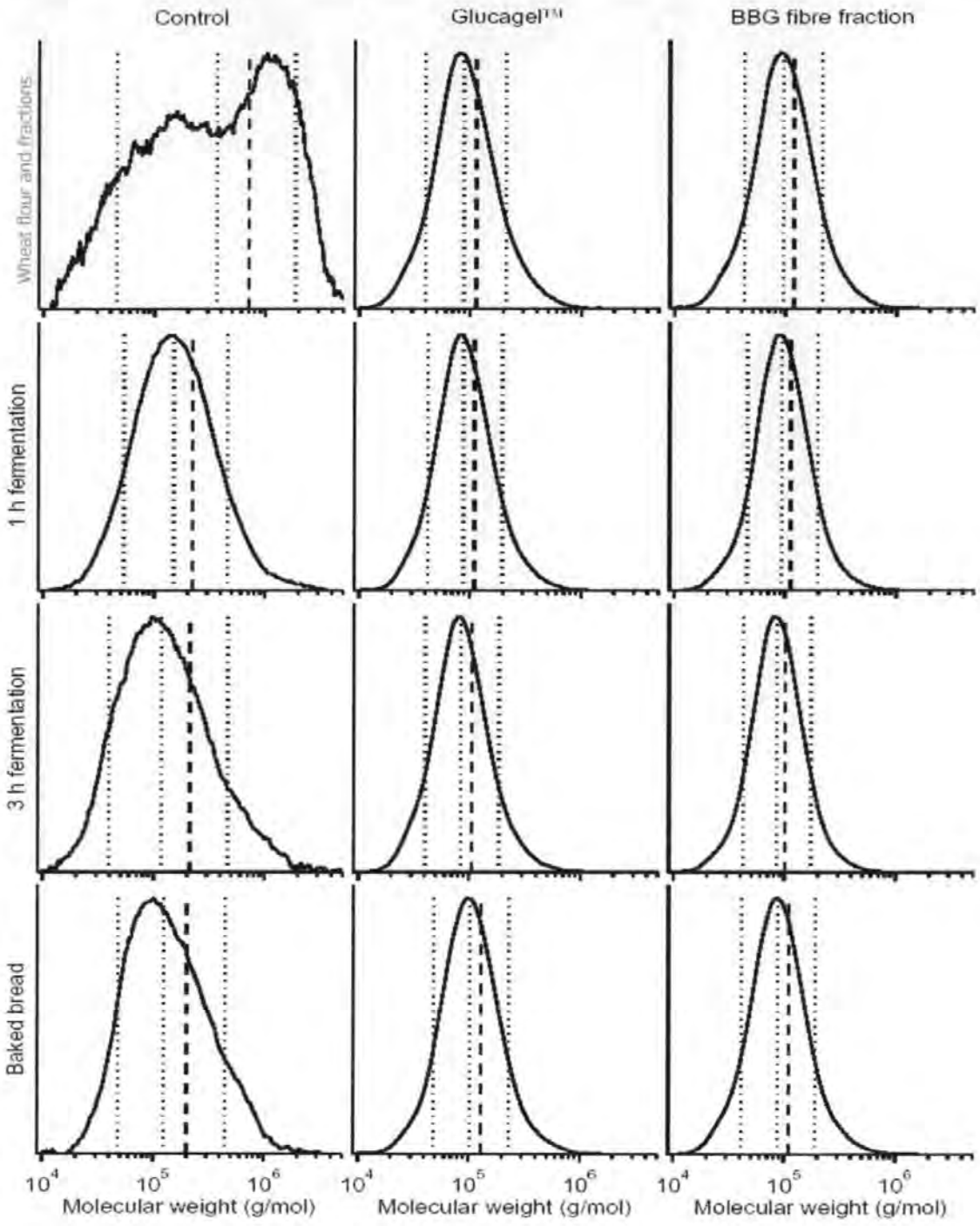


Figure 3.2 MW (g/mol) distribution of β-glucan from bread wheat flour, BBG fibre fraction, Glucagel™ and control, BBG fibre fraction and Glucagel™ (level 3 inclusion) doughs (1 and 3 hours fermentation) and baked breads. Dotted lines represent 10, 50 and 90% percentiles and dashed line represents M_{cf}. Results are from duplicate determinations (samples taken from independent production runs).

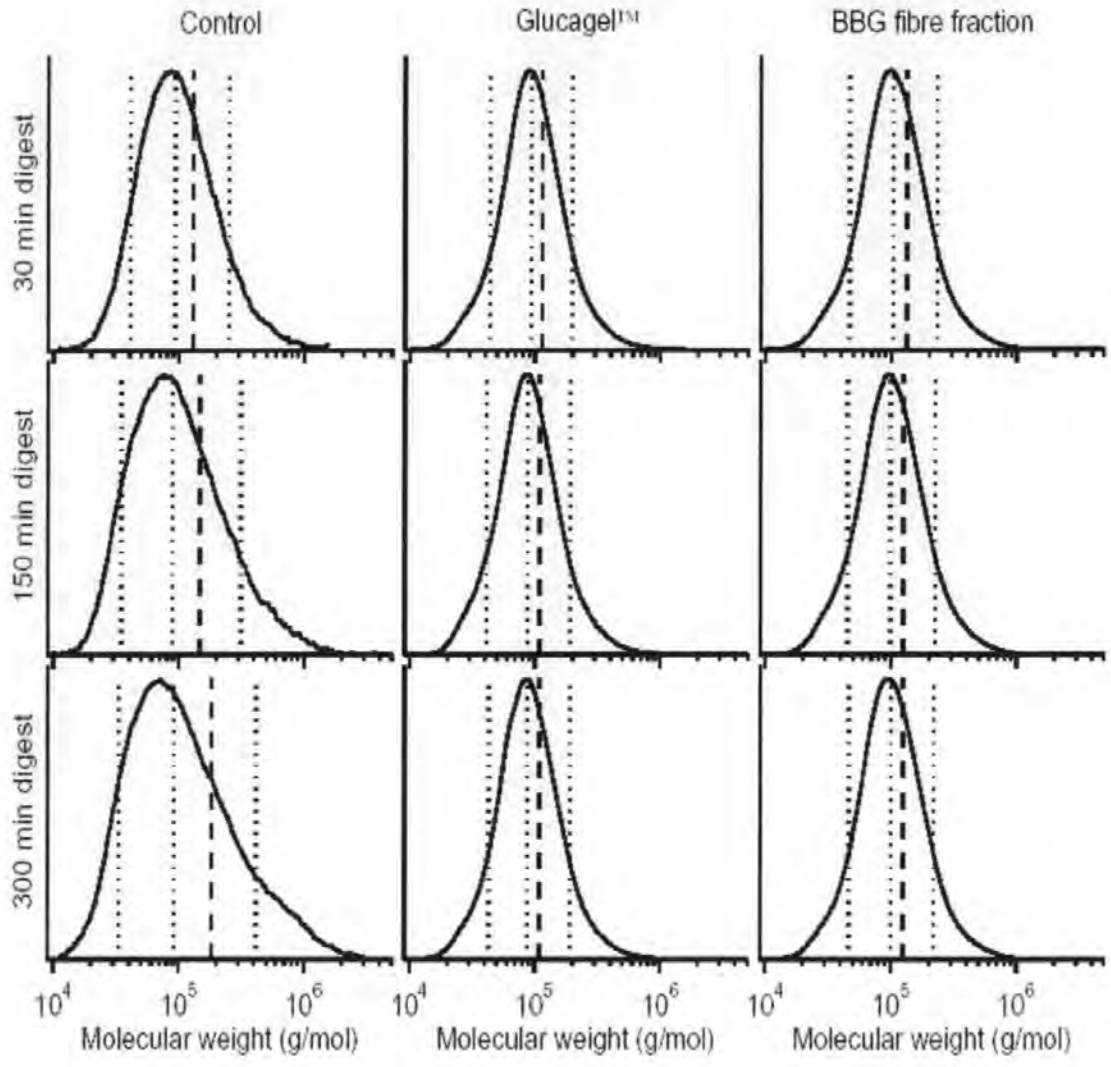


Figure 3.3 MW (g/mol) distribution of β-glucan from *in vitro* digested (30, 150 and 300 minutes) control, BBG fibre fraction and Glucagel™ (level 3 inclusion) breads. Dotted lines represent 10, 50 and 90% percentiles and dashed line represents M_{cf}. Results are from duplicate determinations (samples taken from independent production runs).

Table 3.9 M_{cf} and MW distribution of β-glucan from *in vitro* digested (30, 150 and 300 minutes) control, BBG fibre fraction and Glucagel™ (level 3 inclusion) breads (percentiles describing MW (x 10^4 g/mol) at which 10, 50 and 90% of the distribution fall below that value[1])

Sample	(M_{cf}) (x 10^4 g/mol)	CV[2]	Distribution		
			10%	50%	90%
***In vitro* digest 30 mins**					
Control	$13^{b,c}$	6.8	4.1^{c}	$9.2^{a,b,c}$	25^{c}
BBG	$13^{b,c}$	0.3	4.7^{a}	10^{a}	$23^{c,d}$
Glucagel™	11^{d}	1.1	$4.4^{a,b,c}$	$9.3^{a,b,c}$	$20^{d,e}$
***In vitro* digest 150 mins**					
Control	15^{b}	5.5	3.5^{d}	$8.9^{b,c}$	31^{b}
BBG	$12^{c,d}$	0.4	$4.5^{a,b}$	$9.9^{a,b}$	$22^{c,d,e}$
Glucagel™	11^{d}	0.2	4.2^{c}	8.8^{c}	19^{e}
***In vitro* digest 300 mins**					
Control	18^{a}	4.3	3.3^{d}	$9.2^{a,b,c}$	42^{a}
BBG	$12^{c,d}$	0.1	4.7^{a}	$9.9^{a,b}$	$22^{c,d,e}$
Glucagel™	11^{d}	0.3	$4.3^{b,c}$	8.8^{c}	19^{e}

[1]Results are mean ± SD of duplicate determinations (samples taken from independent production runs).

[2]CV (%) for M_{cf}.

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

3.4 CONCLUSIONS

This study illustrates that the incorporation of both BBG fibre fraction and Glucagel™ in breads significantly reduces the starch digestibility (rate at which reducing sugars are released) in an *in vitro* digestion model, the magnitude of reduction being dependent upon inclusion level and fraction type. This observation may be an indication of the potential of barley β-glucan fractions to regulate *in vivo* sugar release from bread, a traditionally high glycaemic food. In order to deem these fibre enriched breads acceptable to consumers, negative changes in the physico-chemical properties of the doughs and baked breads must be overcome. It is anticipated that other ingredients (i.e. dough conditioners, such as oxidizing agents or emulsifiers) may be incorporated into the breads to counteract the negative effects encountered on baking quality (volume and height loss); however, incorporation of such ingredients must be thoroughly investigated to ensure that the nutritional properties of β-glucans are not compromised. This study also indicates that the different extraction procedures employed in the preparation of BBG fibre fraction and Glucagel™ might result in barley β-glucan fractions with different physico-chemical and compositional properties, despite containing β-glucan with a similar MW. These differences result in a variation of the behaviour of the fractions when included into bread. The lack of β-glucan MW degradation in either BBG fibre fraction or Glucagel™ during fermentation, baking and *in vitro* digestion suggests that at a certain MW β-glucan is not readily degraded by endogenous enzymes and/or higher MW β-glucan (present in the wheat flour) is preferentially degraded. Further investigations are warranted to investigate whether barley β-glucan of a HMW is more readily degraded than LMW β-glucan and what the physico-chemical and potential physiological implications of this are. The production of functional barley β-glucan fractions that are not degraded during processing is of extreme importance when considering the wider application of barley β-glucan in commercial food products.

CHAPTER 4

THE EFFECT OF β-GLUCAN FIBRE FRACTIONS FROM BARLEY ON THE PHYSICO-CHEMICAL PROPERTIES AND *IN VITRO* STARCH DIGESTIBILITY OF DURUM WHEAT SEMOLINA PASTA

4.1 INTRODUCTION

Traditional durum wheat semolina pasta (hereafter referred to as pasta) is a popular cereal commodity in European households and is favoured for its ease of cooking and nutritional qualities. Pastas are generally considered as low GI foods, which elicit low post-prandial blood glucose and insulin responses (Jenkins *et al.* 1983, 1988; Bornet *et al.* 1987; Wolever 1990; Bjorck *et al.* 2000). The low GI of pasta is a result of the progressive liberation of sugars from the pasta matrix during digestion. This progressive sugar release may be attributed to the compact structure of pasta that results from the extrusion process, which brings about a close protein network entrapping starch granules and thereby delaying amylolysis (Pagani *et al.* 1986; Fardet *et al.* 1998, 1999).

Although a low GI food, traditional pasta is a poor source of dietary fibre. Historically, the nutritional improvement of pasta has mainly involved increasing protein contents and fortification with vitamins and minerals. The WHO and the US FDA consider pasta as a good vehicle for added nutrients, and as such pasta was one of the first foods for which the FDA permitted vitamin and iron enrichment (Marconi and Carcea 2001).

The enrichment of pasta with fibre has been subject to an increasing number of investigations, with the greatest number of studies examining the use of soluble fibres such as β-glucan (in the form of enriched oat/barley flour addition) (Dougherty *et al.* 1988;

Knuckles *et al.* 1997a; Yokoyama *et al.* 1997; Bourdon *et al.* 1999; Hallfrish and Behall 2000; Marconi *et al.* 2000), guar gum (Gatti *et al.* 1984; Giorato *et al.* 1986) and arabinoxylans (Ingelbrecht 2001). Enrichment of pasta with fibre material may have multiple nutritional benefits in that not only is the dietary fibre content of the pasta raised, but interactions of starch with fibre may further reduce the rate of starch digestion, thus, lowering glycaemic response (Gatti *et al.* 1984; Yokoyama *et al.* 1997).

Whole durum wheat semolina is employed in traditional pasta manufacture because of the unique rheological properties of its protein (Marconi and Carcea 2001). Partial or complete substitution of durum wheat semolina from pasta with fibre material can often result in negative changes in pasta quality. These negative changes are not only a direct result of removing a proportion of the durum flour but also as a consequence of the physico-chemical properties that fibre materials impart (i.e. high water absorption) into the pasta. Negative changes, such as increased cooking losses and loss of firmness, have been encountered in the manufacture of fibre rich pasta (Kordonowy and Youngs 1985; Edwards *et al.* 1995).

4.1.1 Rationale and Aim

Whilst a number of studies have examined the effect of barley β-glucan rich flour inclusions on the physico-chemical and nutritional properties of pasta, there is a paucity of studies documenting the influence of barley β-glucan rich fractions. As these fractions are a more concentrated source of β-glucan, they may be incorporated in smaller quantities than barley flours but still yield pasta with high β-glucan levels, thus, possibly overcoming the problem of removing high proportions of durum flour and the associated negative changes. As already discussed in Chapter 3, barley β-glucan fractions have unique physico-chemical and compositional properties, which are strongly influenced by the

conditions under which they are extracted. The hypoglycaemic efficacy of these fractions may also be influenced by extraction conditions.

The aim of this study was to assess the influence of the inclusion of two different barley β–glucan fractions, BBG fibre fraction (as prepared in Chapter 2) and a commercial barley β-glucan fraction (Glucagel™), on pasta cooking characteristics, structure, texture and *in vitro* starch digestibility.

4.1.1.1 Objectives

- Incorporate an aqueous-solvent extracted BBG fibre fraction (as prepared in Chapter 2) and a commercial barley β-glucan fraction (Glucagel™) into pasta at different inclusion levels and evaluate and compare effects on the cooking quality (dry matter, cooking loss and swelling index) and textural attributes (hardness and adhesiveness) of cooked pasta.

- Examine and compare the influence of barley β-glucan fraction inclusions on the microstructure of raw, cooked and *in vitro* digested pastas.

- Determine and compare the influence of barley β-glucan fraction inclusions on the digestibility of starch in pasta using a multi-enzymic *in vitro* digestion method.

Such data will provide information on the suitability of different barley β-glucan fractions as potential functional ingredients for pastas and also highlight formulation and process modifications, which may need to be employed in order to make such pastas of an acceptable quality to the consumer.

4.2 MATERIALS AND METHODS

4.2.1 Materials

4.2.1.1 BBG fibre fraction

BBG fibre fraction (69% β-glucan) was prepared from Cindy barley flour using extraction method (a) detailed in Chapter 2 (*2.2.2.1*).

4.2.1.2 Glucagel™

Glucagel™ was supplied as detailed in Chapter 3 (*3.2.1.2*).

The composition and physico-chemical properties of BBG fibre fraction and Glucagel™ are detailed in Chapter 3 (Table 3.1).

4.2.1.3 Durum wheat semolina

Durum wheat semolina was supplied by ADM Milling Ltd (Exeter, UK). Moisture and protein contents were 14% and 13.7% respectively.

4.2.1.4 Reagents

Unless otherwise stated, all general laboratory reagents were purchased from Fisher Scientific (UK) or Sigma Aldrich (UK/Sweden).

4.2.2 Methods

4.2.2.1 Pasta manufacture

Pastas were made using water and durum semolina substituted with BBG fibre fraction or Glucagel™ at four levels (variations in the amount of BBG fibre fraction and Glucagel™ used at each level are a reflection of the difference in the composition of the two fractions and were necessary to achieve pastas with similar levels of β-glucan):

Level 1: BBG (2.5%), Glucagel™ (2.3%).

Level 2: BBG (5%), Glucagel™ (4.6%).

Level 3: BBG (7.5%), Glucagel™ (6.9%).

Level 4: BBG (10%), Glucagel™ (9.2%).

An additional sample with no β-glucan was also prepared as a control. The pasta formulations are illustrated in Table 4.1. Moisture content of the pastas was adjusted on manufacture to produce visually optimum doughs prior to extrusion.

Table 4.1 Control, BBG fibre fraction and Glucagel™ pasta formulations

Pasta	Durum wheat flour (g)	β-glucan (g)	Water (ml)
Control	100.0	0.0	40.0
BBG level 1	97.5	2.5	42.0
BBG level 2	95.0	5.0	47.0
BBG level 3	92.5	7.5	50.0
BBG level 4	90.0	10.0	51.0
Glucagel™ level 1	97.7	2.3	42.0
Glucagel™ level 2	95.4	4.6	47.0
Glucagel™ level 3	93.1	6.9	50.0
Glucagel™ level 4	90.8	9.2	51.0

Pasta dough was prepared in a Kitchen Aid (Kitchen Aid, St Jospeh, Michigan, US). The flour and fibre were mixed for 1 minute using the mixing blade to ensure complete homogeneity. Water was added, and the dough was mixed for a further 2 minutes using a dough hook. The dough was allowed to rest for 15 minutes and then extruded through a spaghetti die (2 mm diameter). The pastas were allowed to air dry for 2 days at ambient

temperature. Dried pastas were broken into 5 cm lengths, placed in sealed bags and stored at −18°C. Proximate composition of the cooked pastas was determined using the methods detailed in Chapter 2 (*2.2.2.2*), with the exception of available starch, which was determined as detailed in Chapter 3 (*3.2.2.1*). Table 4.2 presents the proximate composition of the pastas.

Table 4.2 Proximate composition of control, BBG fibre fraction and Glucagel™ cooked pastas[1]

Pasta	Available starch (%)	Protein (%)	TDF (%)	β-glucan (%)
Control	77.36 ± 0.11	15.20 ± 0.02	5.06 ± 0.80	0.42 ± 0.00
BBG level 1	76.22 ± 0.17	14.88 ± 0.02	7.61 ± 0.31	1.98 ± 0.20
BBG level 2	74.93 ± 1.14	14.66 ± 0.00	8.64 ± 0.29	3.68 ± 0.04
BBG level 3	73.45 ± 0.54	14.71 ± 0.00	10.30 ± 0.83	4.95 ± 0.05
BBG level 4	71.35 ± 0.58	14.50 ± 0.03	12.00 ± 0.39	6.38 ± 0.05
Glucagel™ level 1	77.05 ± 0.31	15.07 ± 0.02	7.12 ± 0.11	1.67 ± 0.03
Glucagel™ level 2	74.83 ± 1.33	14.98 ± 0.02	8.39 ± 0.35	3.30 ± 0.02
Glucagel™ level 3	72.37 ± 0.97	14.88 ± 0.07	10.35 ± 0.26	4.98 ± 0.03
Glucagel™ level 4	71.24 ± 1.14	14.75 ± 0.11	11.60 ± 0.38	6.24 ± 0.03

[1]Results are mean ± SD of duplicate determinations of a composite sample (dwb).

4.2.2.2 Cooking quality

Cooking performance is an important factor in consumer judgement of pasta quality. During cooking, pasta should maintain form without disintegration, increase in volume and exude minimal material to the cooking water (Cole 1991). In this study, the cooking

quality of the pastas was evaluated by determining swelling index, cooking loss and dry matter values.

4.2.2.2.1 Cooking time and procedure

Cooking time (the time necessary to obtain complete gelatinisation of starch as shown by the disappearance of the white central core of the control spaghetti strand) was determined as 7 minutes according to the AACC 'pasta and noodle cooking quality' procedure (Approved Method 66-50, AACC 2000b). Thereafter, samples of pasta (25 g) were boiled in a beaker of 300 ml distilled water (partially covered to help reduce evaporation and maintain a constant temperature) for 7 minutes. After cooking, samples were rapidly drained into a Büchner funnel (cooking water was reserved). Samples were rinsed with a constant stream of distilled water (approximately 50 ml for 30 seconds). Cooking and rinse water were combined to determine cooking loss, and samples of pasta were reserved and analysed for swelling index, dry matter, textural properties, composition, micro-structure and *in vitro* digestibility.

4.2.2.2.2 Cooking loss

Cooking loss was determined according to Approved Method 66-50 (AACC 2000b). Cooking and rinse water (collected from *4.2.2.2.1*) were combined and quantitatively transferred to pre-weighed 500 ml beakers. Samples were evaporated to dryness in an air oven at 100°C (drying time was approximately 20 hours). Beakers were cooled in a desiccator and weighed. Cooking loss was reported as a proportion of the original pasta sample.

4.2.2.2.3 Dry matter

Dry matter was determined according to Approved Method 44-15A (AACC 2000b).

4.2.2.2.4 Swelling index

The swelling index of cooked pasta (swelling index: grams of water per gram of dry pasta) was evaluated according to the procedure used by Fardet *et al.* (1998). Cooked pasta was dried to a constant weight at 105°C and expressed as: ((weight of cooked product)-(weight of pasta after drying)/ (weight of pasta after drying)).

4.2.2.3 Textural attributes (hardness and adhesiveness)

Textural factors, such as uniformity of appearance, structural strength and integrity, absence of a sticky surface and 'al dente' eating properties, as characterised by high degrees of firmness, are predominant characteristics that define the quality of pasta products (Antognelli 1980; Hoseney 1986; Pomeranz 1987). Pasta firmness or hardness represents the degree of resistance to the first bite and can be defined as the force required to penetrate pasta with the teeth and attractive forces among particles opposing disintegration (Kruger *et al.* 1996). Adhesiveness is a measurement of surface condition or extent of disintegration of the cooked product, which determines the extent to which strands adhere to each other (Kruger *et al.* 1996). Traditionally the textural quality of pasta has been determined by sensorial analysis; however, in recent years there is an increasing reliance on instrumental techniques to provide less subjective measurements (Cole 1991).

In this study, the hardness (mean maximum force g) and adhesiveness (mean negative area g s) of cooked pasta strands were determined using a TA (TA.XT2) (Stable Micro Systems, Surrey, UK) with a 35 mm cylinder probe (P/35R) (calibrated for a load cell of 5 kg). Force was measured in compression. Pre-test, test and post-test speeds were 2.0 mm/s. The strain was 75%, trigger type auto 10 g, and the data acquisition rate was 200 pps. Results were obtained from testing six strands per sample. An example of the TA trace obtained can be found in Appendix IX.

4.2.2.4 Starch gelatinisation characteristics

DSC[2] has been used by several authors to characterise the gelatinisation events of starch in the presence of non-starch polysaccharides specifically in pasta (Eerlingen *et al.* 1996; Ferrero *et al.* 1996; Tudorica *et al.* 2002c; Izydorczyk *et al.* 2005).

In this study, a DSC[1] was used to measure the thermal parameters (T_{onset}, T_{peak}, T_{endset} and enthalpy) of raw pasta samples substituted with BBG fibre fraction and Glucagel™, in order to ascertain the influence of β–glucan on the starch fraction. Pasta with no β-glucan addition was used as a control. Prior to analysis, pasta samples were freeze dried and milled to pass a 500 μm mesh screen. Sample preparation, DSC[1] instrumentation and parameters are as described in Chapter 2 (*2.2.2.5*).

4.2.2.5 In vitro digestion

Cooked pasta samples were subjected to an *in vitro* digestion based on the method of Brighenti *et al.* (1995), slightly modified. The method is detailed in Chapter 3 (*3.2.2.4*).

4.2.2.6 Micro-structure

Microscopy techniques have been previously used (Pagani *et al.* 1986; Fardet *et al.* 1998) to gain information about the size, shape and arrangement of particles within pasta; this can be further correlated with other pasta characteristics like texture, cooking behaviour and starch digestibility. The micro-structure of raw, cooked and *in vitro* digested (samples taken at 300 minutes *in vitro* digestion) pastas was determined using SEM[3]. Sample preparation and analysis are detailed in Chapter 3 (*3.2.2.5*).

4.2.2.7 Statistical analysis

Unless otherwise stated, all determinations were made in triplicate (samples taken from three independent production runs), and mean ± SD values are presented. Data was

statistically evaluated by ANOVA as detailed in Chapter 2 (*2.2.2.6*). Significance was defined as $P<0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of BBG Fibre Fraction and Glucagel™ on the Cooking Quality of Pasta

Cooking loss values theoretically reflect the quantity of starch and other bio-chemical components that are released from the pasta protein matrix and subsequently lost to the cooking medium (Cole 1991). Likewise, dry matter contents (also known as total organic matter) may be a reflection of the ability of pasta to retain organic matter during cooking. It is agreed by several authors that the formation of a continuous protein network is of great importance in the entrapment of starch and good cooking quality (Pagani *et al.* 1986), a subject reviewed extensively by Feillet (1988). The continuity and strength of the protein matrix is dependent upon inter and intra-molecular disulphide, hydrogen and hydrophobic bonds. During cooking this matrix gradually disintegrates. If the protein matrix is disrupted, the result is a more rapid disintegration during cooking. A weak or discontinuous protein matrix permits greater amounts of exudates to leach during starch granule gelatinisation; this is reflected in the amount of solids lost to the cooking water.

The cooking quality of pastas with BBG fibre fraction and Glucagel™ inclusions is illustrated in Table 4.3. The dry matter of cooked pastas with BBG fibre fraction level 1 and 2 inclusions was significantly lower than the control ($P<0.05$); however, there was no significant difference between the dry matter of pastas with BBG fibre fraction level 3 and 4 inclusions or any of the Glucagel™ containing pastas and the control ($P>0.05$). With the exception of pasta with Glucagel™ level 4 inclusion, there was no significant difference in the cooking losses encountered between the β-glucan fraction pastas and the control ($P>0.05$).

In the studies of Knuckles *et al.* (1997a) and Marconi *et al.* (2000), increased cooking losses were observed from pastas substituted with 20-40% (4.08 and 8.59% β-glucan dwb respectively) and 50% (4.3-5% β-glucan dwb) barley flour fractions respectively. In these studies, it is likely that the substitution of large amounts of durum wheat semolina with barley flour resulted in a considerable decrease in gluten content. Gluten enables the formation of a strong protein network capable of holding starch during cooking (Marconi and Carcea 2001). In this current study, only a small quantity (≤10%) of durum flour was replaced, thus, there was likely to still be a sufficient quantity of gluten to form a matrix that encompasses and retains starch granules. The increased cooking loss value exhibited by pasta with Glucagel™ level 4 inclusion may be attributed to the ability of Glucagel™ at higher levels of inclusion to form a discrete semi-solid network that disrupts the protein-starch matrix and facilitates leaching of organic matter from the pasta.

The swelling index (Table 4.3) of all BBG fibre fraction pastas was significantly higher than the control ($P<0.05$). The addition of Glucagel™ to pastas did not result in any significant change in swelling index compared to the control ($P>0.05$). There are few studies that have examined the effect of barley β-glucan inclusion, either in the form of high purity fractions or enriched flours, on the swelling properties of pasta. Tudorica *et al.* (2002c) observed increased swelling in pastas substituted with guar gum. The authors attributed increased swelling values to the high WRC of the fibre. The slightly higher swelling index values exhibited by the BBG fibre fraction containing pastas may be related to a greater WRC of the BBG fibre fraction compared to Glucagel™, possibly caused by the presence of co-extracted fibres (Chapter 3, Table 3.1).

Table 4.3 Cooking quality of control, BBG fibre fraction and Glucagel™ pastas[1]

Pasta	Dry matter (cooked) (g/100 g)	Cooking loss (g/100 g raw pasta)	Swelling index (g water/g dry pasta)
Control	$39.05^{a,b} \pm 0.21$	$3.76^{b} \pm 0.06$	$1.54^{b} \pm 0.04$
BBG level 1	$35.75^{d} \pm 0.49$	$4.21^{a,b} \pm 0.16$	$1.76^{a} \pm 0.06$
BBG level 2	$36.36^{c,d} \pm 1.03$	$4.07^{a,b} \pm 0.20$	$1.74^{a} \pm 0.06$
BBG level 3	$37.00^{b,c,d} \pm 0.05$	$4.03^{a,b} \pm 0.02$	$1.69^{a} \pm 0.02$
BBG level 4	$37.00^{b,c,d} \pm 0.38$	$4.11^{a,b} \pm 0.18$	$1.71^{a} \pm 0.03$
Glucagel™ level 1	$40.35^{a} \pm 0.57$	$3.77^{b} \pm 0.28$	$1.61^{a,b} \pm 0.00$
Glucagel™ level 2	$37.23^{b,c,d} \pm 1.10$	$3.83^{b} \pm 0.14$	$1.67^{a,b} \pm 0.06$
Glucagel™ level 3	$38.51^{a,b,c} \pm 0.18$	$4.02^{a,b} \pm 0.15$	$1.62^{a,b} \pm 0.04$
Glucagel™ level 4	$39.00^{a,b} \pm 0.84$	$4.32^{a} \pm 0.21$	$1.62^{a,b} \pm 0.11$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

4.3.2 Effect of BBG Fibre Fraction and Glucagel™ on the Textural Properties of Pasta

Table 4.4 illustrates that the incorporation of BBG fibre fraction in pasta caused a significant loss in hardness compared to the control ($P<0.05$). Glucagel™ inclusion resulted in a significant rise in pasta hardness, which increased with the level of fibre inclusion ($P<0.05$). The adhesiveness of all BBG fibre fraction pastas was not significantly different to the control ($P>0.05$) (Table 4.4). In pastas with Glucagel™ level 1 and 2 inclusions, adhesiveness was similar to the control ($P>0.05$); however, with level 3 and 4 inclusions pasta adhesiveness was significantly higher than the control ($P<0.05$).

Table 4.4 Textural attributes (hardness and adhesiveness) of control, BBG fibre fraction and Glucagel™ pastas[1]

Pasta	Hardness (g)	Adhesiveness (g s)
Control	$701.5^{c} \pm 6.40$	$-2.09^{c} \pm 0.20$
BBG level 1	$457.9^{d} \pm 6.90$	$-1.42^{c} \pm 0.39$
BBG level 2	$338.6^{e} \pm 4.80$	$-1.70^{c} \pm 0.10$
BBG level 3	$354.9^{e} \pm 4.70$	$-1.77^{c} \pm 0.11$
BBG level 4	$368.4^{d,e} \pm 35.10$	$-1.29^{c} \pm 0.21$
Glucagel™ level 1	$691.8^{c} \pm 69.20$	$-1.28^{c} \pm 0.40$
Glucagel™ level 2	$794.5^{b} \pm 25.60$	$-2.91^{c} \pm 1.50$
Glucagel™ level 3	$819.3^{b} \pm 21.10$	$-4.61^{b} \pm 0.21$
Glucagel™ level 4	$1136.9^{a} \pm 16.70$	$-13.28^{a} \pm 2.01$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

The loss of pasta hardness experienced with BBG fibre fraction inclusion may be attributed to the higher moisture contents of the pastas as a result of increased water absorption during cooking. In their studies with guar gum inclusion in pasta, Tudorica *et al.* (2002c) proposed that increased water absorption impacts upon the mechanical properties of pasta, with water acting as a plasticiser of composite materials and increasing flow dynamics of the system. Since pasta firmness can be related to the hydration of starch granules during the cooking process and the subsequent embedding of gelatinised starch granules in a matrix of partially denatured protein, it is also possible that the BBG fibre fraction may withhold water from starch and thereby alter pasta structure and firmness. A similar explanation was proposed in the study of Brennan *et al.* (2004) where loss of firmness was observed in inulin enriched pastas. The increase in pasta firmness encountered with Glucagel™ inclusion may be explained by the ability of Glucagel™ to form a semi-solid network, which contributes to the firmness of the already established protein-starch network. Izydorczyk *et al.* (2005) illustrated that the addition of hull-less barley flour fractions to noodles increased firmness.

The raised adhesiveness values exhibited by Glucagel™ containing pastas may be attributed to the ability of Glucagel™ (at high inclusion levels) to form a semi-solid network. The resultant structure may be discrete from the protein-starch matrix and result in organic matter leaching onto the surface of the pasta, thus, increasing adhesiveness.

4.3.3 Effect of BBG Fibre Fraction and Glucagel™ on the Gelatinisation Characteristics of Pasta

The effect of BBG fibre fraction and Glucagel™ addition on the starch gelatinisation characteristics of raw pasta are presented in Table 4.5. The T_{onset} of pastas with BBG fibre fraction level 1, 2 and 4 inclusions was not significantly different to the control ($P>0.05$);

however, a significant rise was observed in the T_{onset} of pasta with BBG fibre fraction level 3 inclusion ($P<0.05$). The T_{onset} of pastas with Glucagel™ inclusions was similar to the control ($P>0.05$). There was no significant difference between the T_{endset} or T_{peak} of the BBG fibre fraction, Glucagel™ and control pastas ($P>0.05$). There was a general decrease in the enthalpy of BBG fibre fraction pastas, although the difference from the control was only significant in pasta with BBG fibre fraction level 3 inclusion ($P<0.05$). Pastas with Glucagel™ exhibited a general increase in enthalpy, although the difference from the control was only significant in pasta with Glucagel™ level 4 inclusion ($P<0.05$).

Table 4.5 Starch gelatinisation characteristics of control, BBG fibre fraction and Glucagel™ pastas[1]

Pasta	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)	Enthalpy (J/g)
Control	$52.13^{b,c,d} \pm 0.81$	$70.40^{a} \pm 1.04$	$60.77^{a} \pm 0.67$	$4.46^{b,c} \pm 0.13$
BBG level 1	$52.83^{a,b,c,d} \pm 0.12$	$70.10^{a} \pm 0.27$	$60.83^{a} \pm 0.29$	$4.86^{a,b} \pm 0.15$
BBG level 2	$53.10^{a,b,c} \pm 0.30$	$70.03^{a} \pm 0.71$	$60.67^{a} \pm 0.23$	$4.09^{c,d} \pm 0.21$
BBG level 3	$53.67^{a} \pm 0.62$	$70.23^{a} \pm 0.59$	$61.00^{a} \pm 0.61$	$3.89^{d} \pm 0.24$
BBG level 4	$53.53^{a,b} \pm 0.35$	$71.17^{a} \pm 0.29$	$61.47^{a} \pm 0.38$	$4.37^{c,d} \pm 0.20$
Glucagel™ level 1	$52.33^{a,b,c,d} \pm 0.61$	$70.00^{a} \pm 0.72$	$60.67^{a} \pm 0.06$	$4.51^{b,c} \pm 0.37$
Glucagel™ level 2	$51.63^{c,d} \pm 0.67$	$69.83^{a} \pm 0.23$	$60.87^{a} \pm 0.06$	$4.83^{a,b} \pm 0.21$
Glucagel™ level 3	$51.50^{d} \pm 0.46$	$70.53^{a} \pm 0.40$	$61.00^{a} \pm 0.00$	$5.00^{a,b} \pm 0.14$
Glucagel™ level 4	$52.13^{b,c,d} \pm 0.42$	$71.23^{a} \pm 1.02$	$61.29^{a} \pm 0.15$	$5.15^{a} \pm 0.20$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

Elevated starch gelatinisation onset temperatures in the presence of β-glucan have been reported by Kim and Setser (1992) who proposed that amongst other mechanisms, the anti-plasticisation properties of β-glucan are an important factor in altering starch gelatinisation. Water is well established as a plasticiser of the amorphous regions of starch granules and in addition promotes rupture of hydrogen bonds and formation of new hydrogen bonds between itself and the dissociated starch chains (Lelievre 1976; Slade and Levine 1984, 1987, 1988). When non-starch granules are present, they have the capacity to hydrate and consequently restrict the mobility of the plasticiser and hence, delay the initiation of the gelatinisation process. More recently, Tester and Sommerville (2003) illustrated that certain polysaccharides restrict the swelling of starch granules and consequently restrict starch gelatinisation through immobilisation of water, which results in an increase in gelatinisation temperature and a decrease in enthalpy. The results from this current study, although not conclusive, do indicate that BBG fibre fraction alters starch gelatinisation through limiting the amount of available water. The higher enthalpies illustrated by the Glucagel™ level 3 and 4 pastas in comparison to the lower enthalpies exhibited by BBG fibre fraction counterparts may be related to differences in the integration of the β-glucan fractions within the protein-starch matrix. It is possible that the inclusion of Glucagel™ in pasta results in a disruption of the pasta matrix and hence increased availability of starch granules for gelatinisation.

4.3.4 Effect of BBG Fibre Fraction and Glucagel™ on the *In Vitro* Starch Digestibility of Pasta

The low glycaemic response of traditional pasta is attributed to a well-formed protein-starch matrix with strong and continuous protein strands entrapping large starch granules. The entrapment of starch reduces accessibility to enzymic degradation and hence, reduces sugar liberation. Table 4.6 illustrates the effect of BBG fibre fraction and Glucagel™ on

the *in vitro* starch digestibility (as measured by RSR) of pasta. A graphic representation of the *in vitro* starch digestibility of control and BBG fibre fraction and Glucagel™ (level 3) pastas is given in Appendix X to allow a visual appreciation of the differences in the rate of RSR. There was a generally consistent significant decrease in RSR from pastas with BBG fibre fraction level 2 and 3 inclusions between 180-300 minutes *in vitro* digestion ($P<0.05$), and in pasta with BBG fibre fraction level 4 inclusion, a significant decrease in RSR was observed between 210-300 minutes *in vitro* digestion ($P<0.05$). The inclusion of Glucagel™ in pasta did not significantly reduce RSR compared to the control. Generally (although not consistently significant), the release of RSR from the Glucagel™ pastas was higher than that of the control.

Several theories exist to explain the effect of soluble polysaccharides on the starch digestibility of pasta products. Work conducted by Tudorica *et al.* (2002c) on guar gum enriched pasta, using a *in vitro* digestion model, revealed that reduced rates of starch digestion were a result of the formation of a guar gum barrier around starch granules, which protected them from enzymatic degradation (in this study it is not possible to detect from the micrographs whether the β-glucan is in intimate contact with the starch granules). Other authors have proposed that reductions in water available for starch granule hydration limits the degree of gelatinisation and hence, susceptibility to hydrolysis by α-amylase (Jankiewicz and Michniewicz 1987; Holm *et al.* 1988; Tester and Sommerville 2003). Giorato *et al.* (1986) and Leclere *et al.* (1994) have proposed that the lowered glycaemic response of soluble fibre enriched pastas is as a consequence of increased viscosity of the intestinal contents, which results in delayed gastric emptying and slower absorption at the intestinal surface rather than changes in the rate of starch digestibility.

Table 4.6 *In vitro* starch digestibility (RSR, expressed in maltose equivalents as a percentage of total available carbohydrate) of control, BBG fibre fraction and Glucagel™ pastas[1]

Pasta	30	60	90	120	150	180	210	240	270	300
Control	$0.0^{a}\pm0$	$1.61^{b}\pm0.52$	$2.43^{b}\pm0.18$	$4.16^{c,d,e}\pm0.05$	$7.02^{c}\pm0.32$	$10.80^{b,c}\pm0.42$	$13.42^{b,c}\pm0.48$	$17.42^{b}\pm0.56$	$19.91^{b}\pm0.09$	$24.17^{b}\pm0.60$
BBG 1	$0.00^{a}\pm0$	$0.94^{b,c}\pm0.19$	$3.06^{a,b}\pm0.53$	$3.67^{c,d,e}\pm0.12$	$7.42^{b,c}\pm0.50$	$11.60^{a,b}\pm0.15$	$14.01^{b}\pm0.74$	$17.49^{b}\pm0.49$	$20.25^{b}\pm0.31$	$22.14^{c}\pm0.12$
BBG 2	$0.00^{a}\pm0$	$0.50^{c}\pm0.21$	$2.05^{b}\pm0.36$	$3.44^{d,e}\pm0.15$	$6.42^{c}\pm0.24$	$8.73^{d,e}\pm0.70$	$11.90^{c,d}\pm0.76$	$14.38^{c}\pm0.35$	$17.21^{c}\pm0.51$	$20.10^{d}\pm0.51$
BBG 3	$0.00^{a}\pm0$	$0.12^{c}\pm0.13$	$2.18^{b}\pm0.74$	$2.78^{e}\pm0.19$	$6.17^{c}\pm0.55$	$8.37^{e}\pm0.50$	$10.70^{d}\pm0.45$	$13.46^{c}\pm0.18$	$16.73^{c}\pm0.06$	$17.71^{e}\pm0.10$
BBG 4	$0.00^{a}\pm0$	$0.78^{b,c}\pm0.33$	$2.37^{b}\pm0.43$	$4.51^{c,d}\pm0.65$	$7.00^{c}\pm0.63$	$9.32^{c,d,e}\pm0.38$	$11.80^{d}\pm0.64$	$14.47^{c}\pm0.28$	$16.58^{c}\pm0.49$	$18.62^{e}\pm0.13$
GLU 1	$0.00^{a}\pm0$	$2.94^{a}\pm0.44$	$3.61^{a,b}\pm0.97$	$4.96^{b,c}\pm0.40$	$9.07^{a,b}\pm0.64$	$11.32^{b}\pm0.87$	$14.03^{b}\pm0.49$	$19.11^{a}\pm0.49$	$20.99^{a,b}\pm0.49$	$25.80^{a}\pm0.47$
GLU 2	$0.00^{a}\pm0$	$1.02^{b,c}\pm0.38$	$4.09^{a}\pm0.75$	$6.06^{a,b}\pm1.09$	$9.13^{a}\pm0.55$	$10.13^{b,c,d}\pm0.52$	$14.36^{a,b}\pm0.2$	$18.47^{a,b}\pm0.28$	$19.96^{b}\pm0.62$	$20.89^{c,d}\pm0.27$
GLU 3	$0.00^{a}\pm0$	$0.40^{c}\pm0.44$	$3.38^{a,b}\pm0.43$	$5.82^{a,b}\pm0.55$	$9.94^{a}\pm0.91$	$13.20^{a}\pm1.04$	$14.56^{a,b}\pm0.41$	$18.80^{a,b}\pm0.67$	$22.18^{a}\pm0.45$	$24.52^{a,b}\pm0.75$
GLU 4	$0.00^{a}\pm0$	$2.63^{a,b}\pm0.24$	$4.07^{a}\pm0.40$	$6.87^{a}\pm0.30$	$9.52^{a}\pm0.65$	$10.89^{b,c}\pm0.51$	$15.74^{a}\pm0.46$	$18.69^{a,b}\pm0.84$	$20.24^{b}\pm0.70$	$23.96^{b}\pm0.81$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

The mechanism(s) behind the ability of the BBG fibre fraction to reduce starch digestibility in pasta is unclear, although results do indicate that the restriction of starch gelatinisation (as observed by DSC[2]) and slight modifications to pasta structure (as observed by SEM[3]) may be involved. The delay in consistent attenuation of RSR in pastas with level 2, 3 and 4 BBG fibre fraction inclusions may be attributed to a slow and/or uneven hydration of the polysaccharide matrix, which delays/hinders integration of the β-glucan within the protein-starch matrix until the later stages of digestion. In a similar study, Tudorica *et al.* (2002c) observed a delay in the attenuation of glucose release from guar gum containing pasta until the latter stages of *in vitro* digestion. In addition to reductions in starch granule availability, it is also plausible that as time elapses and as the BBG fibre fraction is thoroughly hydrated there is a cumulative leaching of β-glucan into the digesta, which increases viscosity and hinders the diffusion of reducing sugars to the dialysate. Further studies to characterise the viscous influence of the fibres on *in vitro* digesta may be warranted; however, as discussed in Chapter 3, such data should be viewed conservatively since correlation with the *in vivo* situation is poor.

The inability of Glucagel™ to attenuate the *in vitro* starch digestibility of pasta in comparison to BBG fibre fraction may be because of poor solubility and/or slow/partial hydration of the preparation. It is also possible that Glucagel™ does not incorporate thoroughly and forms a discrete polysaccharide network within the pasta matrix, offering no protection to the starch granules. This discrete polysaccharide network may also disrupt the protein-starch matrix of the pasta, resulting in starch granules more readily exposed for amylolytic attack, thus, explaining the raised RSR from the Glucagel™ pastas. Tudorica *et al.* (2002c) observed increased *in vitro* glucose release from pea and inulin fibre containing pastas compared to a control and attributed the increase to disruption and weakening of the protein-starch network in the overall pasta structure.

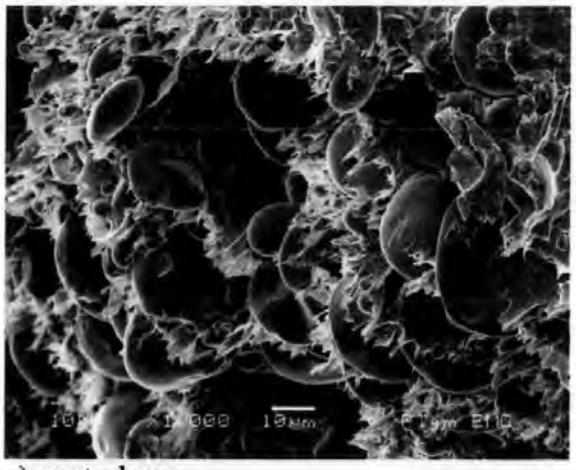
Authors investigating the effect of β-glucan addition on the glycaemic response from pasta have reported variable outcomes. Yokoyama *et al.* (1997) observed that pastas produced from durum wheat partially substituted with barley β-glucan enriched flour (17.5 g/100 g TDF (of which 7.7 g was β-glucan)) significantly lowered glycaemic and insulin responses of healthy subjects. Conversely, when Holm *et al.* (1992) incorporated oat bran concentrate enriched with β-glucan into fettuccini in partial replacement (29%) of durum wheat, despite a raise in TDF from 3-12 g/100 g (of which 5.2 g was β-glucan), blood glucose and insulin responses in healthy volunteers were only marginally reduced compared with a reference fettuccini. Similarly, Bourdon *et al.* (1999) observed that barley β-glucan enriched spaghetti (15.7 g TDF/100 g pasta (of which 5.2 g β-glucan)), manufactured by substituting 40% standard durum flour with a barley β-glucan rich flour, did not significantly lower the post-prandial blood glucose responses of healthy men compared to a control durum pasta. Both authors attributed the lack of any significant reductions in glycaemic response to a reduction in pasta gluten contents and weakening of the protein matrix.

4.3.5 Effect of BBG Fibre Fraction and Glucagel™ on the Micro-Structure of Pasta

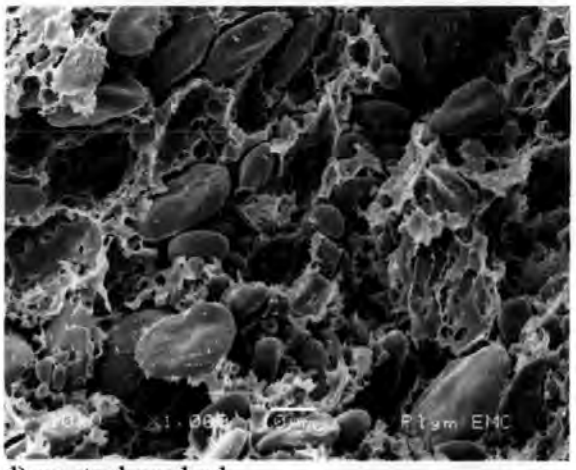
Figure 4.1 illustrates SEMs[1] of raw, cooked and *in vitro* digested (300 minute) control, BBG fibre fraction and Glucagel™ (level 3 inclusion) pastas. Additional SEMs[1] of all raw, cooked and *in vitro* digested BBG fibre fraction and Glucagel™ pastas are contained in Appendix XI. The raw control pasta (Figure 4.1a) has a well-formed starch protein matrix with an abundance of protein strands entrapping large starch granules. With the inclusion of BBG fibre fraction and Glucagel™, there appears to be a difference in protein-starch binding patterns, both pastas exhibiting a slight loss of profuse protein network (Figures 4.1b and 4.1c respectively).

The cooked control pasta (Figure 4.1d) has a developed and coagulated protein network, which entraps large swollen starch granules. The cooked BBG fibre fraction pasta (Figure 4.1e) exhibits a slight loss of protein network, a more compact structure and a greater quantity of less swollen starch granules than the control pasta. The cooked Glucagel™ (Figure 4.1f) pasta exhibits a distinct loss of protein network and increased starch granule exposure. The *in vitro* digested control and Glucagel™ pastas (Figures 4.1g and 4.1i respectively) are similar in that they have webbed structures. The *in vitro* digested BBG fibre fraction pasta (Figure 4.1h) also has a webbed structure but appears more compact in comparison to the *in vitro* digested control and Glucagel™ pastas.

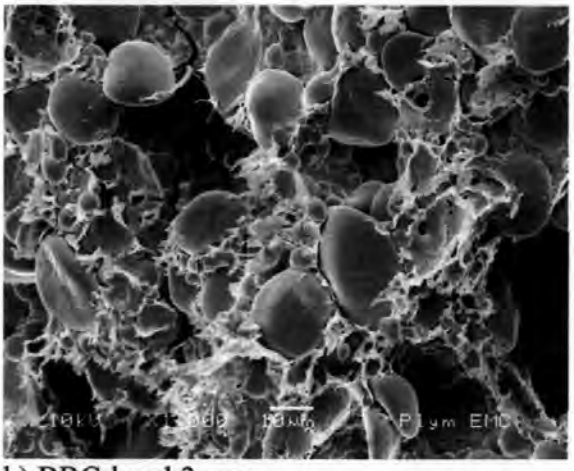
The micrographs highlight the importance of internal structure on the cooking, textural and *in vitro* starch digestibility of pasta. The decreased firmness values exhibited by the BBG fibre fraction pastas may be in part attributed to the slight loss of protein network and reduced starch granule swelling exhibited by the pasta (Figure 4.1e). The increased adhesiveness and slightly higher enthalpy values exhibited by the Glucagel™ pastas (level 3 and 4 inclusions) may be explained by the loss of profuse protein network and increased starch granule exposure (Figure 4.1f). Marconi and Carcea (2001) provide a thorough discussion of the technologies, formulations and added ingredients available for counteracting negative changes in the rheological properties of pasta caused by the incorporation of non-durum materials. Marconi *et al.* (2000) illustrated that barley β-glucan rich pastas required gluten to overcome changes in the rheology of the pasta systems on fibre addition. High temperature drying has also been illustrated to be a key factor in obtaining good quality β-glucan rich pasta. High temperature drying treatments promote the formation of a diffused and coagulated protein network, which contributes to the firmness of pasta (Resmini and Pagani 1983; Cubadda and Acquistucci 1987).



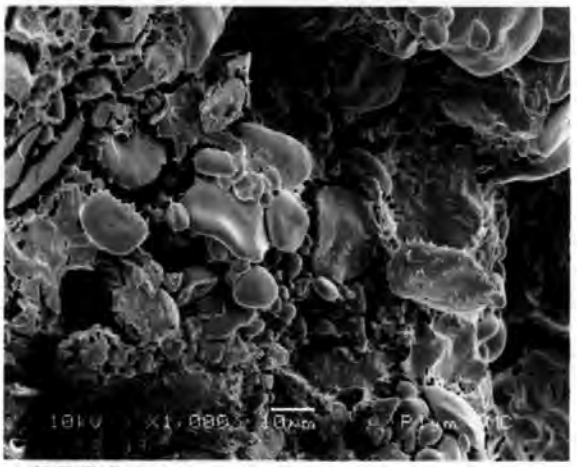
a) control raw



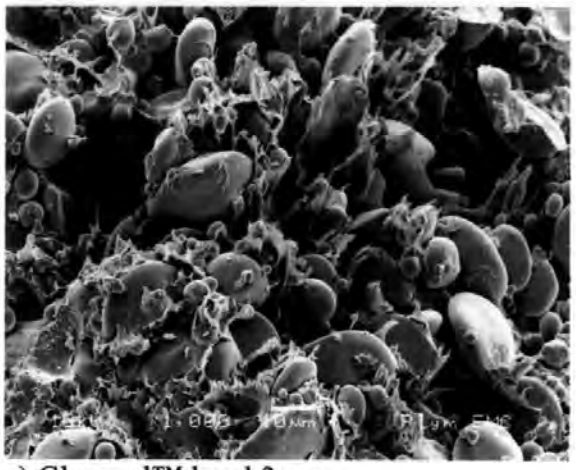
d) control cooked



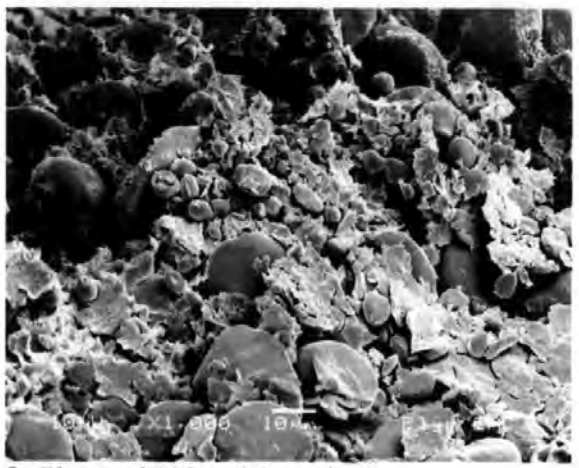
b) BBG level 3 raw



e) BBG level 3 cooked

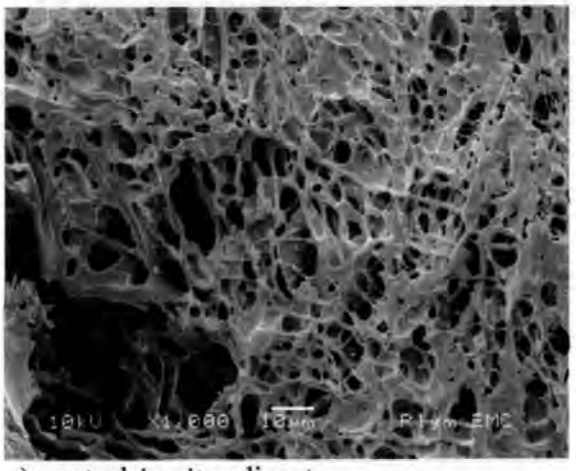


c) Glucagel™ level 3 raw

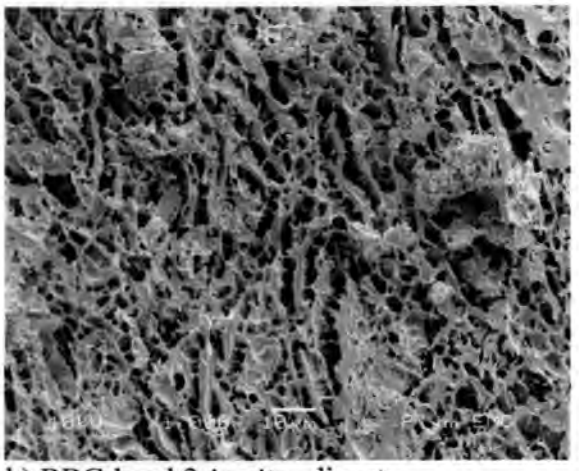


f) Glucagel™ level 3 cooked

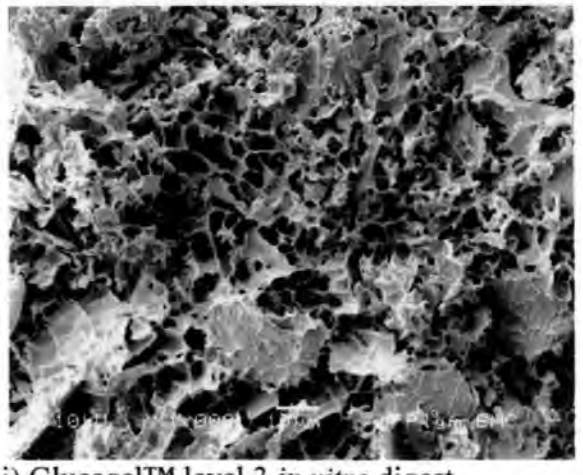
Figure 4.1 SEMs[1] of raw, cooked and *in vitro* digested (300 minutes) pastas (x 1000): (a) control raw; (b) BBG level 3 raw; (c) Glucagel™ level 3 raw; (d) control cooked; (e) BBG level 3 cooked; (f) Glucagel™ level 3 cooked; (g) control *in vitro* digest; (h) BBG level 3 *in vitro* digest; and (i) Glucagel™ level 3 *in vitro* digest.



g) control *in vitro* digest



h) BBG level 3 *in vitro* digest



i) Glucagel™ level 3 *in vitro* digest

Figure 4.1 SEMs[1] continued.

4.4 CONCLUSIONS

This study illustrates that the addition of barley β-glucan fractions to pasta significantly improves nutritional quality by increasing dietary fibre contents and by reducing *in vitro* starch digestibility, although the latter is dependent upon the type of barley β-glucan fraction added. This observation may be an indication of the potential of certain barley β-glucan fractions to reduce *in vivo* sugar release from pasta, an already low glycaemic food.

Changes to the cooking and textural qualities of the pasta were also observed with barley β-glucan fraction addition, the nature and magnitude of change varying with fraction type.

It is anticipated that these changes are in part a result of reduced gluten contents, disruption of the protein-starch matrix and the physico-chemical properties of the barley β-glucan (i.e. high WRC). Other researchers have shown that some of the negative changes encountered on non-durum material addition to pasta can be negated by changing formulations, for example the addition of gluten or gluten like whey proteins and/or by the use of suitable processing technologies, for example high temperature drying. Such treatments may enable the production of barley β-glucan rich pastas that are capable of retaining structure and shape during cooking, have an acceptable degree of stickiness and have satisfactory sensory properties when eaten; however, any treatment or combination of treatments employed must be investigated to ensure that the physico-chemical and physiological properties of barley β-glucan are not compromised.

CHAPTER 5

THE EFFECT OF HIGH AND LOW MOLECULAR WEIGHT BARLEY β-GLUCAN FRACTIONS ON THE PHYSICO-CHEMICAL AND *IN VITRO* STARCH DIGESTIBILITY OF BREAD AND PASTA

5.1 INTRODUCTION

Although barley β-glucan enriched cereal foods have been reported to have beneficial nutritional properties (hypoglycaemic and hypoinsulinaemic capacities), incorporation of β-glucan in a cereal system, in the form of native grain or fraction, can often result in undesirable changes to product quality (as observed from the work conducted in Chapter 3 and 4 of this study). For example, in bread this may be increased water absorption and mixing time, reductions in loaf height and volume and changes to crumb structure (Knuckles *et al.* 1997a; Cavallero *et al.* 2002; Gill *et al.* 2002). In pasta, increased cooking loss and loss of firmness have been observed with barley β-glucan inclusions (Marconi *et al.* 2000). Ultimately, these negative changes may result in reduced consumer acceptance of barley β-glucan enriched cereal products. It is likely that these changes are in part related to MW, viscosity and water retaining capacities of the β-glucans, although few studies have confirmed this.

Incorporating reduced or lower MW barley β-glucan preparations may limit negative changes to cereal food quality; however, the MW of β-glucan has an important influence upon hypoglycaemic and hypoinsulinaemic capacity. Mathematical correlations of blood glucose level to MW of β-glucan have been illustrated by Wood *et al.* (1994a, 2000) who demonstrated an inverse linear relationship between log (viscosity) of oat β-glucan in a drink model (varying MW/dose) and the magnitude of 50 g oral load. Similar studies with

solid foods, such as bread and pasta where the interaction of β-glucan with other macro-components (protein and starch) and rate of digestibility are of importance, are limited. In the studies of Ellis *et al.* (1991), consumption of breads with guar gum of both HMW and LMW resulted in a significant decrease in post-prandial plasma insulin response compared to a control bread. There was no significant difference observed between the two types of guar gum. In addition, the sensory qualities of guar gum bread were significantly improved by the use of LMW guar gum.

The MW of β-glucan may be furthered lowered by the conditions of food processing, such as bread making (Andersson *et al.* 2004), which may possibly result in loss of physiological activity. Frank *et al.* (2004) illustrated that oat breads containing HMW or LMW β-glucan did not differ in their effects on blood concentrations of lipids, insulin or glucose in humans. The conditions of the gastrointestinal tract have also been reported to favour the degradation of β-glucan and result in loss of viscous effect (Johansen *et al.* 1997). Such loss of MW has been reported to be particularly marked in HMW materials (Bedford *et al.* 1991).

5.1.1 Rationale and Aim

Whilst studies have been conducted to evaluate the effects of barley β-glucan on the physico-chemical and nutritional properties of cereal products, few studies have addressed the impact of differing MW. This study is an extension to Chapter 3 and 4 where the effects of incorporating relatively LMW barley β-glucan fractions from different extraction procedures in bread and pasta were investigated. Thus, the overall aim of this study was to explore and compare the behaviour of HMW and LMW barley β-glucan fractions in bread and pasta by examining physico-chemical changes in the products and effects on *in vitro*

starch digestibility. The susceptibility of these two barley β-glucan fractions to degradation during bread baking and *in vitro* digestion was also investigated.

5.1.1.1 Objectives

- Incorporate HMW and LMW barley β-glucan fractions into bread and pasta and compare effects on the physico-chemical properties of bread dough (resistance to extension and extensibility), baked bread (height, volume, firmness and crust and crumb colour) and the cooking quality (dry matter, cooking loss and swelling index) and textural attributes (hardness and adhesiveness) of cooked pasta.

- Determine and compare the influence of HMW and LMW barley β-glucan fractions on the digestibility of starch in bread and pasta using a multi-enzymic *in vitro* digestion method.

- Examine and compare the influence of HMW and LMW barley β-glucan fractions on the micro-structure of baked and *in vitro* digested bread and raw, cooked and *in vitro* digested pastas.

- Profile and compare the MW of barley β-glucan fractions during bread manufacture and *in vitro* digestion.

5.2 MATERIALS AND METHODS

5.2.1 Materials

5.2.1.1 HMW and LMW barley β-glucan fractions

HMW and LMW barley β-glucan fractions were purchased from Megazyme™ International Ireland Ltd (Wicklow, Ireland). Composition, MW and viscosity (as specified by Megazyme™) are detailed in Table 5.1.

Table 5.1 Composition and physical properties of HMW and LMW barley β-glucan fractions

	HMW	LMW
% Component (dwb)		
β-glucan	~95	~95
Starch	0.21	<0.12
Protein	1.5	<0.1
Moisture	3.6	2.0
Physical properties		
MW (Daltons)	510,000	160,000
Viscosity (cSt)	>80	10

5.2.1.2 Bread and pasta making materials

Bread and pasta making materials are as detailed in Chapter 3 (*3.2.1.3*) and Chapter 4 (*4.2.1.3*).

5.2.1.3 Reagents

Unless otherwise stated, all general laboratory reagents were purchased from Fisher Scientific (UK) or Sigma Aldrich (UK/Sweden).

5.2.2 Methods

5.2.2.1 Bread making

Breads were manufactured with bread wheat flour substituted with either 5% HMW or LMW barley β-glucan fraction as detailed in Chapter 3 (*3.2.2.1*). Bread with no β-glucan was prepared as the control. Formulations for the breads are contained in Table 5.2. Proximate composition of the breads was determined using the methods detailed in Chapter 2 (*2.2.2.2*), with the exception of available starch, which was determined as detailed in Chapter 3 (*3.2.2.1*). Table 5.3 presents the proximate composition of the breads.

Table 5.2 Control, HMW and LMW barley β-glucan fraction bread formulations

Bread	White bread flour (g)	β-glucan (g)	Distilled water (ml)
Control	125.00	0.00	70.00
HMW	118.75	6.25	75.00
LMW	118.75	6.25	75.00

*yeast (6g), salt (6g), sugar (1g) and vegetable fat (6.25g) were constant for all breads.

Table 5.3 Proximate composition of control, HMW and LMW barley β-glucan fraction breads[1]

Bread	Available starch (%)	Protein (%)	TDF (%)	β-glucan (%)
Control	69.52 ± 0.25	16.09 ± 0.02	5.88 ± 0.78	0.14 ± 0.00
HMW	64.82 ± 1.31	15.09 ± 0.06	10.56 ± 0.51	4.30 ± 0.10
LMW	64.55 ± 0.26	15.25 ± 0.01	11.40 ± 0.29	4.47 ± 0.04

[1]Results are mean ± SD of duplicate determinations of a composite sample reported on a dwb.

5.2.2.2 Bread dough rheology

Resistance to extension (mean maximum force g) and extensibility (mean distance at maximum force mm) of the bread doughs were determined as detailed in Chapter 3 (*3.2.2.2*).

5.2.2.3 External, internal and texture quality evaluation of bread

Loaf height, volume, crumb texture (firmness) and crust and crumb colour were determined as detailed in Chapter 3 (*3.2.2.3*).

5.2.2.4 Pasta manufacture

Pasta was manufactured with durum wheat semolina substituted with 5% HMW or LMW barley β-glucan fraction as detailed in Chapter 4 (*4.2.2.1*). Pasta with no β-glucan was prepared as the control. Formulations are contained in Table 5.4. Proximate composition of cooked pastas was determined as detailed in Chapter 2 (*2.2.2.2*), with the exception of available starch, which was determined as detailed in Chapter 3 (*3.2.2.1*). Table 5.5 presents the proximate composition of the pastas.

Table 5.4. Control, HMW and LMW barley β-glucan fraction pasta formulations

Pasta	Durum wheat flour (g)	β-glucan (g)	Distilled water (ml)
Control	100.0	0.0	40
HMW	95.0	5.0	50
LMW	95.0	5.0	50

Table 5.5 Proximate composition of control, HMW and LMW barley β-glucan fraction cooked pastas[1]

Pasta	Available starch (%)	Protein (%)	TDF (%)	β-glucan (%)
Control	77.36 ± 0.11	15.20 ± 0.02	5.07 ± 0.80	0.42 ± 0.00
HMW	73.61 ± 0.91	14.56 ± 0.09	10.14 ± 0.21	4.65 ± 0.01
LMW	73.01 ± 0.82	14.54 ± 0.07	9.65 ± 0.23	4.87 ± 0.05

[1]Results are mean ± SD of duplicate determinations of a composite sample reported on a dwb.

5.2.2.5 Cooking quality and textural attributes of pasta

Cooking time and quality and textural attributes of the pastas were determined according to the procedures detailed in Chapter 4 (*4.2.2.2* and *4.2.2.3* respectively).

5.2.2.6 Starch gelatinisation characteristics of pasta

DSC[2] was used to measure the gelatinisation characteristics of raw pasta samples substituted with HMW and LMW barley β-glucan fractions, to ascertain the influence of β–glucan on the starch fraction. Pasta with no β-glucan addition was used as a control. Prior to analysis, pasta samples were freeze dried and milled to pass a 500 μm mesh screen. Sample preparation and DSC[1] parameters are as described in Chapter 2 (*2.2.2.5*).

5.2.2.7 In vitro digestion of bread and pasta

Bread and cooked pasta samples were subjected to an *in vitro* digestion based upon the method of Brighenti *et al.* (1995), slightly modified. The method is described in Chapter 3 (*3.2.2.4*)

5.2.2.8 Micro-structure of bread and pasta

The micro-structure of baked and *in vitro* digested (samples taken at 300 minutes *in vitro* digestion) bread and raw, cooked and *in vitro* digested (samples taken at 300 minutes *in vitro* digestion) pasta was determined as detailed in Chapter 3 (*3.2.2.5*).

5.2.2.9 Extraction and analysis of β-glucan M_{cf} and MW distribution

The M_{cf} and MW distribution of β-glucan within bread wheat flour, HMW and LMW barley β-glucan fractions, and baked and *in vitro* digested (samples taken at 30, 150 and 300 minutes *in vitro* digestion) control, HMW and LMW barley β-glucan fraction breads were determined using the procedure detailed in Chapter 3 (*3.2.2.6*).

5.2.2.10 Statistical analysis

Unless otherwise stated, all determinations were made in triplicate (samples taken from three independent production runs), and mean ± SD values are presented. Data was statistically evaluated by ANOVA as detailed in Chapter 2 (*2.2.2.6*). Significance was defined as $P<0.05$.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of HMW and LMW Barley β-Glucan Fractions on the Rheological Properties of Bread Dough

Table 5.6 illustrates the effect of HMW and LMW barley β-glucan fraction inclusions on the rheological properties of bread dough. The resistance to extension of dough containing both types of barley β-glucan fraction was significantly higher than the control dough ($P<0.05$). The extensibility of doughs containing the HMW and LMW weight barley β-glucan fractions was also significantly reduced compared to the control dough ($P<0.05$). In particular, the dough with HMW barley β-glucan fraction inclusion yielded the greatest

resistance to extension and also the lowest extensibility of the samples ($P<0.05$). These results illustrate that barley β-glucan fractions of both high and low MW significantly alter the rheology of bread dough. The production of doughs with greater resistance to extension may be attributed to the viscous influence of the β-glucan. A proportion of dough viscosity can be attributed to the concentration of macro-molecules in the aqueous phase, which in turn is strongly dependent upon MW (Stauffer 1998). Thus, the combined MW of gluten and β-glucan may be higher than that of gluten alone, which results in a greater viscous effect within the dough matrix; this might explain the highest resistance to extension exhibited by the HMW barley β-glucan fraction containing dough. Loss of dough extensibility may be caused by the excessive retention of water by the β-glucans, which in turn leads to an impaired gluten network. If this is the mechanism, it suggests that the HMW barley β-glucan fraction has the greatest WRC and results in the greatest loss in dough extensibility. In a similar study, Courtin and Delcour (1998) observed that wheat doughs prepared with HMW arabinoxylan had a greater water absorption than wheat doughs prepared with LMW arabinoxylan.

Table 5.6 Control, HMW and LMW barley β-glucan fraction dough rheology and baked bread evaluation[1]

Dough/Bread	Extension (g)	Distance (mm)	Height (cm)	Volume (ml)	Firmness (g)
Control	$33.34^{c} \pm 0.65$	$-29.83^{a} \pm 0.47$	$6.18^{a} \pm 0.08$	$212^{a} \pm 2.0$	$5.40^{b} \pm 0.02$
HMW	$74.28^{a} \pm 1.03$	$-22.30^{c} \pm 0.69$	$3.65^{c} \pm 0.11$	$100^{c} \pm 0.0$	$5.44^{a,b} \pm 0.21$
LMW	$49.18^{b} \pm 2.98$	$-23.75^{b} \pm 0.32$	$4.03^{b} \pm 0.13$	$118^{b} \pm 2.89$	$6.07^{a} \pm 0.40$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs). [a]means values in the same column followed by the same letter are not significantly different ($P>0.05$).

5.3.2 Effect of HMW and LMW Barley β-Glucan Fractions on Baked Bread Quality

The inclusion of HMW and LMW barley β-glucan fractions in bread resulted in a significant decrease in loaf volume and height (Table 5.6). The reduction in height and volume was greatest in the HMW barley β-glucan fraction bread ($P<0.05$). In comparison to the control sample, breads containing HMW and LMW barley β-glucan fractions exhibited higher values in compression force measurements; however, the difference was only significant between the control and LMW barley β-glucan fraction bread ($P<0.05$).

As reported by a number of authors, incorporation of barley β-glucan in wheat bread results in loss of height, volume and increased firmness (Knuckles *et al.* 1997a; Cavallero *et al.* 2002; Gill *et al.* 2002). As discussed in Chapter 3 (3.3.2), loss of height and volume may be a result of disruption to the starch-gluten matrix and distortion of the gas cell structure, excessive retention of water by β-glucan, which leads to an underdeveloped gluten network and/or a reduction in steam production. These results indicate that the HMW barley β-glucan fraction may have the greatest starch-gluten disrupting and/or WRC, which results in the greatest loss of bread quality. The higher compression value exhibited by the LMW barley β-glucan fraction bread is difficult to explain but may be related to the differing viscoelastic characteristics of the β-glucan fractions (Vaikousi *et al.* 2004).

Figures 5.1a and 5.1b illustrate the effect of HMW and LMW barley β-glucan fractions on the crust and crumb colour of bread respectively. The L* crust colour values of HMW and LMW barley β-glucan fraction breads were significantly lower than the control (darker) ($P<0.05$). The a* crust colour values of the HMW and LMW barley β-glucan fraction breads were significantly higher than the control (more red) ($P<0.05$); however, the b* crust colour values of the breads were similar to the control ($P>0.05$).

The L* and b* crumb colour values of the HMW and LMW barley β-glucan fraction breads were similar to the control; however, a* crumb colour values of the barley β-glucan fraction breads were significantly higher than the control (more red) ($P<0.05$). There was no significant difference between the L*a*b* crust and colour values of the HMW and LMW barley β-glucan fraction breads ($P>0.05$).

a) b)

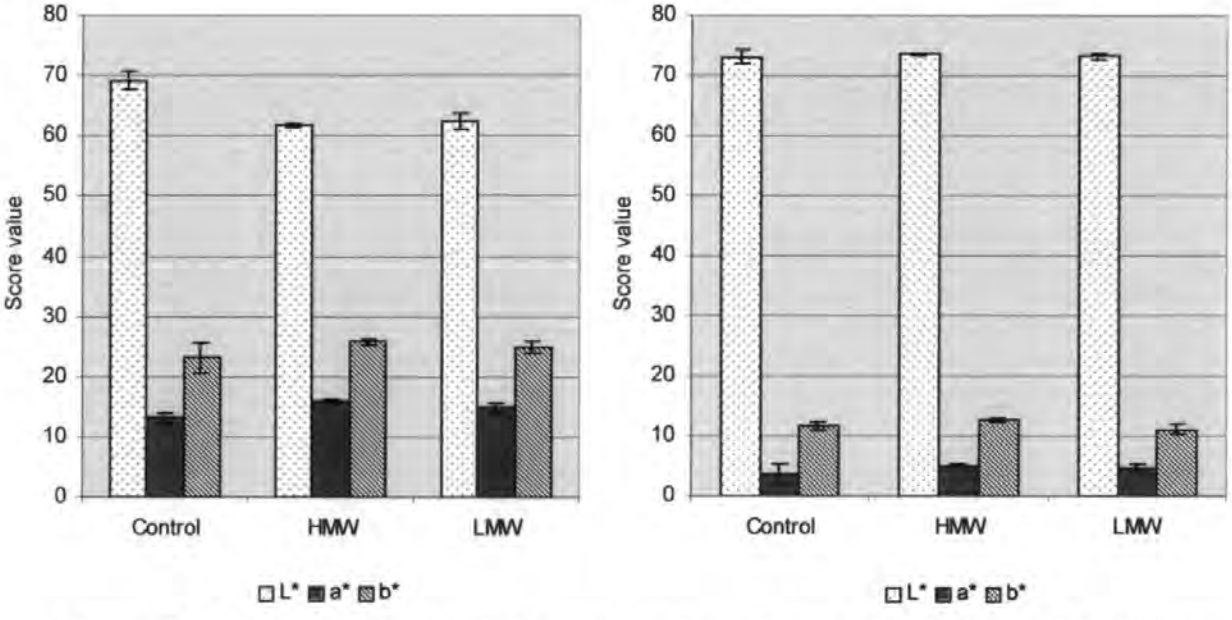


Figure 5.1 (a) Crust colour and (b) crumb colour of control, HMW and LMW barley glucan fraction breads. Results are mean of triplicate determinations (samples taken from three independent production runs). SD is presented as error bars.

5.3.3 Effect of HMW and LMW Barley β-Glucan Fractions on the Cooking Quality and Textural Attributes of Pasta

Table 5.7 illustrates the cooking qualities of control, HMW and LMW barley β-glucan fraction pastas. The dry matter contents of the cooked control and HMW barley β-glucan fraction pastas was similar ($P>0.05$); however, a significantly lower dry matter contents was observed in the LMW barley β-glucan fraction pasta ($P<0.05$). Despite a small rise in

the cooking loss from the LMW barley β-glucan fraction pasta, the value was similar to the control ($P>0.05$). A significantly lower cooking loss was observed from the HMW barley β-glucan fraction pasta compared to the control and LMW barley β-glucan fraction pastas ($P<0.05$). Swelling index of the control and HMW barley β-glucan fraction pastas was similar ($P>0.05$); however, a significantly increased swelling index was observed with the LMW barley β-glucan fraction pasta ($P<0.05$).

Table 5.7 Cooking quality of control, HMW and LMW barley β-glucan fraction pastas[1]

Pasta	Dry matter (g/100 g)	Cooking loss (g/100 g raw pasta)	Swelling index (g water/g dry pasta)
Control	$39.05^{a} \pm 0.21$	$3.76^{a} \pm 0.06$	$1.54^{b} \pm 0.04$
HMW	$38.88^{a} \pm 0.01$	$3.28^{b} \pm 0.13$	$1.59^{b} \pm 0.03$
LMW	$37.17^{b} \pm 0.47$	$3.96^{a} \pm 0.27$	$1.73^{a} \pm 0.08$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

These results indicate that pasta made with LMW barley β-glucan fraction has reduced cooking tolerance and swells excessively during cooking in comparison to the control and HMW barley β-glucan fraction pastas. It is plausible that the LMW barley β-glucan fraction increases starch granule availability and swelling due to the formation of a weak polysaccharide gel/network, which disrupts the protein network and entrapment of starch. Simultaneously, this disrupted network may permit greater amounts of exudate to leach to the cooking medium (as observed by the decreased dry matter contents and increased cooking loss values).

The increased cooking tolerance exhibited by the HMW barley β-glucan fraction pasta may be attributed to the swelling of HMW barley β-glucan fraction particles and the formation of a viscous network that restricts the excessive swelling and movement of starch polymers and subsequent leaching into the cooking medium. A similar explanation has been postulated by Tudorica *et al.* (2002c) and Izydorczyk *et al.* (2005) examining the effects of soluble fibres in pasta.

Table 5.8 illustrates the textural attributes of control, HMW and LMW barley β-glucan fraction pastas. The hardness of HMW and LMW barley β-glucan fraction pastas was significantly lower than the control, the LMW barley β-glucan fraction pasta exhibiting the greatness loss of hardness ($P<0.05$). Adhesiveness of the control and LMW barley β-glucan fraction pastas was similar ($P>0.05$); however a significantly higher adhesiveness value was observed from the HMW barley β-glucan fraction pasta ($P<0.05$).

Table 5.8 Textural attributes (hardness and adhesiveness) of control, HMW and LMW barley β-glucan fraction pastas[1]

Pasta	Hardness (g)	Adhesiveness (g s)
Control	$701.5^{a} \pm 6.39$	$-2.09^{b} \pm 0.20$
HMW	$500^{b} \pm 19.44$	$-4.85^{a} \pm 0.72$
LMW	$408.2^{c} \pm 6.04$	$-1.63^{b} \pm 0.23$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

The loss of firmness exhibited by the HMW and LMW barley β-glucan fraction pastas may be attributed to a loss in gluten contents and disruption to the protein-starch network, which has been illustrated by other authors to be of extreme importance in dictating the firmness of pastas (Marconi *et al.* 2000). The increased adhesiveness of the HMW barley β-glucan fraction pasta may be explained by a leaching of viscous β-glucan material onto the pasta surface.

5.3.4 Effect of HMW and LMW Barley β-Glucan Fractions on the Gelatinisation Characteristics of Pasta

The inclusion of HMW and LMW barley β-glucan fractions in pasta did not have any significant effect on starch gelatinisation characteristics in comparison to the control ($P>0.05$) (Table 5.9). This suggests that no interaction between starch granules and β-glucans occurs within the pasta matrix.

Table 5.9 Starch gelatinisation characteristics of control, HMW and LMW barley β-glucan fraction pastas[1]

Pasta	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)	Enthalpy (J/g)
Control	$52.13^a \pm 0.81$	$70.40^a \pm 1.04$	$60.77^a \pm 0.67$	$4.46^a \pm 0.13$
HMW	$52.17^a \pm 0.74$	$70.13^a \pm 0.78$	$60.87^a \pm 0.06$	$4.43^a \pm 0.10$
LMW	$53.40^a \pm 1.22$	$70.33^a \pm 0.15$	$61.23^a \pm 0.23$	$4.48^a \pm 0.08$

[1]Results are mean ± SD of triplicate determinations (samples taken from three independent production runs).

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

5.3.5 Effect of HMW and LMW Barley β-Glucan Fractions on the *In Vitro* Starch Digestibility of Bread and Pasta

5.3.5.1 Bread

Figure 5.2. illustrates the effect of HMW and LMW barley β-glucan fraction inclusion on the RSR from the bread matrix during an *in vitro* digestion process. The results reveal a significant decrease in RSR from both breads (LMW 90-300 minutes *in vitro* digestion and HMW 120-300 minutes *in vitro* digestion) compared to the control ($P<0.05$). Generally, there was no significant difference between the RSR values from HMW and LMW barley β-glucan fraction breads ($P>0.05$).

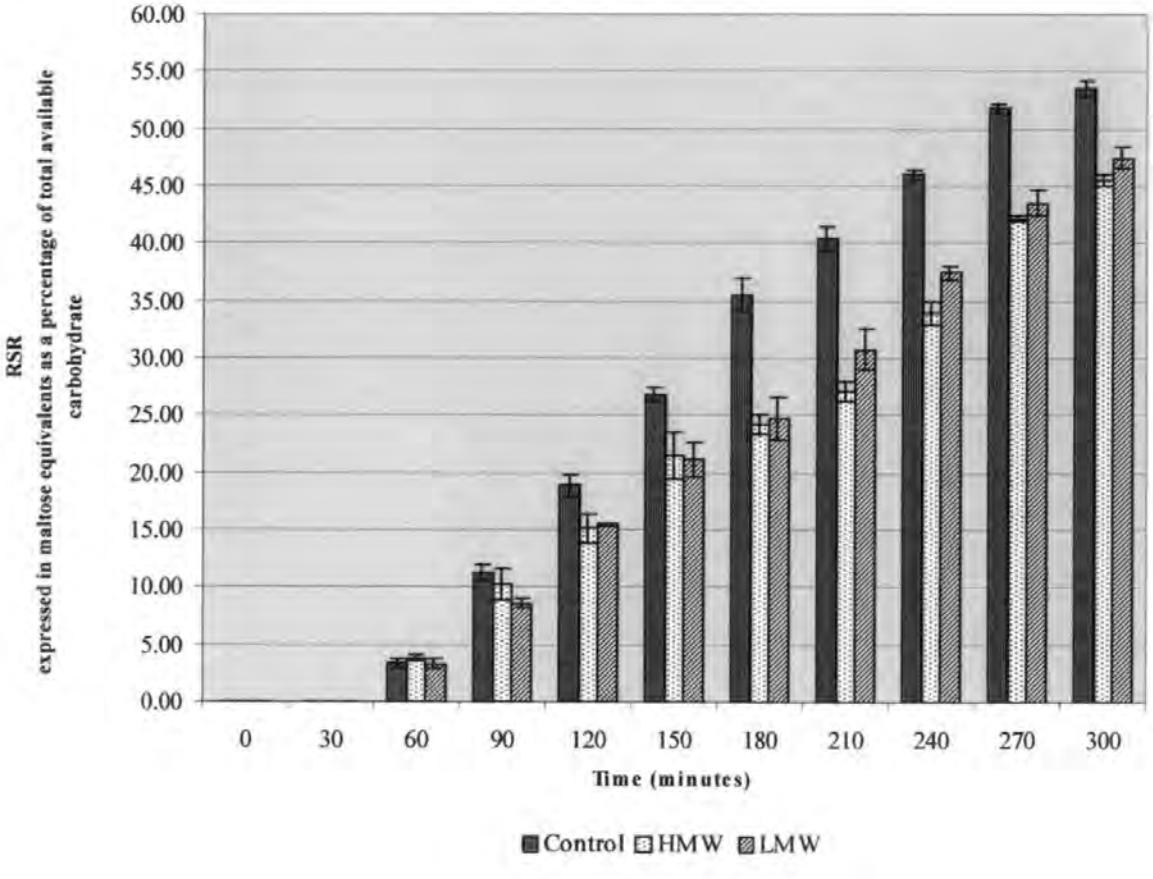


Figure 5.2 *In vitro* starch digestibility (RSR, expressed in maltose equivalents as a percentage of total available carbohydrate) of control, HMW and LMW barley β-glucan fraction breads. Results are mean of triplicate determinations (samples taken from three independent production runs). SD is presented as error bars.

The general similarity of RSR from the HMW and LMW barley β-glucan fraction breads is surprising since the relationship between MW, viscosity and reductions in sugar diffusion has been illustrated by other workers (Wood *et al.* 1994a, 2000); however, the majority of these studies are with homogenous solutions of soluble fibre and glucose as opposed to foods with a solid matrix where other macro-components, such as starch and protein, exist. The similarity and ability of both the HMW and LMW barley β-glucan fractions to reduce RSR might be explained by a different mechanism of action of β-glucan in solid foods than in liquids, with MW and viscosity having a lesser role in a solid matrix (as observed and discussed in *5.3.6.1*). Brennan *et al.* (1996a) illustrated that guar gum (physico-chemically similar to β-glucan) had the ability to modify the micro-structure of wheat breads, which resulted in a significant reduction in starch hydrolysis compared with the control; this effect was independent of the MW of guar gum contained in the wheat bread. The ability of soluble fibres to reduce starch granule hydrolysis regardless of MW may partly explain why wheat bread containing guar gum of a LMW reduced post-prandial glycaemia and plasma insulin concentrations in diabetic (Gatenby *et al.* 1996) and non diabetic (Ellis *et al.* 1991) human subjects. It is also possible that the decreased RSR from the HMW and LMW barley β-glucan breads is a result of increased digesta viscosity and attenuated sugar diffusion to the dialysate. The similarity in RSR from the β-glucan breads may be explained by the MW degradation of the HMW barley β-glucan fraction, which may have resulted in a reduction in viscous effect similar to that of LMW barley β-glucan fraction.

5.3.5.2 Pasta

Figure 5.3 illustrates the effect of HMW and LMW barley β-glucan fractions on the RSR from the pasta matrix during an *in vitro* digestion process. There was no consistent significant reduction in the RSR from HMW and LMW barley β-glucan fraction pastas compared to the control ($P>0.05$).

Between 120-210 minutes *in vitro* digestion, the HMW barley β-glucan fraction pasta exhibited a significantly higher RSR compared to the control ($P<0.05$), thereafter there was no difference between the RSR from the control and the HMW barley β-glucan fraction pasta ($P>0.05$). The LMW barley β-glucan fraction pasta exhibited a continuously higher RSR compared to the control pasta ($P<0.05$). The RSR from the HMW and LMW barley β-glucan fraction pastas were similar 60-240 minutes *in vitro* digestion ($P>0.05$), thereafter the LMW barley β-glucan pasta exhibited a significantly higher RSR than the HMW barley β- glucan fraction pasta ($P<0.05$).

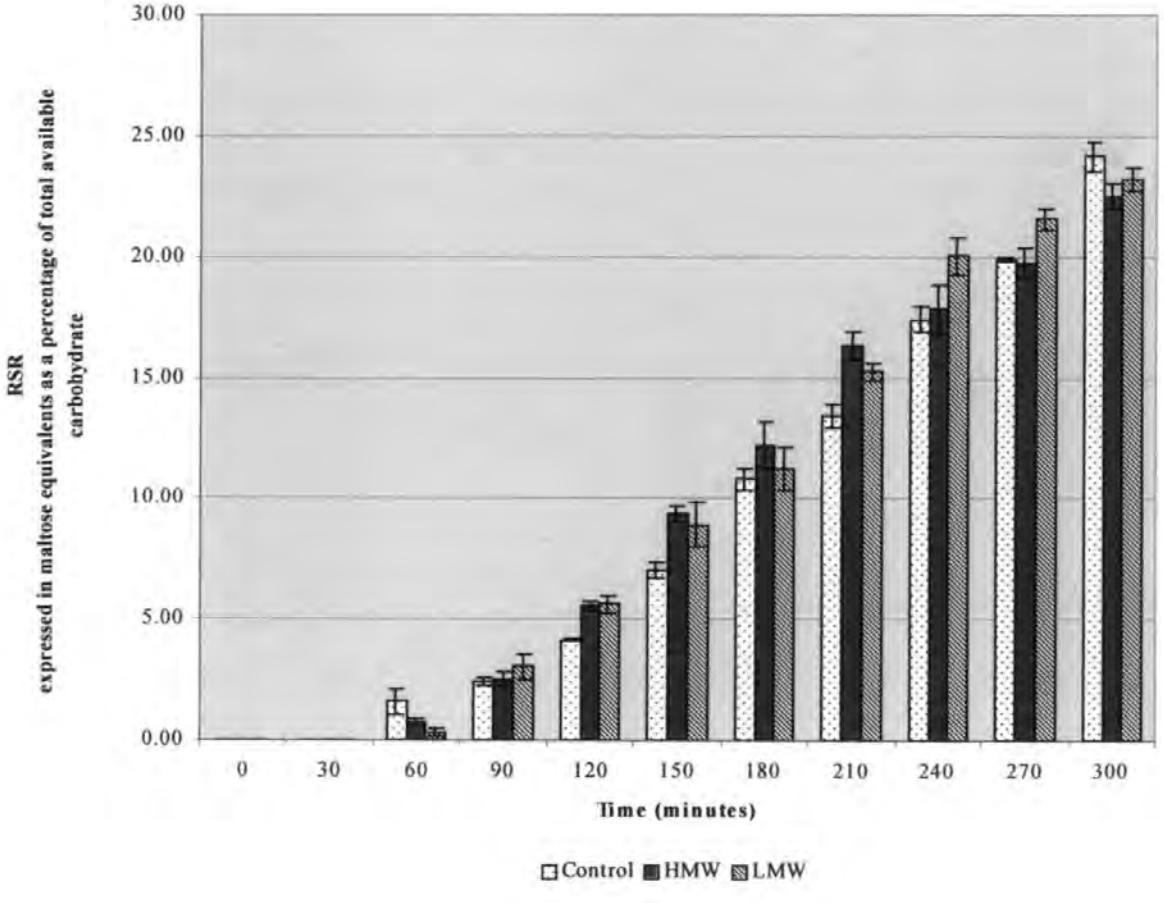


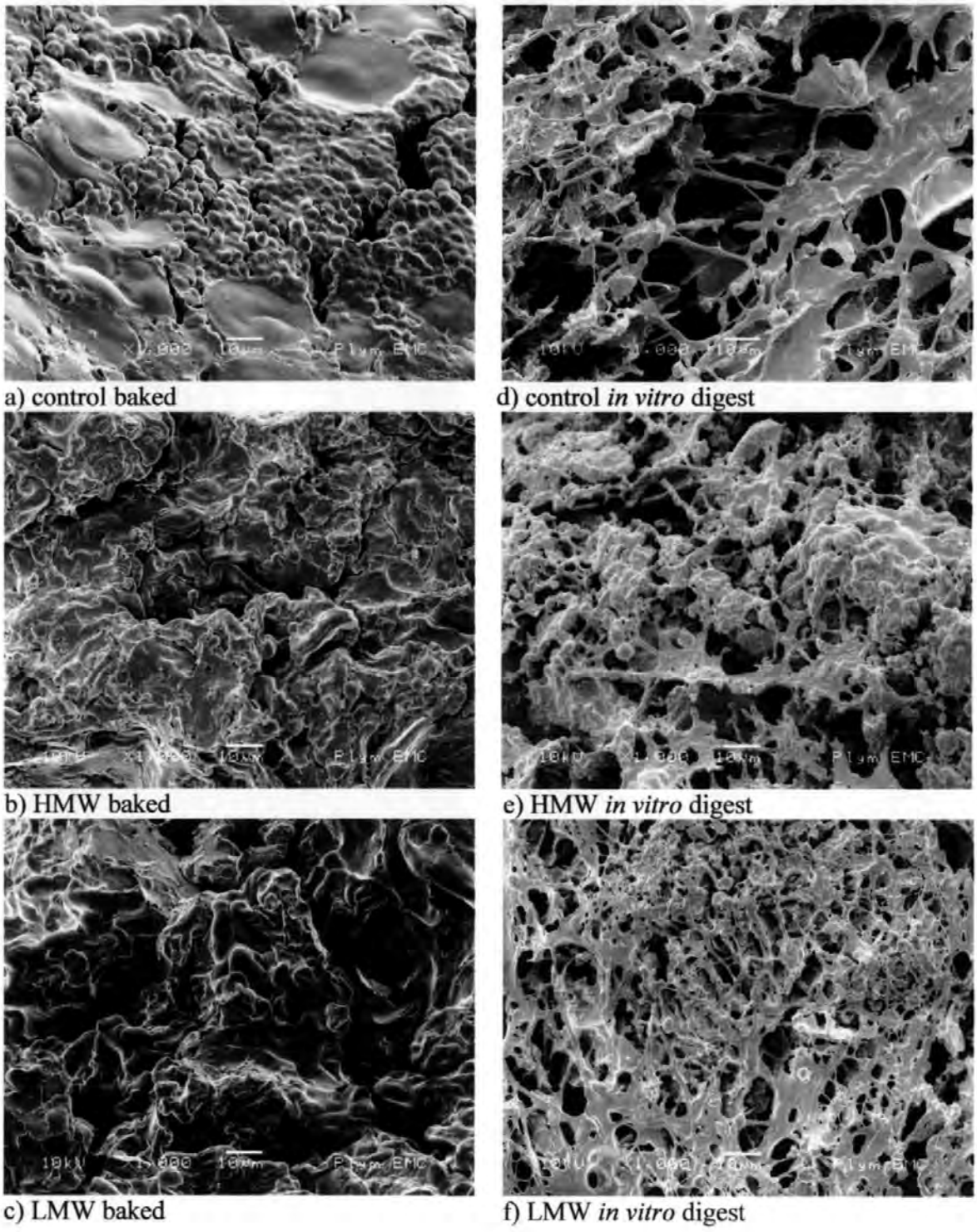
Figure 5.3 *In vitro* starch digestibility (RSR, expressed in maltose equivalents as a percentage of total available carbohydrate) of control, HMW and LMW barley β-glucan fraction pastas. Results are mean of triplicate determinations (samples taken from three independent production runs). SD is presented as error bars.

The increased RSR exhibited by the LMW barley β-glucan fraction pasta may be attributed to the formation of a weak gel, which disrupts the pasta protein-starch matrix and results in increased exposure of starch granules for amylolysis. Tudorica *et al.* (2002c) reported disruption to the pasta protein-starch matrix and increased sugar release (*in vitro*) on addition of inulin and pea fibre. Disruption of the protein-starch matrix may not appear as such a likely explanation for the lack of RSR attenuation from the HMW barley β-glucan fraction pasta since cooking loss was lower than the control; however, it may be hypothesised that the HMW barley β-glucan fraction forms a viscous polysaccharide matrix that prevents leaching of starch molecules from a disrupted protein-network but does not encapsulate them, thus, offering no protective effect from amylolytic attack.

5.3.6 Effect of HMW and LMW Barley β-Glucan Fractions on the Micro-Structure of Baked and *In Vitro* Digested Bread and Raw, Cooked and *In Vitro* Digested Pasta

5.3.6.1 Bread

Figure 5.4 contains SEMs[1] of baked and *in vitro* digested (300 minutes) control, HMW and LMW barley β-glucan fraction breads. Figure 5.4a, the baked control bread, has an even structure with the presence of relatively exposed large and small starch granules. The baked HMW and LMW barley β-glucan fraction breads (Figures 5.4b and 5.4c respectively) have a more compact and uneven structure with fewer starch granules exposed. Figure 5.4d, the *in vitro* digested control bread (300 minutes), has a very porous appearance with relatively few undigested starch granules. The *in vitro* digested HMW and LMW barley β-glucan fraction breads (Figures 5.4e and 5.4f respectively) have a more compact appearance and retention of undigested starch granules.



a) control baked
d) control *in vitro* digest
b) HMW baked
e) HMW *in vitro* digest
c) LMW baked
f) LMW *in vitro* digest

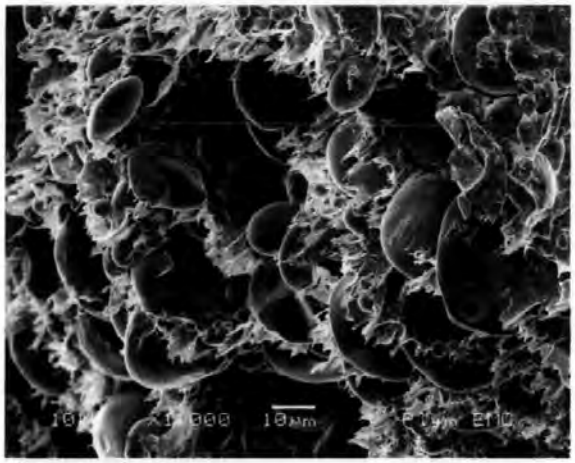
Figure 5.4 SEMs[1] of baked and *in vitro* digested (300 minutes) breads (x 1000): (a) control baked; (b) HMW baked; (c) LMW baked; (d) control digest; (e) HMW digest; and (f) LMW digest.

This study clearly illustrates that the inclusion of barley β-glucan fractions of both high and low MW within bread has an impact upon structure, which in turn may change the physico-chemical properties of breads and the rate of amylolytic activity and starch hydrolysis. The change in bread structure does not appear to vary with the MW of the barley β-glucan fractions, this consistent with the findings of Brennan *et al.* (1996a).

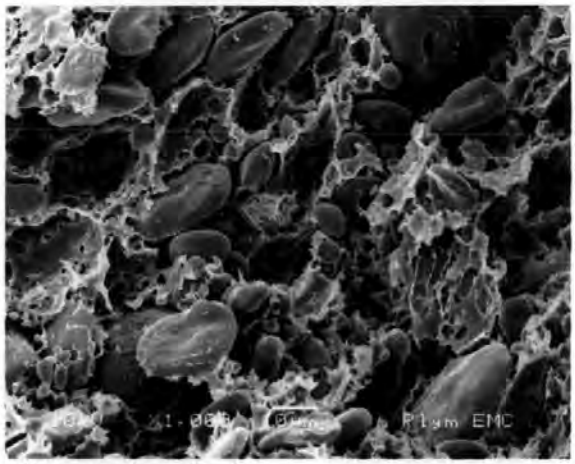
5.3.6.2 Pasta

Figure 5.5. illustrates SEMs[1] of raw, cooked and *in vitro* digested pastas. The raw control pasta (Figure 5.5a) has a well-formed protein-starch matrix with an abundance of protein strands entrapping large starch granules. With HMW and LMW barley β-glucan fraction incorporation (Figures 5.5b and 5.5c respectively) there appears to be a difference in protein-starch binding patterns and a loss of profuse protein network within the raw pastas. The cooked control pasta (Figure 5.5d) has a developed and coagulated protein network that entraps swollen starch granules. The cooked HMW barley β-glucan fraction pasta (Figure 5.5e) appears to have higher quantity of less swollen starch granules than the control, these granules embedded in a weak protein network. The cooked LMW barley β-glucan fraction pasta (Figure 5.5f) has a definite loss of protein network and what appears like a webbed network. The *in vitro* digested control, HMW and LMW barley β-glucan fraction pastas (Figures 5.5h, 5.5i, and 5.5g respectively) are all similar in having webbed structures with an absence of visible starch granules.

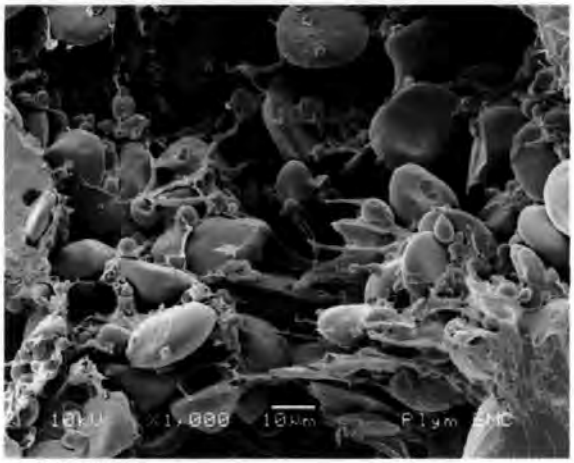
The images gathered in this study might help to explain the significant losses in pasta firmness and lack of RSR attenuation exhibited by the HMW and LMW barley β-glucan fraction pastas. As discussed in Chapter 4, the loss of rich protein matrix on fraction inclusion may be completely or partially remedied by changes to formulation or processing conditions (i.e. addition of vital gluten/high temperature drying treatments).



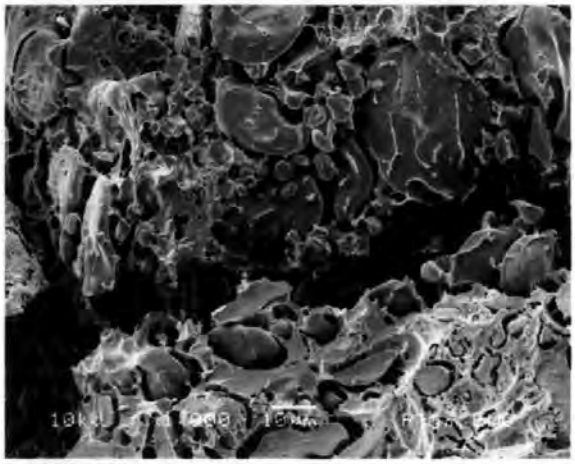
a) control raw



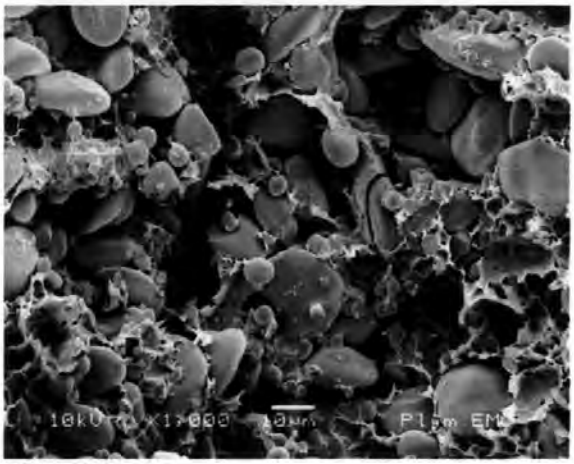
d) control cooked



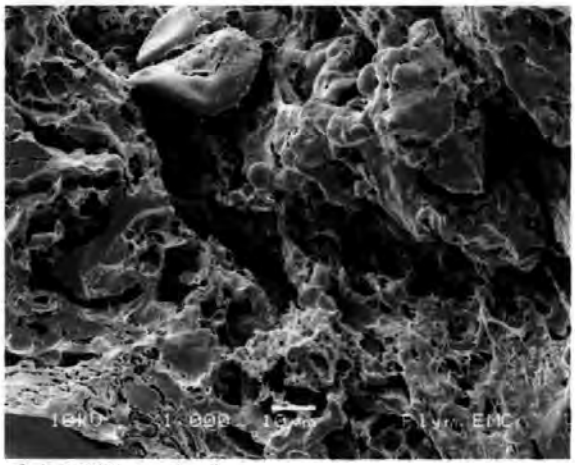
b) HMW raw



e) HMW cooked

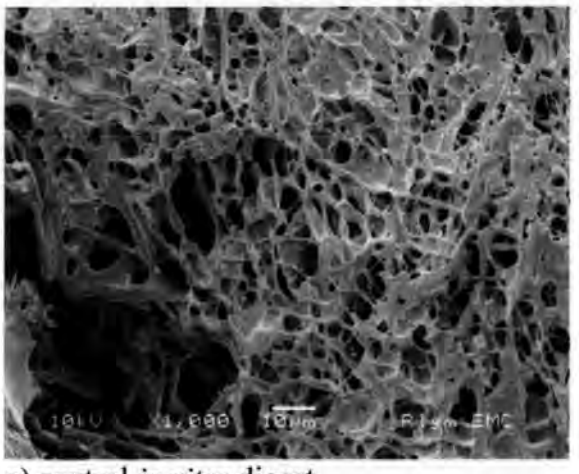


c) LMW raw

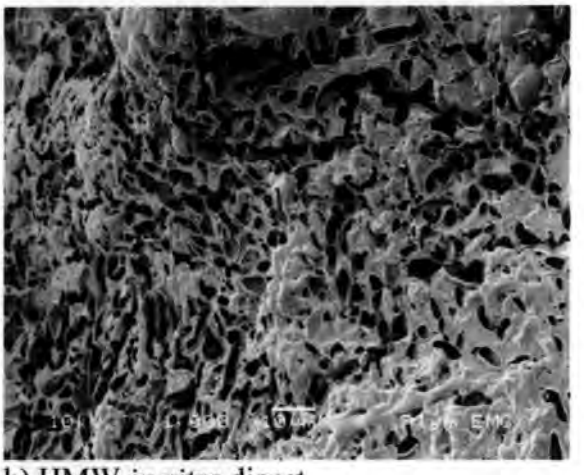


f) LMW cooked

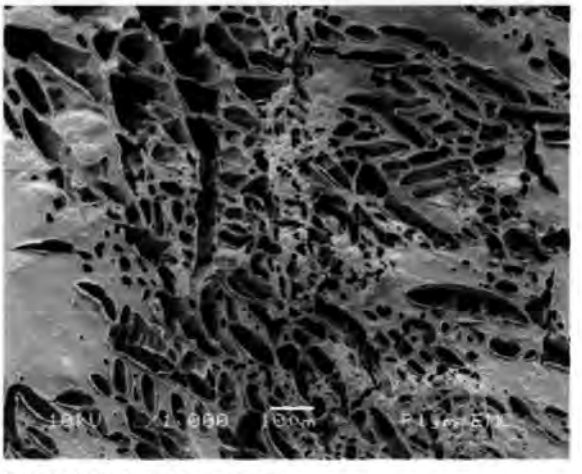
Figure 5.5 SEMs[1] of raw, cooked and *in vitro* digested (300 minutes) pastas (x 1000): (a) control raw; (b) HMW raw; (c) LMW raw; (d) control cooked; (e) HMW cooked; (f) LMW cooked; (g) control digest; (h) HMW digest; and (i) LMW digest.



g) control *in vitro* digest



h) HMW *in vitro* digest



i) LMW *in vitro* digest

Figure 5.5 SEMs[1] continued.

5.3.7 Effect of Baking and *In Vitro* Digestion on the M_{cf} and MW Distribution of HMW and LMW Barley β-Glucan

M_{cf} and MW distribution of the β-glucans within the wheat flour, barley β-glucan fractions and baked and *in vitro* digested (30, 150 and 300 minutes) control, HMW and LMW barley β-glucan fraction breads were determined. The M_{cf} at which 10, 50 and 90% of the distribution fall below are illustrated in Table 5.10 and 5.11. The effects of bread manufacture on the MW distributions differ between the HMW and LMW barley β-glucan fractions (Figure 5.6). The M_{cf} of β-glucan from the HMW fraction decreased from 64 x 10^4 to 31 x 10^4 g/mol during bread manufacture; this observation is consistent with those of Knuckles *et al.* (1997b), Andersson *et al.* (2004) and Trogh *et al.* (2004).

These studies clearly demonstrate hydrolysis of the β-glucan most likely from enzymes present in the flour or in added yeast. The fact that the MW of β-glucan from the LMW fraction doesn't change at all during fermentation and baking is interesting but difficult to explain; however, it does support the observations of Chapter 3 that HMW β-glucans are more readily degraded by β-glucanases than those with a LMW. The distributions in the control are of less importance because of the very low β-glucan content compared to the breads with added β-glucan; however, they do illustrate degradation of HMW β-glucan.

Table 5.10 M_{cf} and MW distribution of β-glucans from bread wheat flour, HMW and LMW barley β-glucan fractions and baked control, HMW and LMW barley β-glucan fraction breads (percentiles describing MW (x 10^4 g/mol) at which 10, 50 and 90% of the distribution fall below that value[1])

Sample	(M_{cf})		Distribution		
	(x 10^4 g/mol)	CV[2]	10%	50%	90%
Wheat flour	70^a	2	4.5^d	36^b	186^a
Fractions					
HMW	64^b	0.4	17^a	59^a	118^b
LMW	21^d	1.4	$7.2^{b,c}$	17^d	40^d
Baked bread					
Control	20^d	8.2	4.9^d	13^e	44^d
HMW	31^c	2.5	7.6^b	23^c	64^c
LMW	20^d	1.5	7.0^c	16^d	38^d

[1]All measurements are mean values of duplicate determinations (samples taken from independent production runs). [2]CV (%) for M_{cf}.

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

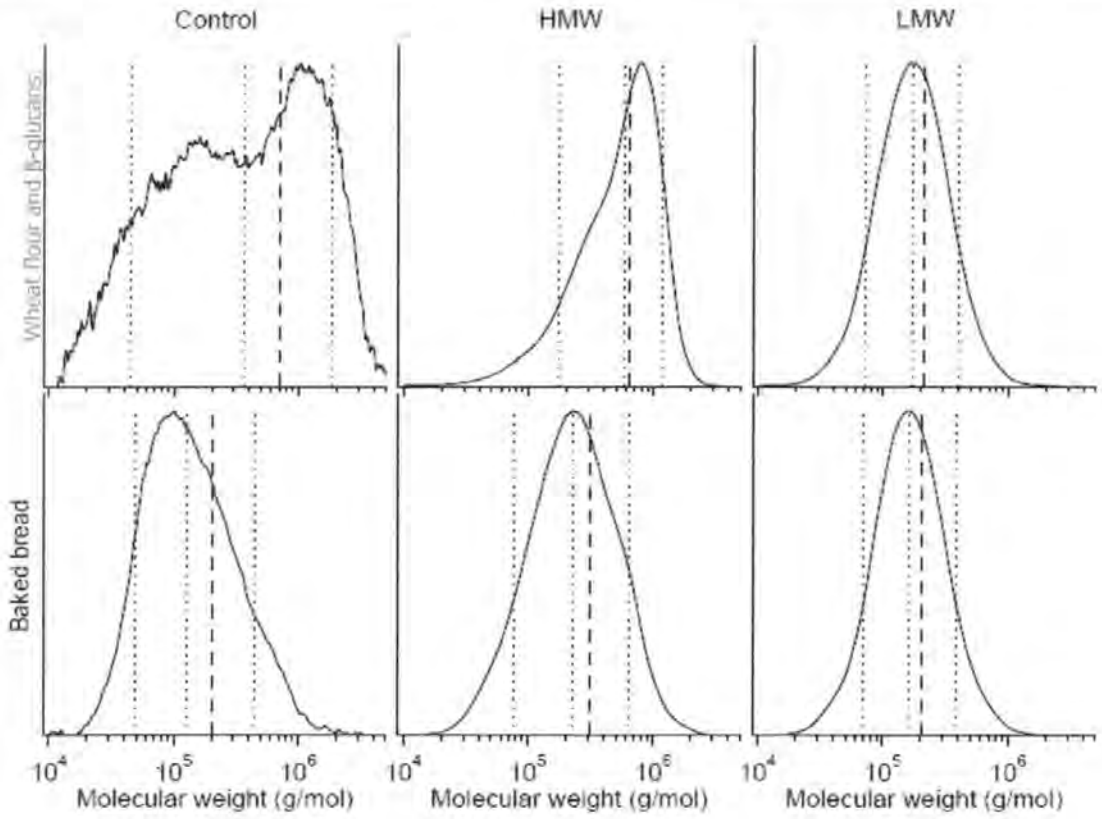


Figure 5.6 MW (g/mol) distribution of β-glucan from bread wheat flour, HMW and LMW barley β-glucan fractions and baked control, HMW and LMW barley β-glucan fraction breads. Dotted lines represent 10, 50 and 90% percentiles and dashed line represents M_{cf}. Results are from duplicate determinations (samples taken from independent production runs).

The distributions in the *in vitro* digests do not change drastically during treatment (Figure 5.7), and this is probably because the enzymes responsible for β-glucan degradation were inactivated during baking. The slight increase in M_{cf} (Table 5.11) might be explained by an increase in extractability or a reduction in bread components as reducing sugars are released.

Table 5.11 M_{cf} and MW distribution of β-glucans from *in vitro* digested (30, 150 and 300 minutes) control, HMW and LMW barley β-glucan fraction breads (percentiles describing MW (x 10^4 g/mol) at which 10, 50 and 90% of the distribution fall below that value[1])

Sample	(M_{cf})		Distribution		
	(x 10^4 g/mol)	CV[2]	10%	50%	90%
30 minutes					
Control	13^{e}	6.8	4.1^{c}	9.3^{c}	25^{e}
HMW	26^{b}	4.2	5.4^{b}	17^{b}	58^{a}
LMW	21^{c}	1.6	6.8^{a}	16^{b}	$40^{b,c}$
150 minutes					
Control	15^{e}	5.5	3.5^{c}	8.9^{c}	31^{d}
HMW	30^{a}	0.8	6.9^{a}	21^{a}	62^{a}
LMW	$20^{c,d}$	0.0	6.6^{a}	16^{b}	$38^{b,c}$
300 minutes					
Control	18^{d}	4.3	3.3^{c}	9.2^{c}	42^{b}
HMW	30^{a}	1.0	7.1^{a}	21^{a}	61^{a}
LMW	$20^{c,d}$	2.3	6.6^{a}	16^{b}	37^{c}

[1]All measurements are mean values of duplicate determinations (samples taken from independent production runs).

[2]CV (%) for M_{cf}.

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

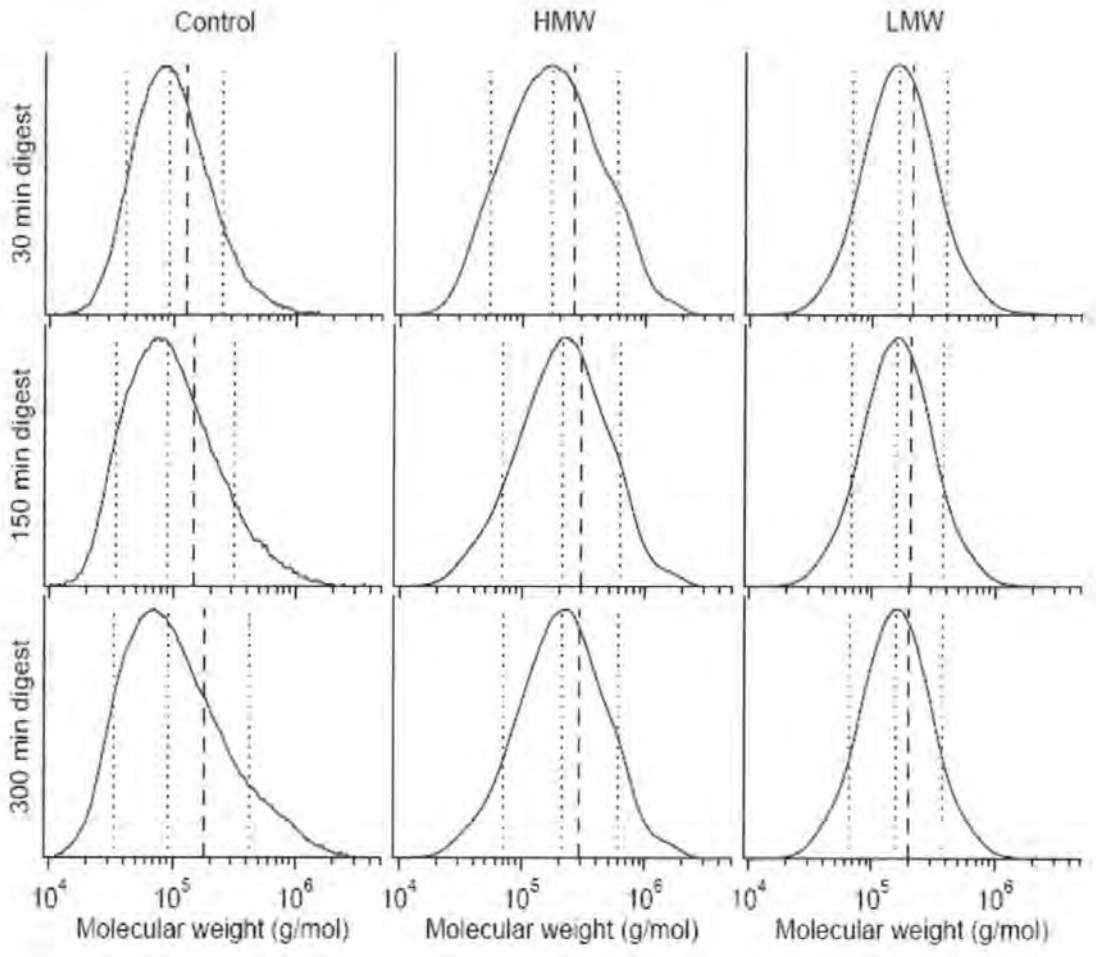


Figure 5.7 MW (g/mol) distribution of β-glucan from *in vitro* digested (30, 150 and 300 minutes) control, HMW and LMW barley β-glucan fraction breads. Dotted lines represent 10, 50 and 90% percentiles and dashed line represents M_{cf}. Results are from duplicate determinations (samples taken from independent production runs).

5.4 CONCLUSIONS

This study clearly illustrates that the behaviour of HMW and LMW barley β-glucan fractions varies in different cereal systems, and the behaviour of barley β-glucan fractions within a cereal food system varies with MW. Both HMW and LMW barley β-glucan fractions improve the nutritional quality of white breads by attenuating *in vitro* starch digestibility, which in turn may have potential in the regulation of *in vivo* sugar release from white bread, a traditionally high glycaemic food. The results illustrate that loss of

dough and bread quality is related to MW, with the HMW barley β-glucan fraction bringing about the greatest changes to dough and bread characteristics. HMW barley β-glucan appears to be more susceptible to MW degradation during bread processing than LMW β-glucan. Collectively, these results suggest that it may be technologically easier to incorporate LMW barley β-glucan fractions to breads, which may make barley β-glucan more appealing to the food industry.

With regard to pasta, neither the HMW nor LMW barley β-glucan fraction reduced the *in vitro* starch digestibility of pasta. This is surprising when taking into consideration the attenuated RSR from pasta with BBG fibre fraction, as observed in Chapter 4. This study suggests that the lack of effect is a result of the poor integration of the HMW and LMW barley β-glucan fractions in the pasta matrix and disruption to the protein-starch network, with the greatest disruption occurring in LMW barley β-glucan fraction pasta. Loss of cooking quality was greatest in the LMW barley β-glucan fraction pasta, and it is hypothesised that this is due to the formation of a weak gel, which disrupts the protein-starch network. HMW barley β-glucan fraction pastas had increased cooking tolerance, and it is postulated that although there is a disruption of the protein–starch network, organic matter within the pasta is withheld in a viscous network rather than leaching to the cooking medium. It is possible that with modifications to pasta formula and changes to processing the loss in protein-starch matrix quality may be greatly improved, thus, reducing loss in cooking quality; however, this must be confirmed by further studies, which simultaneously evaluate whether such treatments improve the ability of these barley β-glucan fractions to attenuate *in vitro* starch digestibility.

CHAPTER 6

CONCLUDING DISCUSSION

6.1 REVIEW OF STUDY RATIONALE AND AIMS

Chronic diseases are now the major causes of death and disability worldwide. Relatively few risk factors are responsible for the majority of the chronic disease burden. Improving dietary habits (increasing intakes of fruit, vegetables, nuts and wholegrain foods) can have a significant impact upon reducing the rates of chronic disease. In western countries, daily consumption of wholegrain foods is far from the desired quantities, and dietary fibre intakes are also significantly lower than those recommended by national and international health authorities (Mathers and Wolever 2002). Wholegrain foods, rich in dietary fibre, are often rejected due to a consumer preference for more refined products; this is exemplified by the heavy consumption of white breads and pasta in European countries (Bjorck *et al.* 2000). A potential solution to low dietary fibre intakes lie in the enrichment of popular cereal foods with concentrated sources of dietary fibre.

β-glucans from barley (and oat) are soluble dietary fibres widely known for their hypoglycaemic (Wood *et al.* 1990, 1994a) and hypocholesterolemic capacity (Braaten *et al.* 1994; Beer *et al.* 1995). These soluble fibres have also been shown to have satiation (Bourdon *et al.* 1999), prebiotic (Dongowski *et al.* 2002) and immunostimulatory (Causey *et al.* 1998; Fulcher *et al.* 2000) effects.

The incorporation of barley β-glucan in cereal foods (bread and pasta) is relatively limited to the use of enriched flours from the barley grain (Table 6.1). The inclusion of such flours often compromises the organoleptic properties of the products (Knuckles *et al.* 1997a;

Marconi *et al.* 2000), thus, reducing consumer appeal and consumption. There is potential to use barley as an extraction source for β-glucan fractions and to subsequently incorporate these fractions into cereal foods. As these fractions are a concentrated source of β-glucan, they may be incorporated into foods in lower quantities than barley flours, which may reduce negative effects on product quality. At present, there has been little work conducted on the physico-chemical and nutritional effects of barley β-glucan fractions in cereal foods, and such a lack of information has prevented the food industry use of barley β-glucan as a functional food ingredient.

Table 6.1 Examples of barley β-glucan material used in cereal products

Barley β-glucan material	**Product**	**Reference**
Prowashonupana barley flour (18% β-glucan dwb)	Bread	Liljeberg *et al.* (1996)
Milled and sieved fractions from Waxbar barley flour (20.11% β-glucan dwb)	Pasta	Yokoyama *et al.* (1997)
Whole, sieved and water fractions from Zacinto barley flour (4.6, 8.5 and 33.2% β-glucan dwb respectively)	Bread	Cavallero *et al.* (2002)
Fractions from commercial Italian and English barley (9.1 and 10.5% β-glucan dwb respectively)	Pasta	Marconi *et al.* (2000)
Roller milled fractions produced from SR9315 and CDC-92-55-06 barley (22.02 and 22.14% β-glucan dwb respectively)	Pasta	Izydorczyk *et al.* (2005)

In an attempt to address this lack of information, this study investigated the potential of barley β-glucan fractions as functional food ingredients for use in cereal foods, with particular emphasis on how the composition, physico-chemical properties and MW of barley β-glucan fractions affects behaviour in cereal food systems. Specific aims of the study were to:

1. Investigate different extraction treatments for the isolation of β-glucan fractions from barley and the effects of their inclusion in wheat starch (Chapter 2).

2. Investigate and compare the influence of different barley β-glucan fractions on the physico-chemical properties, micro-structure and *in vitro* starch digestibility of white wheat bread (Chapter 3).

3. Investigate and compare the influence of different barley β-glucan fractions on the physico-chemical properties, micro-structure and *in vitro* starch digestibility of durum wheat semolina pasta (Chapter 4).

4. Investigate and compare the effects of differing MW barley β-glucan fractions (high and low) on the physico-chemical properties, microstructure and *in vitro* starch digestibility of white wheat bread and durum wheat semolina pasta (Chapter 5).

5. Investigate the susceptibility of barley β-glucan fractions to MW degradation during fermentation, baking and *in vitro* digestion (Chapter 3 and 5).

The essentially commercial nature of this study has required a highly practical and applied interdisciplinary approach to research where not only has the behaviour of barley β-glucan fractions been investigated in very different food systems, but where certain individual physico-chemical properties of barley β-glucan fractions have also been examined. In addition to answering the main question proposed at the outset of the study, that is "the potential of barley β-glucan as a functional food ingredient for cereal foods", other questions for discussion have also been generated. Whilst this thesis has aimed to address most of these questions, it is acknowledged that further in depth research is justified to provide clarity. Thus, in this conclusion key findings of the research are summarised alongside indication and suggestions for where further research is required.

6.2 STUDY OUTCOMES

In the first stage of the study, BBG fibre fractions from four different aqueous-solvent based extraction treatments (water only, refluxed, purified and alkali) were evaluated in terms of yield and β-glucan recovery, WRC and composition. The subsequent effects of these fractions on wheat starch gelatinisation and pasting properties (as determined by DSC[2] and RVA[2]) were also investigated (Aim 1). Chapter 2 details the results of these experiments.

The investigation supported the observations of other workers indicating that the composition and functional behaviour of barley β-glucan fractions may be influenced by choice of extraction treatment (Beer *et al.* 1996; Temelli 1997; Burkus and Temelli 1998). The study also indicates that substitution of wheat starch with BBG fibre fractions may result in a change to gelatinisation characteristics and pasting properties. At a low level of inclusion (1%), all fractions appear to raise the PV and FV of wheat starch pastes. At a higher concentration of BBG fibre fraction (5%), there appears to be a decrease in the PV,

BD, and FV of wheat starch pastes. At 5% inclusion, there appears to be an associated reduction in the enthalpy of gelatinisation. Mechanisms behind the behaviour of the BBG fibre fractions in the starch system are unconfirmed. It is plausible that the apparent increase in paste viscosity at low BBG fibre fraction concentrations (1%) is a result of changes to the viscosity of the continuous phase of the starch dispersions and/or the formation of polymer complexes, as reported by Alloncle and Doublier (1991) and Bahnassey and Breene (1994) in their studies of NSP and starch interactions. Whilst it is acknowledged that the apparent reduction in viscosity and enthalpy of starch pastes with a higher BBG fibre fraction concentration (5%) may be a result of starch replacement, it is also possible that the reduction is a result of the 'anti-plasticisation' capacity of BBG fibre fractions, that is the ability in a starch-water system to decrease the free volume of water and hinder mobility, which in turn effects the plasticisation of amorphous regions and the dissociation of double helices during the gelatinisation process, thus reducing starch gelatinisation. Further studies are justified to clarify the behaviour of barley β-glucan fractions in starch pastes and to further characterise the hydration and water holding properties of barley β-glucan.

The observations from this study make a contribution to the current knowledge on the effects of barley β-glucan in starch systems, which as reported in Chapter 1 is relatively limited, despite the plethora of data existing on the effects of other NSPs in starch systems. The information gathered in this study has both technological and nutritional value, for example the possible ability of low BBG fibre fraction concentrations to increase viscosity may be of importance in technological applications, such as use as thickening and stabilising ingredients, whilst the possible ability of high BBG fibre fraction concentrations to reduce gelatinisation of starch may result in a reduced rate of starch digestion, both *in vitro* and *in vivo*, and this has relevance to human nutrition in the regulation of *in vivo*

glycaemic response in carbohydrate rich diets where the degree of starch gelatinisation can affect the post-prandial sugar availability from foods.

In the second stage of the study, a BBG fibre fraction (as produced in Chapter 2) and a commercial barley β-glucan fraction, Glucagel™, were incorporated into bread at different levels of inclusion. Changes to the physico-chemical properties, *in vitro* starch digestibility and micro-structure of breads were evaluated, and comparisons were drawn between the effects of the two fractions (Aim 2). The results of this investigation are documented in Chapter 3.

The rheological properties of bread doughs and baked bread quality were significantly affected by BBG fibre fraction and Glucagel™ inclusions, the nature and magnitude of change dependent upon the level of inclusion and fraction type. The results are in agreement with other authors observing significant changes to dough rheology and baking performance with concentrated dietary fibre inclusions (Wang *et al.* 2002; Gomez *et al.* 2003). Whilst BBG fibre fraction inclusions resulted in the greatest change to the rheological properties of doughs, Glucagel™ inclusions resulted in the greatest loss of baking performance. It is thought that this difference in behaviour might be a result of contamination of the BBG fibre fraction with co-extracted fibres (arabinose and xylose), which have been shown to have a significant impact on the quality of bread dough and baking performance (Hoseney and Faubion 1981; Delcour *et al.* 1991). It has been postulated that the viscous nature (Cawley 1964), strong water holding capacity (Michniewicz *et al.* 1991,1992), gelling capacity (Neukom and Markwalder 1978) and hydrogen bonding capacity (Patil *et al.* 1975) of arabinoxylans may contribute to the enhanced volume of breads. There is growing interest amongst the scientific community regarding the combined use of β-glucan and arabinoxylans, particularly those from barley,

in cereal products, not only in relation to their effect on product quality but with regard to their nutritional properties (Trogh *et al.* 2004). This study indicates that further investigation of a possible 'synergistic effect' between barley β-glucan and arabinoxylan is justified.

The results of this study also illustrate that even at relatively low levels of inclusion (≤7.5%), barley β-glucan fractions still negatively affect dough and bread quality, the results being comparable to the studies of Knuckles *et al.* (1997a), Cavallero *et al.* (2002), and Gill *et al.* (2002) where significant changes to bread dough rheology and loss of bread height and volume were observed with barley β-glucan rich flour inclusions of up to 50%. The results of this study suggest that the physico-chemical properties (i.e. WRC) of the barley β-glucan within the fractions might be of greater/equal importance to dilution of the gluten network, which is more significant when a large quantity of wheat flour is substituted. Further investigations may be warranted to further characterise the WRC properties of barley β-glucan fractions in relation to their impact upon the sensory quality of bread.

Changes in both the rheological properties of the dough and the loss of baking quality on barley β-glucan fraction inclusion may be partially negated by optimisation of the bread recipes (i.e. farinographic determination of water absorption and mixing time), which may counteract the negative changes caused by the WRC of the fractions; however, it remains unclear whether the changes to gluten structure and function that these fractions bring about can be negated. Changes to formulations, for example the use of dough conditioners (i.e. emulsifiers, which enhance dough extensibility and loaf volume) may improve dough and bread quality. Further investigations are necessary to evaluate the optimisation of the bread recipes and the employment of recipe modifications on product quality, whilst

simultaneously ensuring that such treatments do not compromise the physico-chemical and physiological functionality of the barley β-glucan.

The *in vitro* starch digestibility (as measured by RSR) of bread was significantly attenuated by the inclusion of BBG fibre fraction and Glucagel™, the magnitude of reduction dependent upon level of inclusion and fraction type. The results are in agreement with Pick *et al.* (1998) and Cavallero *et al.* (2002) who observed reductions in post-prandial glycaemia after consumption of barley β-glucan rich breads. The reductions are most likely as a result of a combination of changes to bread matrix structure and starch granule availability (as observed by SEM^3) and reductions in starch granule hydration and gelatinisation. It is also possible that the reductions are in part a result of increased digesta viscosity and reduced sugar diffusion to the dialysate. This study did not directly examine the effects of the fractions on digesta viscosity, thus, further studies both *in vitro* and *in vivo* may be necessary to characterise the viscous influence of the fractions.

The ability of the BBG fibre fraction to attenuate RSR from the breads earlier in *in vitro* digestion than Glucagel™ may be due to a difference in the rate of fraction hydration and incorporation within the bread matrix. As reported in Chapter 3, differences in the rate of soluble fibre (gum) hydration have been reported by Ellis *et al.* (1991) to be importance of in hypoglycaemic efficacy and may partly explain the variable responses (effect and no effect) reported in studies investigating the same soluble fibre but in different forms of preparation (Wursch and Pi-Sunyer 1997). Thus, further studies may be warranted to examine the hydration characteristics of the two barley β-glucan fractions and factors, for example temperature and β-glucan concentration, which may impact upon rate and degree of hydration.

This study has made a significant contribution to the current knowledge on the effects of barley β-glucan fractions in bread, an area only recently being explored. The study illustrates that barley β-glucan fractions from different extraction procedures not only have different effects on the physico-chemical properties of doughs and breads but also the *in vitro* starch digestibility. It is anticipated that with further recipe optimisation these barley β-glucan fractions may be used as functional food ingredients and offer a potential solution for the nutritional improvement of bread by increasing overall dietary fibre content and potentially attenuating the *in vivo* glycaemic response of a traditionally high GI product.

In the third stage of the study, BBG fibre fraction from barley (as produced in Chapter 2) and a commercial barley β-glucan fraction, Glucagel™, were incorporated into another popular cereal food, pasta. Changes to the physico-chemical properties of the pasta, *in vitro* starch digestibility and micro-structure were evaluated, and comparisons were drawn between the effects of the two fractions (Aim 3). The results of this investigation are documented in Chapter 4.

The inclusion of both BBG fibre fraction and Glucagel™ resulted in significant changes to the cooking and textural qualities of pasta, the nature and magnitude of change being dependent upon level of inclusion and fraction type. The results are consistent with the observations of other authors investigating the effect of concentrated fibre sources on the pasta matrix (Tudorica *et al.* 2002c; Brennan *et al.* 2004). The increased swelling index and reductions in firmness of BBG fibre fraction pastas were attributed to the high WRC of the BBG fibre fraction. The increased firmness and cooking loss (at high levels of inclusion only) exhibited by the Glucagel™ pastas is possibly a result of the ability of Glucagel™ to form a semi-solid gel.

Only BBG fibre fraction inclusions resulted in a significant decrease in the *in vitro* starch digestibility (as measured by RSR) of pasta, with Glucagel™ having no reducing effect and a tendency to increase RSR compared to the control pasta. Results suggest that restriction of starch gelatinisation (as observed by DSC[2]) and slight modifications to pasta structure (as detected via SEM[1]) are the mechanisms behind the ability of the BBG fibre fraction to attenuate RSR, mechanisms also experienced by Izydorczyk *et al.* (2005) studying the effect of hull-less barley fractions in pasta. The lack of reducing effect observed with Glucagel™ inclusions is attributed to disruption of the protein-starch matrix, which leads to greater starch granule exposure and increased amylolytic attack, as experienced by Tudorica *et al.* (2002c) examining the effects of soluble fibre on the *in vitro* starch digestibility of pasta.

This study has made a significant contribution to the current knowledge on the use of barley β-glucan fractions within pasta, clearly illustrating that choice of fraction has an influence upon not only physico-chemical qualities but also the *in vitro* starch digestibility of pasta. From this investigation it appears that BBG fibre fraction would be more suitable for use as a functional food ingredient in pasta than Glucagel™. The ability of BBG fibre fraction to decrease the *in vitro* starch digestibility of pasta may be an indication of the potential of BBG fibre fraction to further lower the *in vivo* glycaemic response to pasta, an already low GI food. Further studies are justified to examine the effect of changes in formulation and novel treatments on the quality of β-glucan enriched pastas. Such studies should simultaneously ensure that the physico-chemical and physiological properties of the barley β-glucan are retained.

In the fourth stage of the study, the effects of high and low MW barley β-glucan fractions on the physico-chemical and *in vitro* starch digestibility of bread and pasta were examined and compared (Aim 4). The results of this investigation are documented in Chapter 5.

Both HMW and LMW barley β-glucan fractions brought about negative changes to dough and bread quality. The HMW barley β-glucan fraction brought about the greatest changes to dough and bread characteristics, and this was thought to be a result of the greater viscous effect and higher WRC of the HMW β-glucan fraction. Both HMW and LMW barley β-glucan fractions attenuated *in vitro* starch digestibility (as measured by RSR) of bread, with generally no difference observed between the two fractions. It is possible that the similarity between the behaviour of the two fractions is a result of the ability of barley β-glucan fractions, regardless of MW, to alter the micro-structure of the bread matrix (as observed by SEM^3) and reduce starch granule availability for amylolysis. Similar observations have been made by Ellis *et al.* (1991) and Brennan *et al.* (1996a) in their studies with guar gum. It is also accepted that the reductions in RSR may be a result of increased digesta viscosity and reduced sugar diffusion to the dialysate, the similarity between the fractions a result of the degradation of HMW fraction and loss of viscous capacity. Further investigations are necessary to evaluate and compare the effect of the fractions on the viscosity of digesta.

Inclusion of HMW and LMW barley β-glucan fractions to pasta did not reduce the *in vitro* starch digestibility (as measured by RSR), this is in contrast to the attenuated starch digestibility of pastas with BBG fibre fraction (Chapter 4). The lack of effect was attributed to poor hydration/integration of the HMW/LMW barley β-glucan fractions in the pasta matrix and subsequent disruption to the protein-starch network (as detected from SEM^3), which increased starch granule exposure for amylolysis. The greatest disruption

occurred with LMW barley β-glucan fraction addition to pasta, which also resulted in a greater loss of cooking quality. Pastas with HMW barley β-glucan fraction had increased cooking tolerance, and it is postulated that although there is a disruption of the protein–starch network, organic matter within the pasta is withheld in a viscous network rather than leaching to the cooking medium. Further studies to investigate the effect of pre-gum hydration, modifications to pasta formula and changes to processing on negating protein-starch matrix disruption are necessary; however, such investigations must simultaneously evaluate whether these treatments improve the ability of either the high or low MW barley β-glucan fractions to attenuate *in vitro* starch digestibility.

The results from the study indicate that the MW of barley β-glucan fractions has a significant impact upon physico-chemical behaviour in cereal foods; however, MW does not impact greatly upon *in vitro* starch digestibility. The study has illustrated that the behaviour of β-glucans in a solid food matrix is different to that of a homogenous solution where MW and high viscosity are of extreme importance. Integration of barley β-glucan within the cereal food matrix and interaction with macro-components (starch and protein) appears to be of greater importance than MW in controlling rates of *in vitro* starch digestibility.

In the final stage of the study, the susceptibility of β-glucan (from BBG fibre fraction, Glucagel™ and HMW and LMW barley β-glucan fractions) to MW degradation during the conditions of bread fermentation, baking and *in vitro* digestion was evaluated (Aim 5). The results of this investigation are documented in Chapters 3 and 5.

The investigations revealed that the MW of barley β-glucan is degraded during bread processing (most likely from endogenous enzymes within wheat flour or added yeast),

thus, supporting the observations of Knuckles *et al.* (1997b), Andersson *et al.* (2004) and Trogh *et al.* (2004); however, the study also revealed that barley β-glucan with a HMW is more susceptible to MW degradation during bread processing than that with a LMW, and that LMW barley β-glucan is not readily degraded by endogenous enzymes. Thus, this study suggests it may be technologically easier (and more economical) to incorporate barley β-glucan with a lower MW into bread. The study also revealed that *in vitro* digestion does not degrade the MW of barley β-glucan.

6.2.1 Summary of Key Findings

This study illustrates:

- Different extraction treatments may affect the composition and physico-chemical properties of barley β-glucan fractions, these different properties may result in differences in behaviour when incorporated into a model cereal food system.

- The inclusion of barley β-glucan fractions in bread and pasta results in significant changes to product quality, the nature and magnitude of change dependent upon inclusion level and fraction type. Factors such as composition, WRC, integration within the cereal food matrix and MW may be of importance in dictating the behaviour of these fractions.

- The inclusion of barley β-glucan fractions in bread and pasta results in significant changes to *in vitro* starch digestibility, the nature and magnitude of change dependent upon inclusion level and fraction type. Factors such as composition, WRC and integration within the cereal food matrix may influence the behaviour of

the fractions. MW appears to have a lesser importance on the ability of barley β-glucan fractions to attenuate the *in vitro* starch digestibility of bread and pasta.

- The conditions of bread manufacture result in degradation of barley β-glucan MW, although only HMW barley β-glucan is susceptible to degradation. This degradation does not result in loss of ability to attenuate RSR from the bread matrix. There is no significant degradation of barley β-glucan MW during *in vitro* digestion.

6.3 STRENGHS AND WEAKNESSES OF STUDY

One of the major limiting factors in this study has been the restriction of investigations to a micro-scale and *in vitro*. Whilst Glucagel™ is commercially produced, BBG fibre fractions had to be manufactured on a laboratory scale, which involved a great deal of time and ethanol consumption, thus, amounts produced had to be restricted. At present, there are no bulk commercial HMW and LMW barley β-glucan fractions available, therefore, these fractions had to be purchased as chemicals, and their relative expense limited the amount available. Ideally in Chapter 3 and 5 evaluations of baking performance (farinographic determinations of water absorption and mixing time) and recipe optimisation would have been performed, and in Chapter 3, 4, and 5 starch digestibility investigations *in vivo* would have been performed as well as sensory analysis to judge the organoleptic properties and acceptance of the barley β-glucan fraction enriched products.

It is acknowledged that the analysis and discussions regarding the influence of arabinoxylans contained within the barley β-glucan fractions on dough, bread and pasta quality (Chapters 3 and 4) is very limited; however, due to technical limitations, analyses had to be conducted (with the generosity of Professor Roger Andersson) in Sweden

(Department of Food Science, Uppsala). Financial and time limitations meant that the depth of the investigations had to be limited; however, it is hoped that in further studies, arabinoxylans contained within the barley β-glucan fractions will be characterised (i.e. MW analysis) and their exact role within bread and pasta evaluated.

As emphasised earlier in the discussion, the nature of this study has required a multi-dimensional research approach, that is a balance of investigations, which collectively evaluated the physico-chemical and physiological functionality of barley β-glucan fractions in cereal products, as well investigation of appropriate levels of barley β-glucan fraction inclusion required to have nutritional significance. Such an approach has meant that it has not been possible to conduct in depth investigations required to provide affirmative answers to a number of questions generated (in addition to the main research question) during the study; however, it is hoped that with further research (see recommendations in section 6.4) these questions will be fully answered.

Despite its limitations, this study has made a significant contribution to knowledge on the potential of barley β-glucan fractions as functional food ingredients and has provided a foundation for further development and optimisation of barley β-glucan enriched cereal foods. Therefore, this study has contributed to the development of practical and realistic measures to increase human dietary fibre intakes, which may potentially contribute to a reduction in the global chronic disease burden.

6.4 RECOMMENDATIONS FOR FUTURE WORK

The use of barley β-glucan fibre fractions as functional food ingredients definitely warrants further research. This study has generated a number of areas to which further study is justified:

- It is suggested that further studies aim to examine the extent of starch granule swelling and retrogradation, gelation behaviour and hydration kinetics of starch-barley β-glucan pastes. Such research would clarify the mechanisms behind the behaviour of barley β-glucan in starch systems.

- Further studies are required to evaluate the WRC of barley β-glucan fractions, in relation to their impact on macro-components (i.e. starch and protein) within cereal food matrices and the sensory properties of products.

- An investigation of the optimisation of barley β-glucan fraction bread and pasta recipes (i.e. farinographic determinations of water absorption and mixing time) and formula/processing modifications may allow for further development of barley β-glucan fractions as functional ingredients in cereal foods. Simultaneous investigations must examine the effect of such treatments on product quality and the physico-chemical properties and MW of barley β-glucan fractions.

- Investigations of the effect of barley β-glucan fractions on the viscosity of both *in vitro* and *in vivo* digesta may enable a greater understanding of the mechanisms behind the behaviour of the fractions during digestion.

- The study identified a possible relationship between barley β-glucan and co-extracted fibres (i.e. arabinoxylans) in bread, the co-extracted fibres possibly counteracting some of the negative effects of barley β-glucan inclusion. Thus, further studies may be justified to investigate the potential of exploiting this interaction.

APPENDIX I

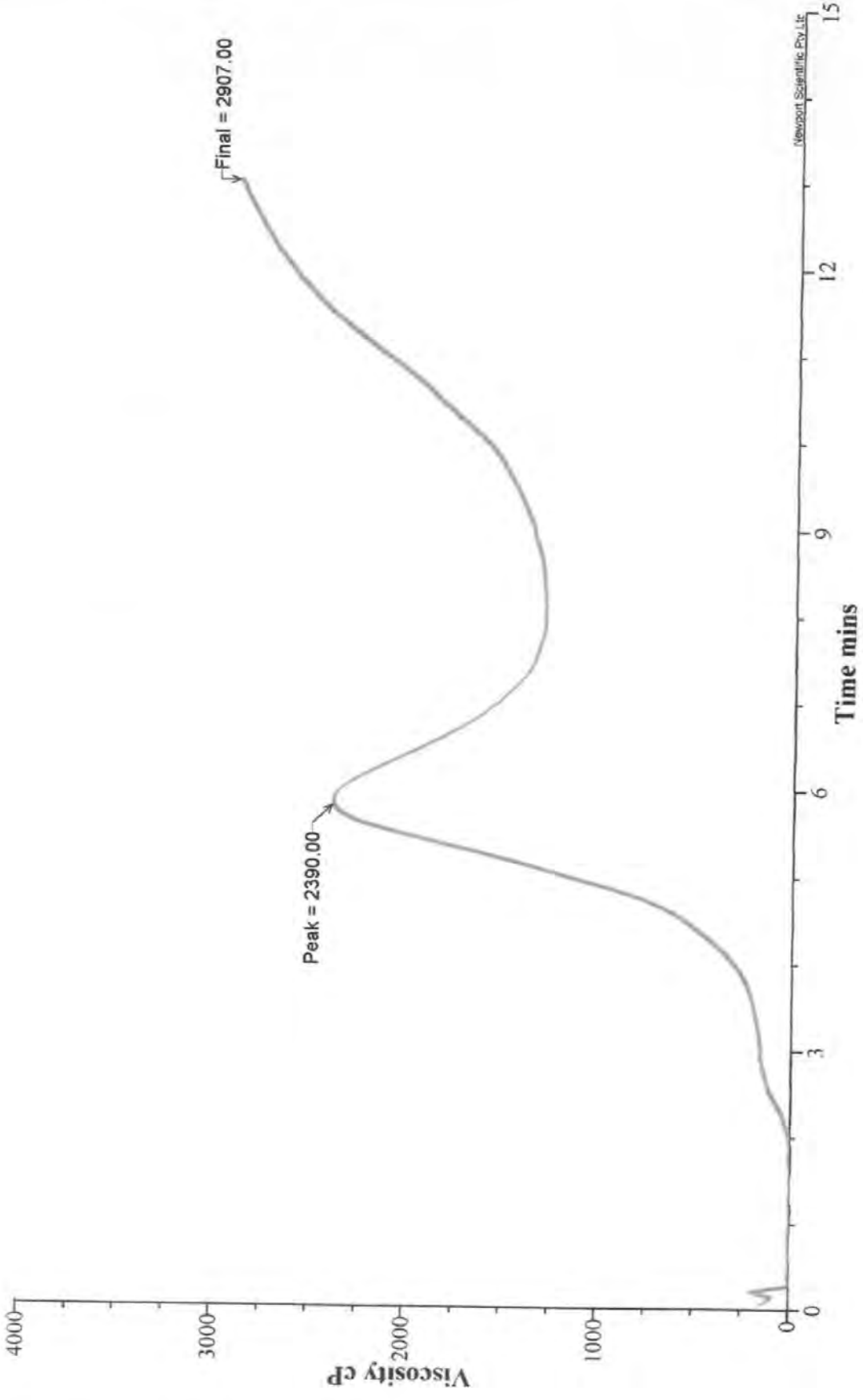


Figure AI.1 Example RVA[1] pasting curve.

APPENDIX II

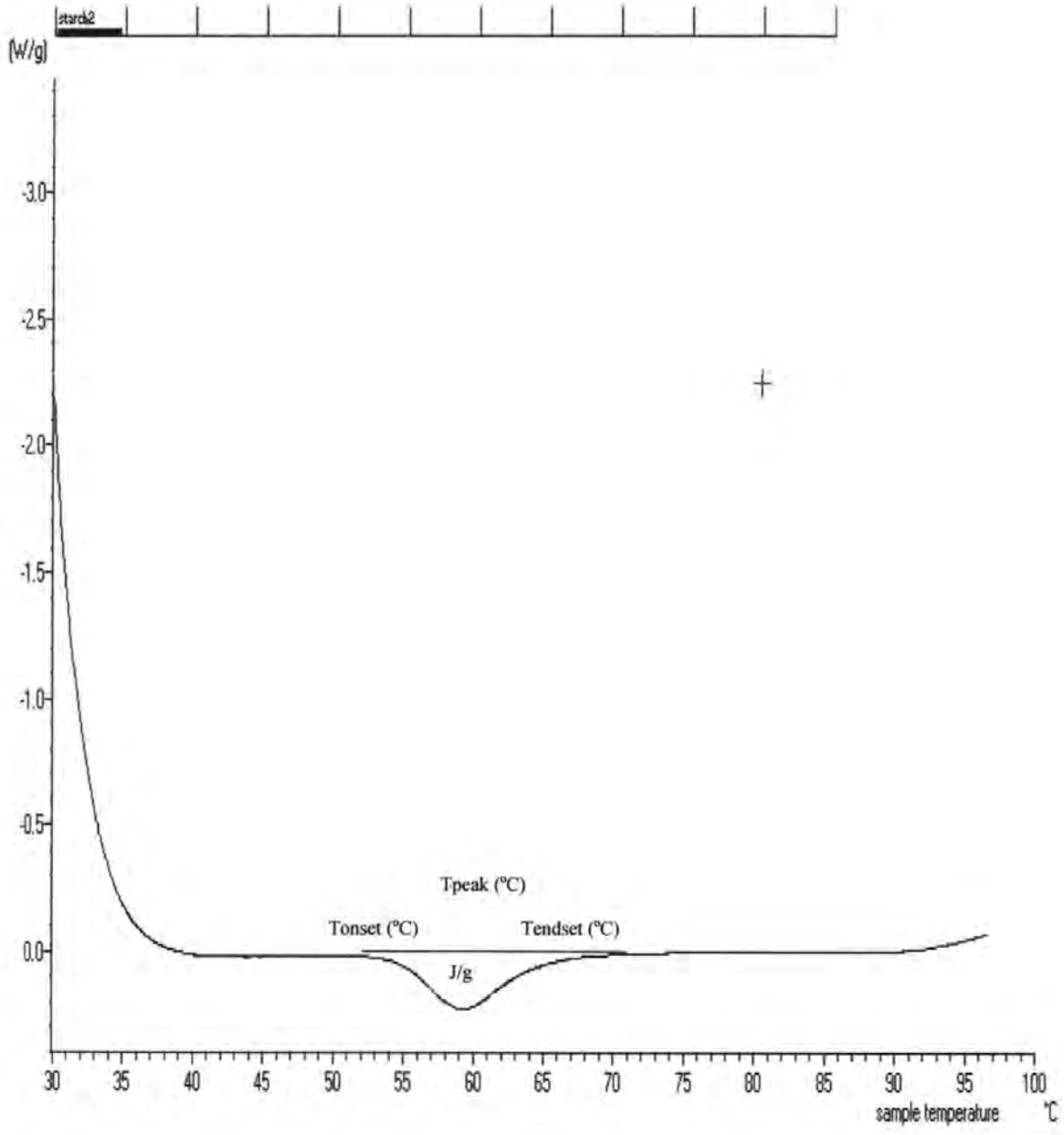


Figure AII.1 Example DSC[1] trace.

APPENDIX III

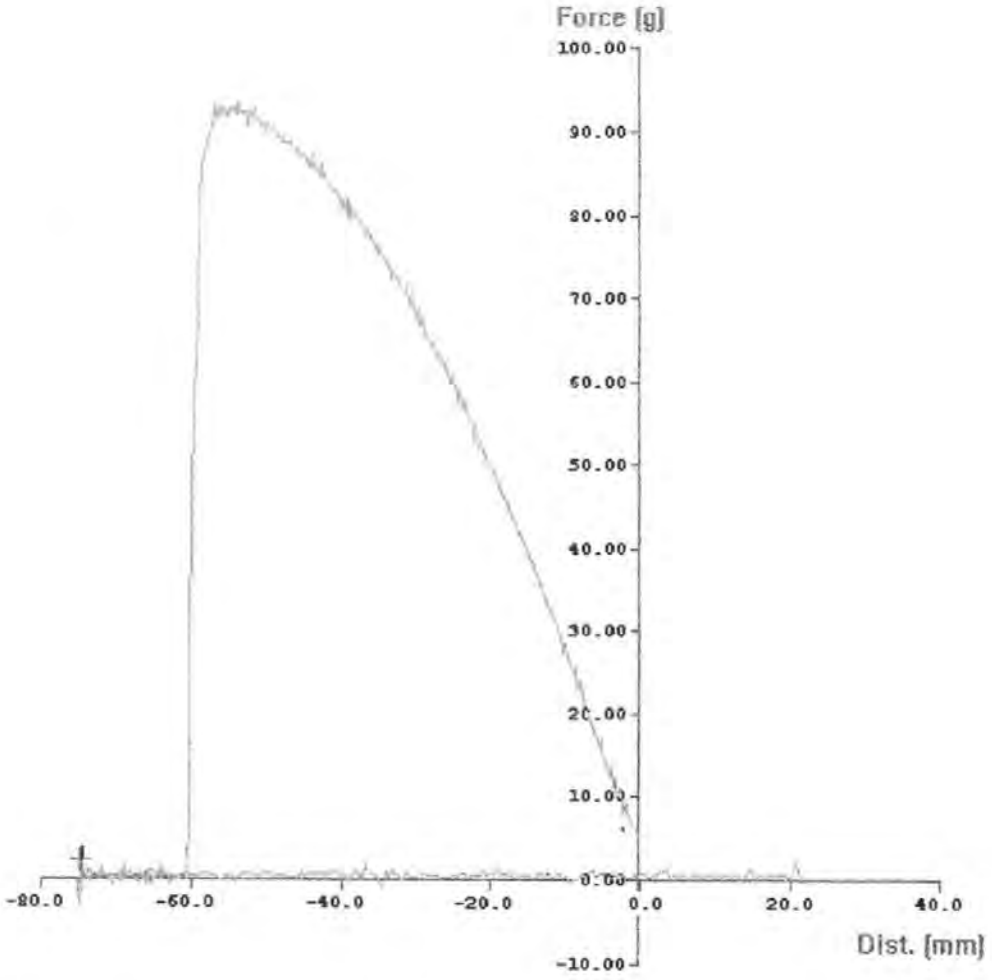


Figure AIII.1 Example TA trace (dough resistance to extension and extensibility).

APPENDIX IV

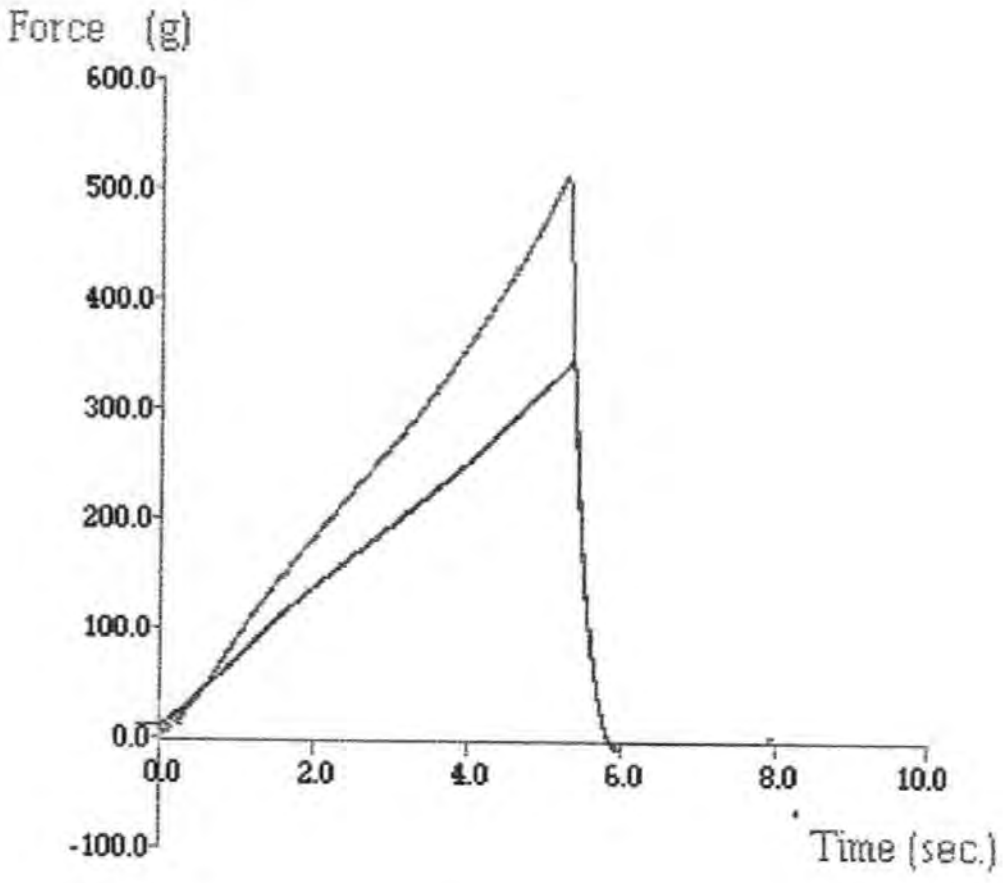


Figure AIV.1 Example TA trace (crumb firmness).

APPENDIX V

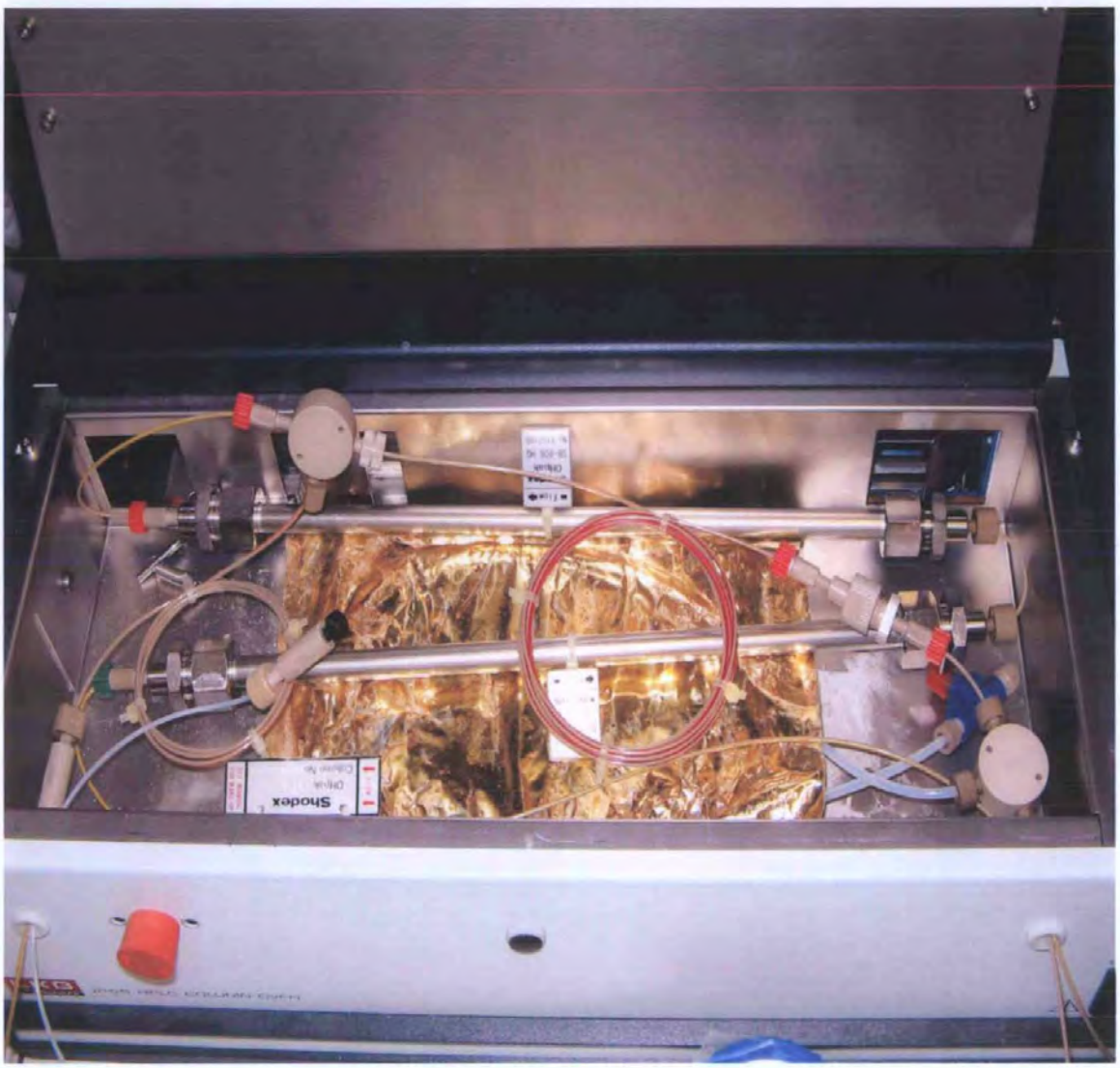
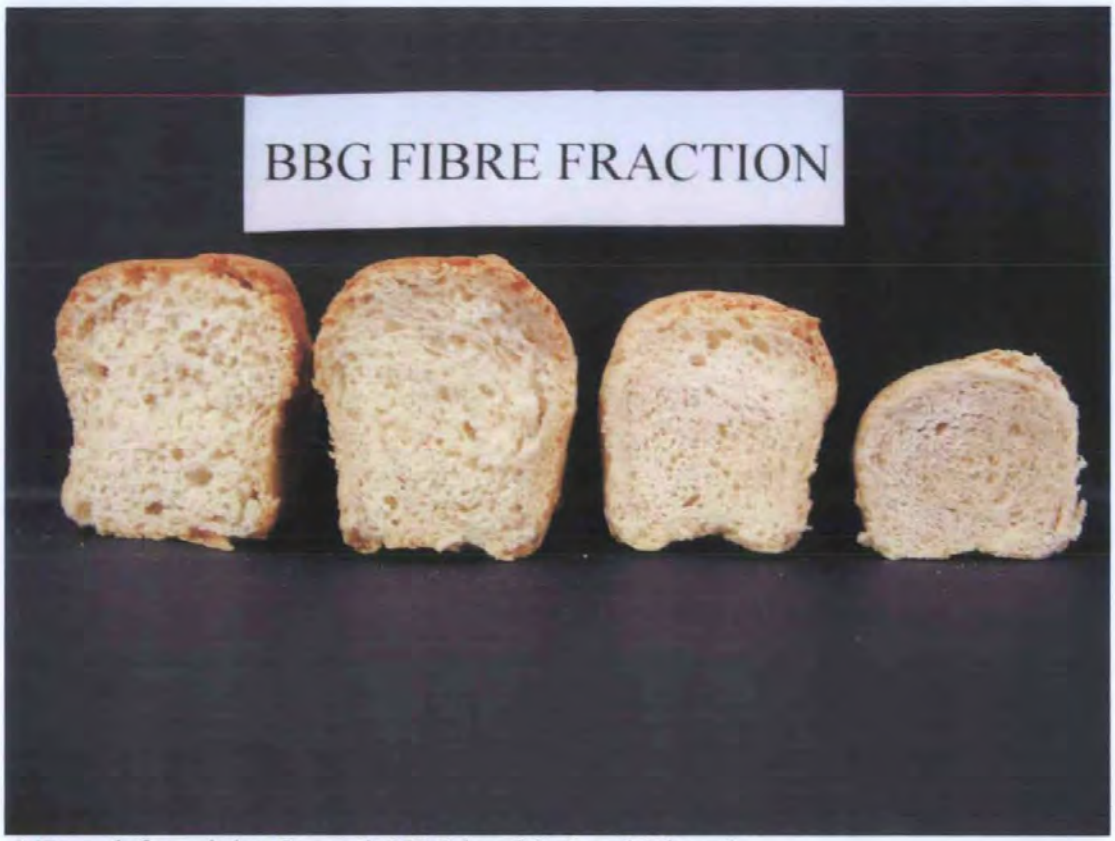
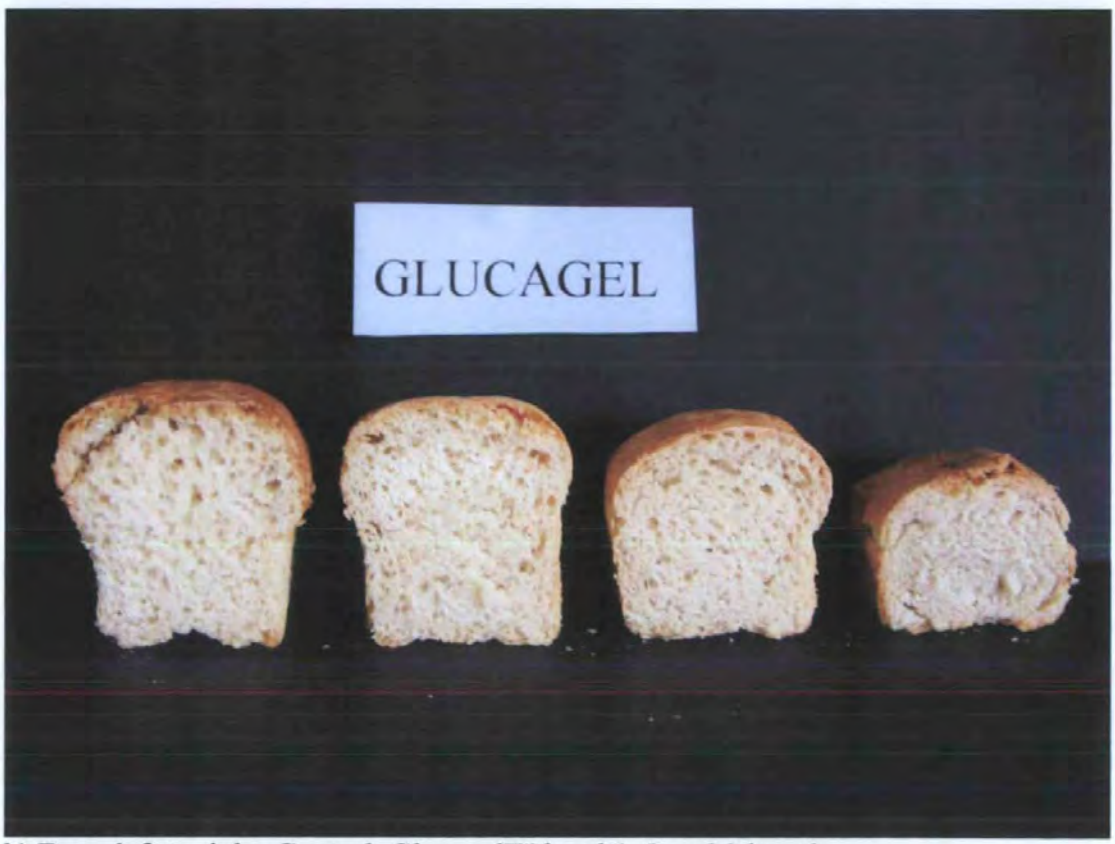


Figure AV.1 Internal view of a HPSEC-FD system.

APPENDIX VI



a) From left to right: Control, BBG level 1, 2 and 3 breads.



b) From left to right: Control, Glucagel™ level 1, 2 and 3 breads.

Figure AVI.1 Cross sectional views of a) BBG fibre fraction and b) Glucagel™ breads.

APPENDIX VII

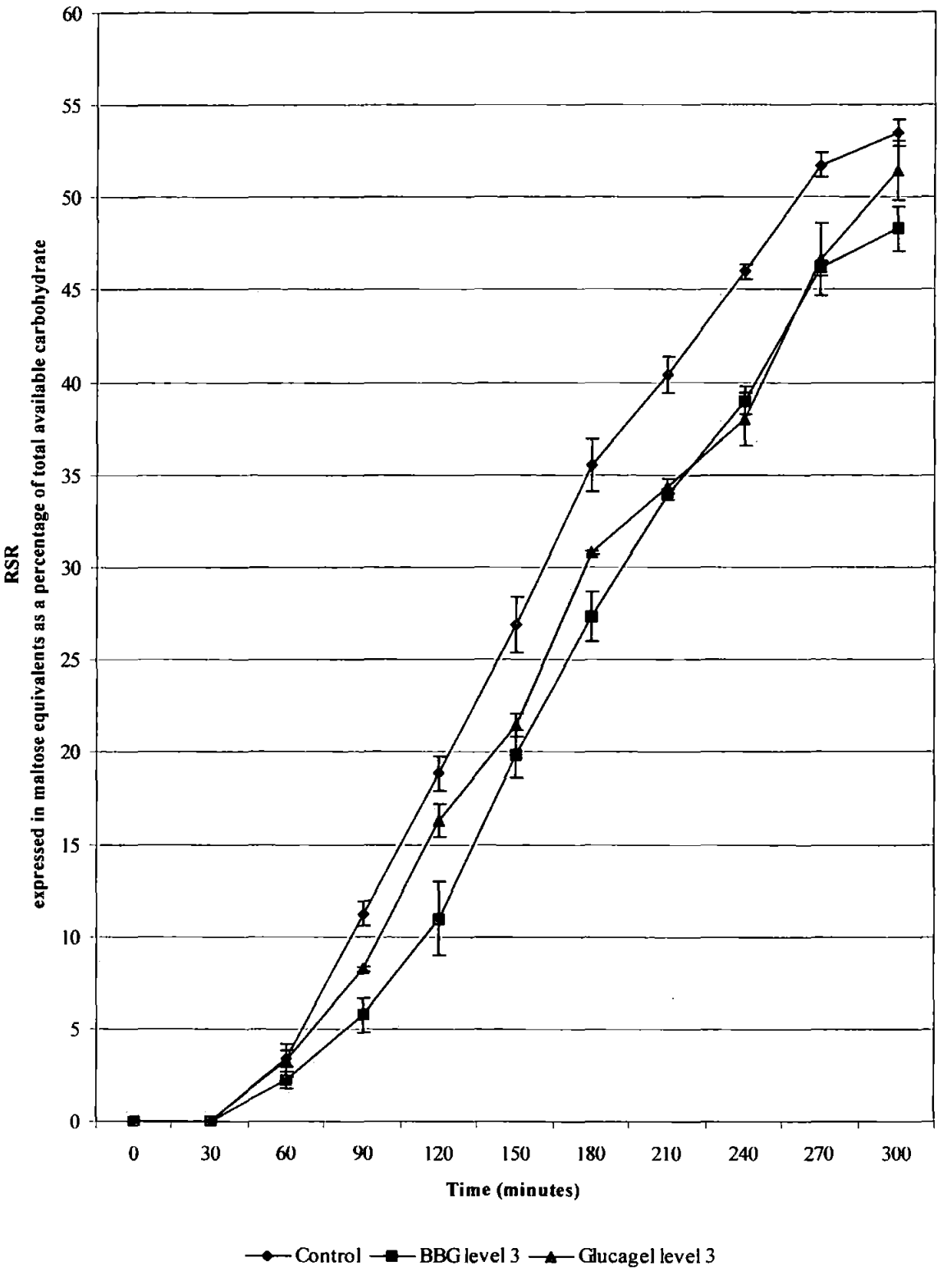
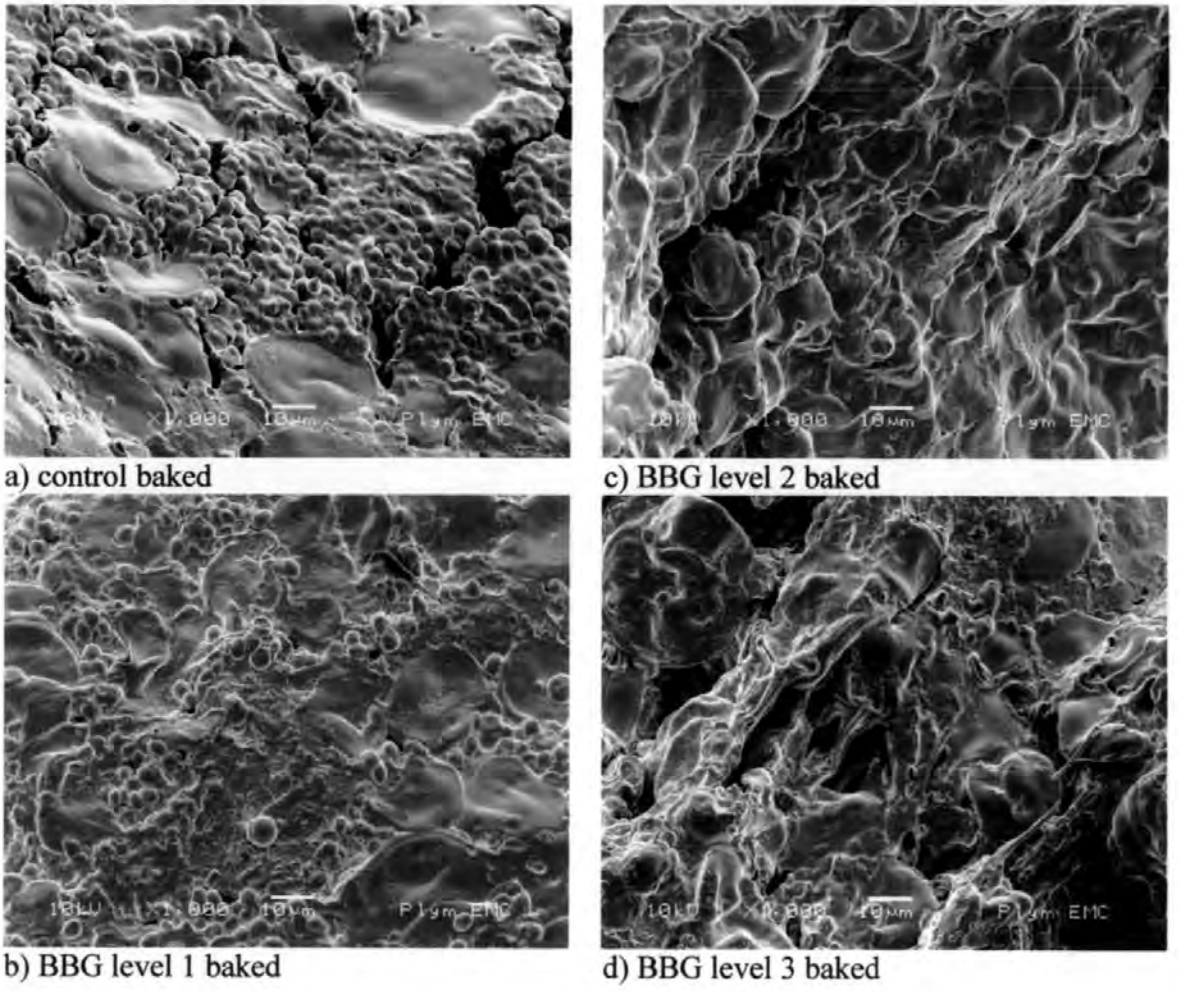


Figure AVII.1 *In vitro* starch digestibility (RSR) of control, BBG fibre fraction and Glucagel™ (level 3) breads.

APPENDIX VIII



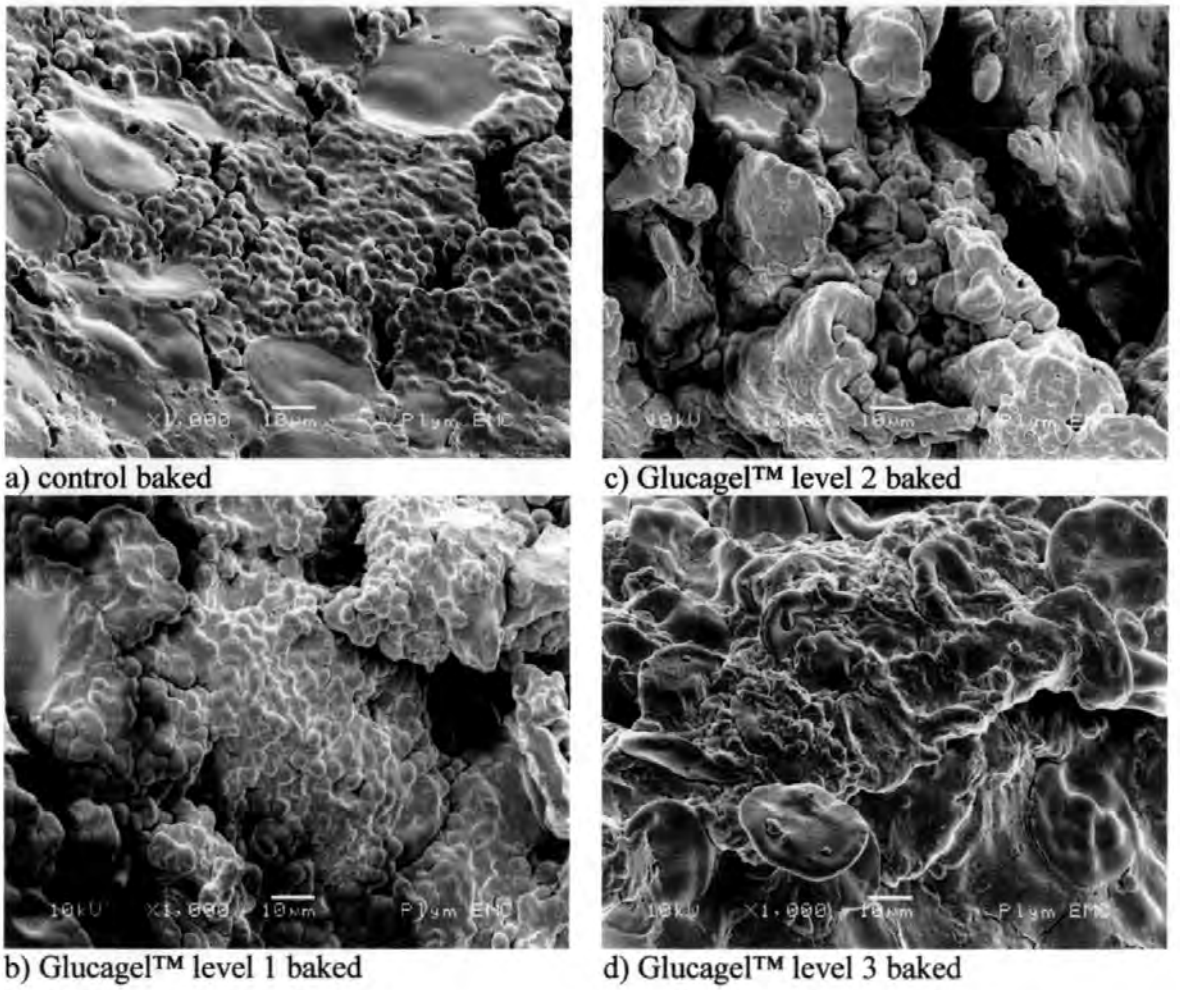
a) control baked

c) BBG level 2 baked

b) BBG level 1 baked

d) BBG level 3 baked

Figure AVIII.1 SEMs[1] (x 1000) of baked control and BBG fibre fraction breads.



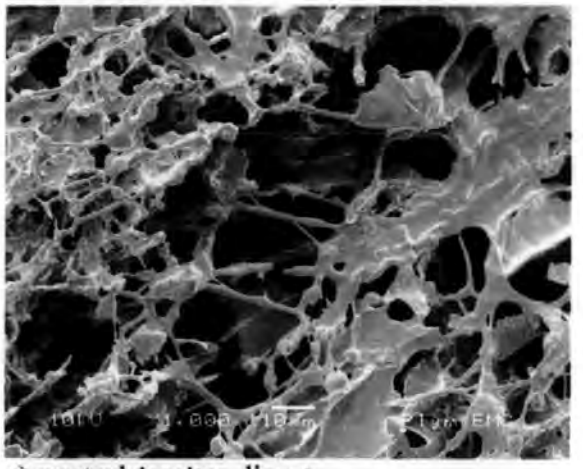
a) control baked

c) Glucagel™ level 2 baked

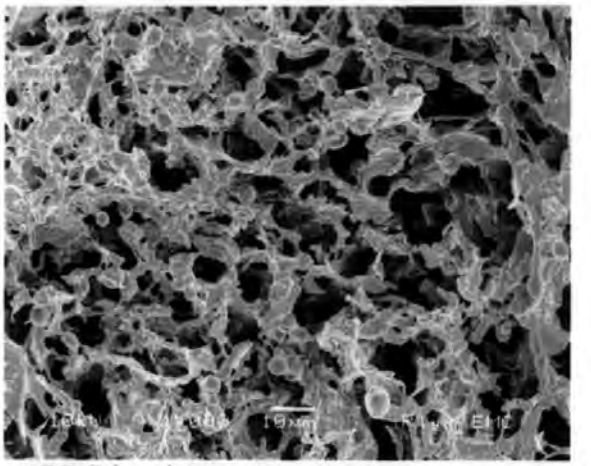
b) Glucagel™ level 1 baked

d) Glucagel™ level 3 baked

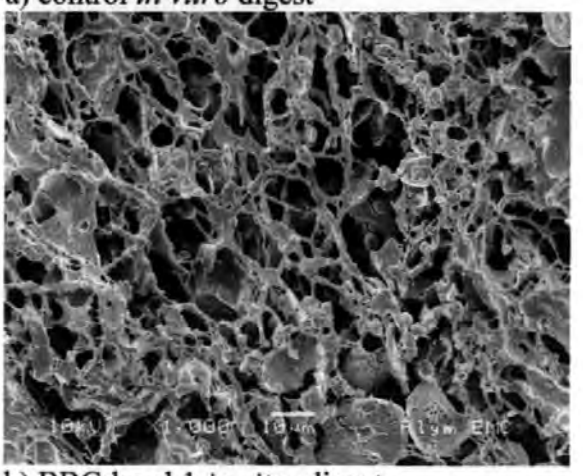
Figure AVIII.2 SEMs[1] (x 1000) of baked control and Glucagel™ breads.



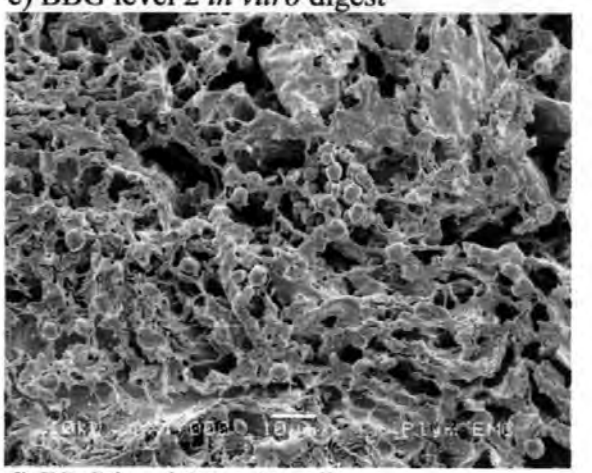
a) control *in vitro* digest



c) BBG level 2 *in vitro* digest

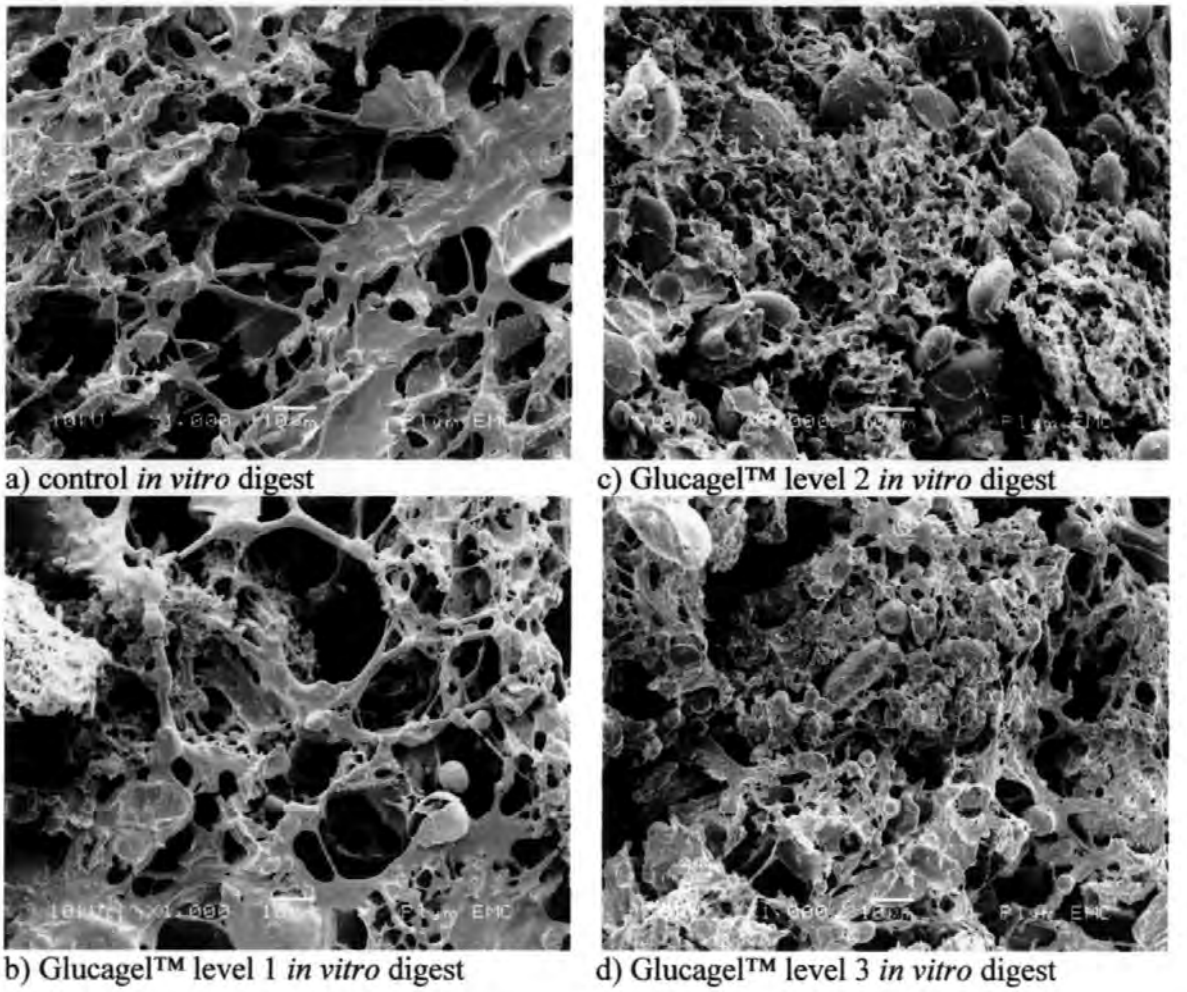


b) BBG level 1 *in vitro* digest



d) BBG level 3 *in vitro* digest

Figure AVIII.3 SEMs[1] (x 1000) of *in vitro* digested control and BBG fibre fraction breads.



a) control *in vitro* digest

c) Glucagel™ level 2 *in vitro* digest

b) Glucagel™ level 1 *in vitro* digest

d) Glucagel™ level 3 *in vitro* digest

Figure AVIII.4 SEMs[1] (x 1000) of *in vitro* digested control and Glucagel™ breads.

APPENDIX IX

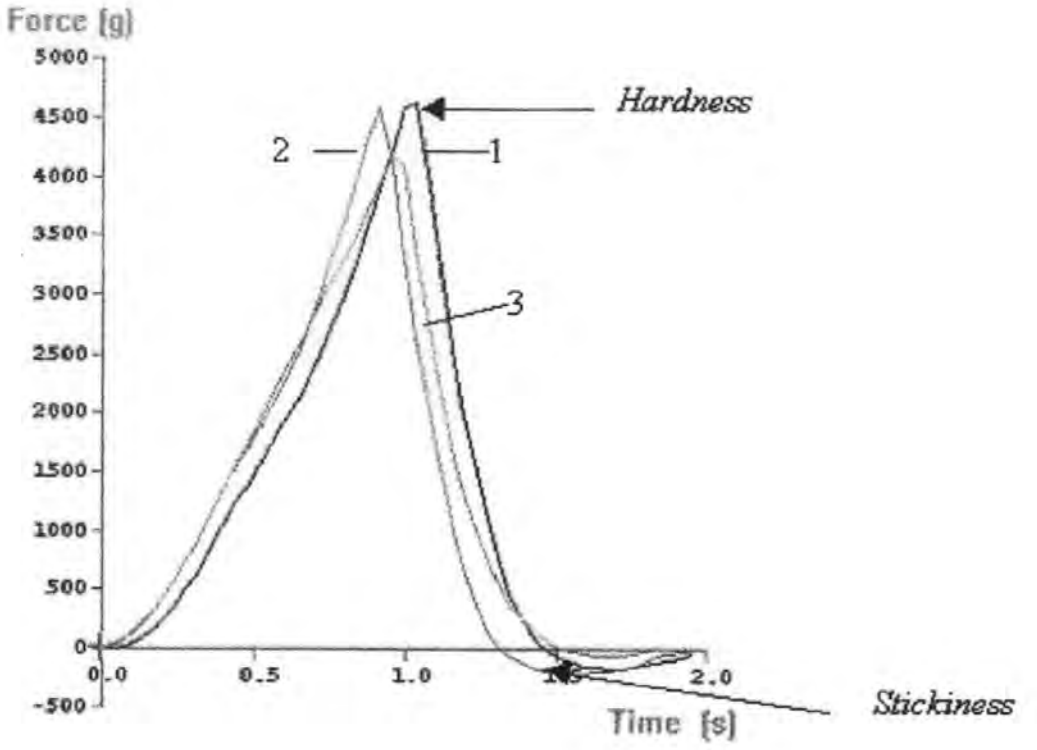


Figure AIX.1 Example TA trace (pasta hardness and adhesiveness).

APPENDIX X

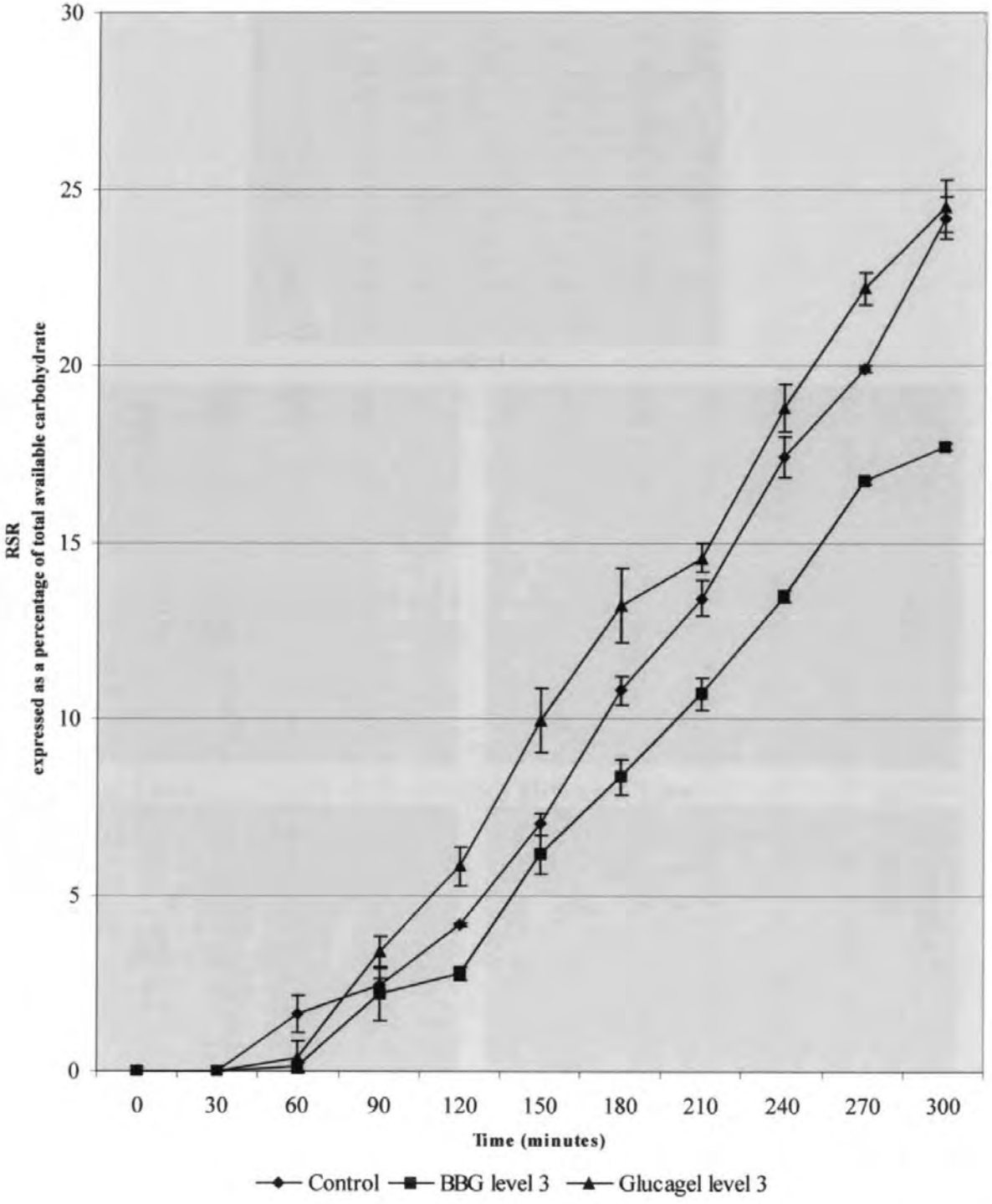
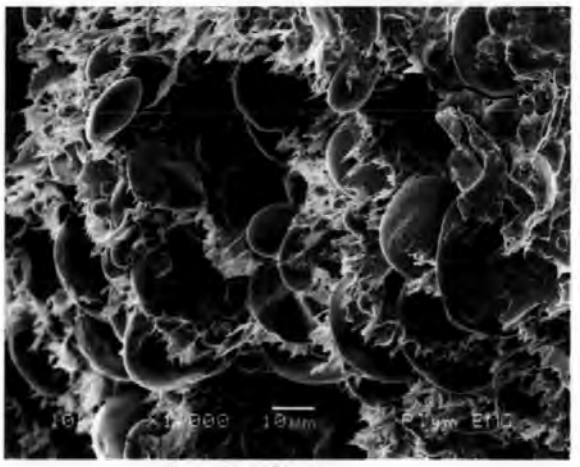
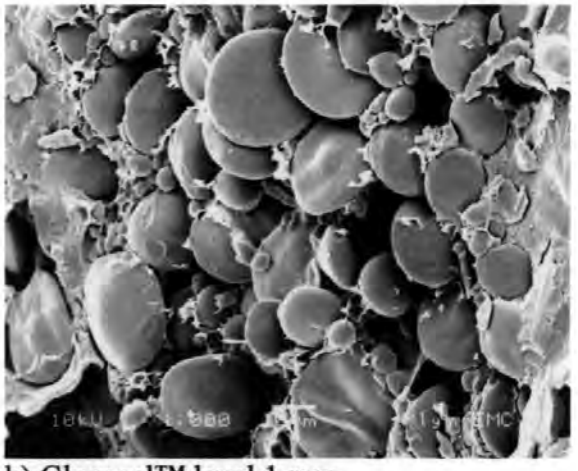


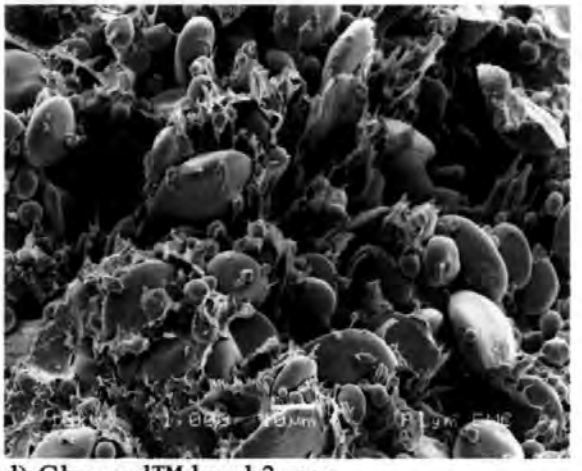
Figure AX.1 *In vitro* starch digestibility (RSR) of control, BBG fibre fraction and Glucagel™ (level 3) pastas.



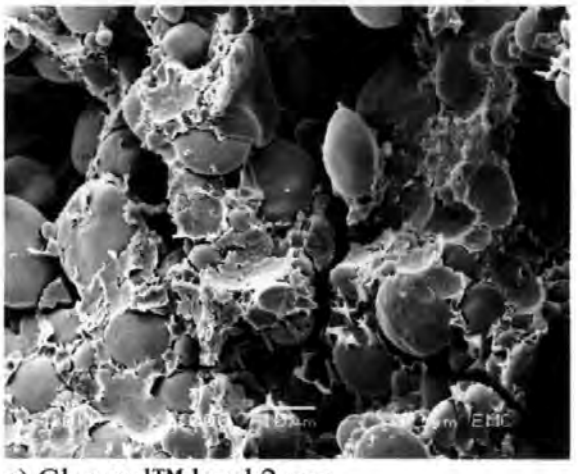
a) control raw



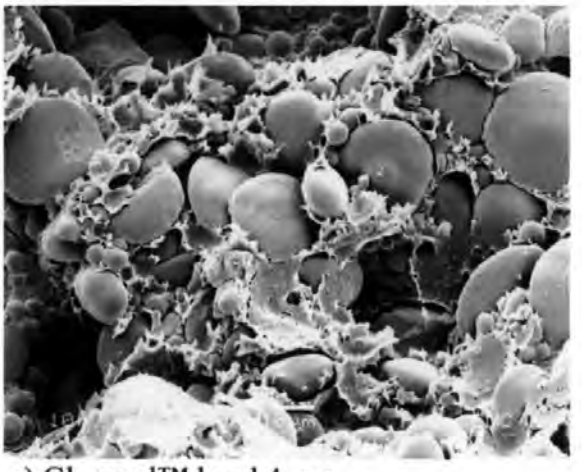
b) Glucagel™ level 1 raw



d) Glucagel™ level 3 raw

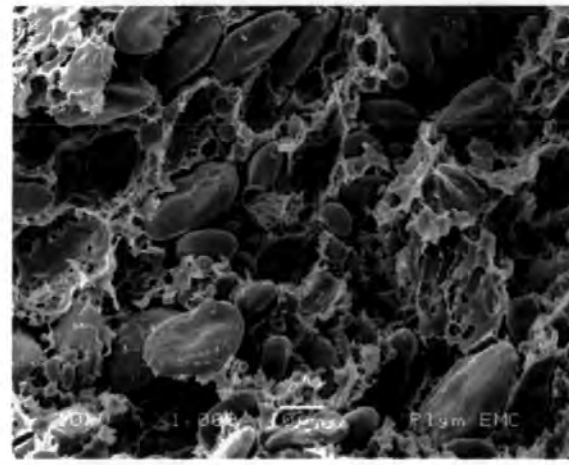


c) Glucagel™ level 2 raw

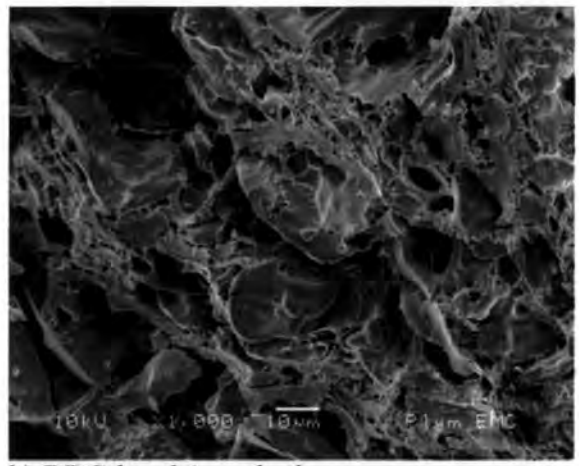


e) Glucagel™ level 4 raw

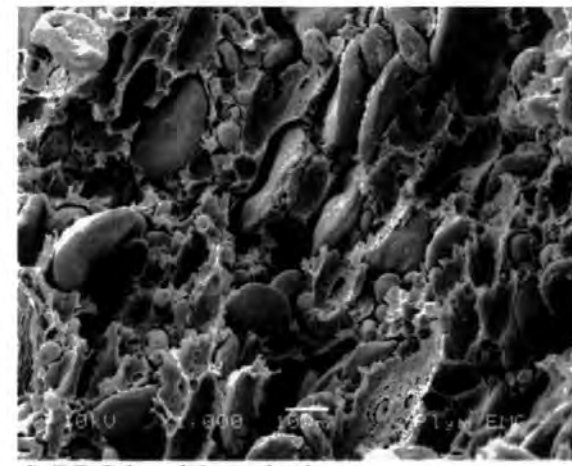
Figure AXI.2 SEMs[1] (x 1000) of raw control and Glucagel™ pastas.



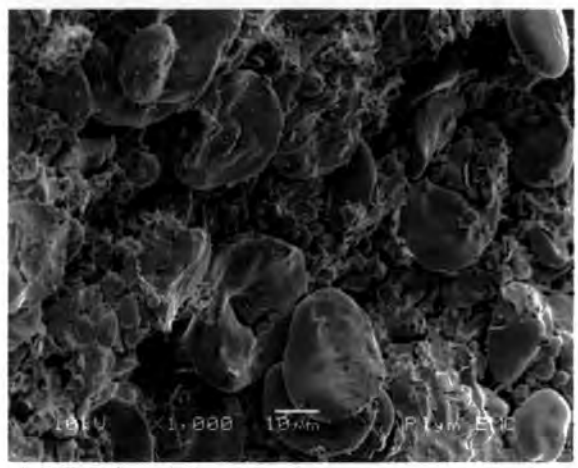
a) control cooked



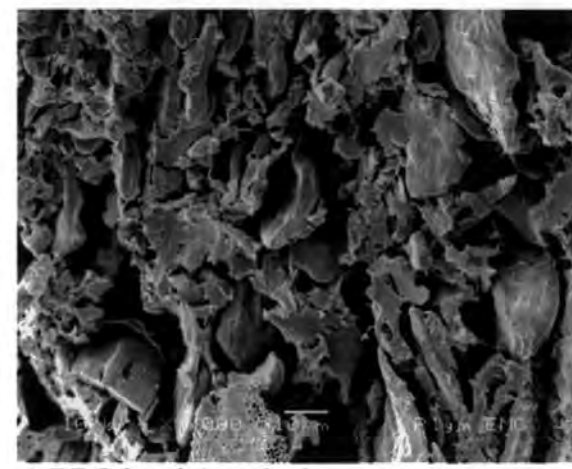
b) BBG level 1 cooked



d) BBG level 3 cooked

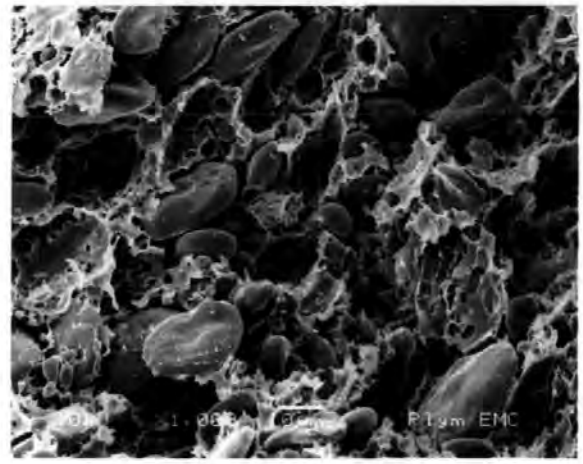


c) BBG level 2 cooked

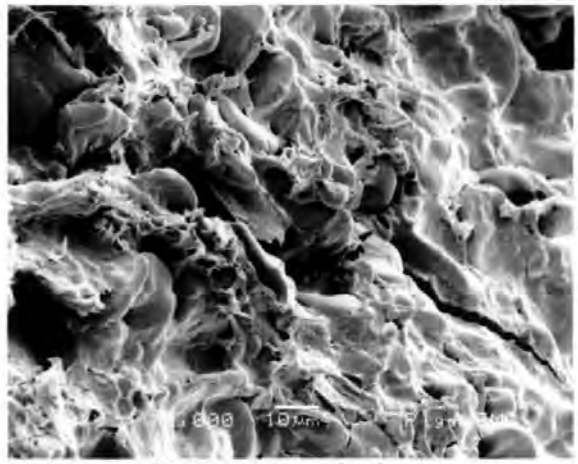


e) BBG level 4 cooked

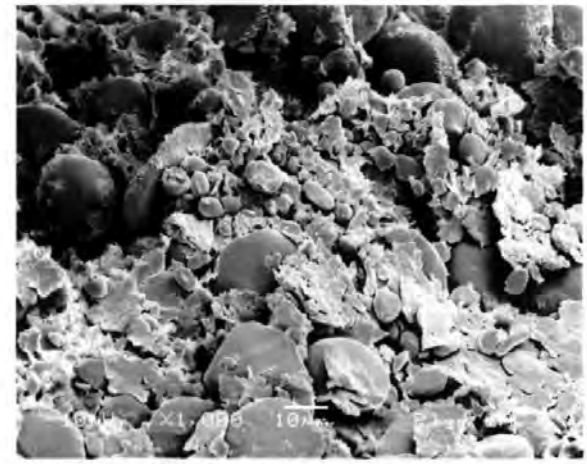
Figure AXI.3 SEMs[1] (x 1000) of cooked control and BBG fibre fraction pastas.



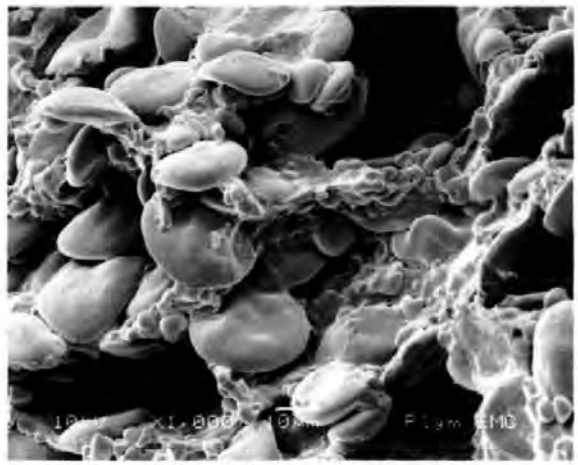
a) control cooked



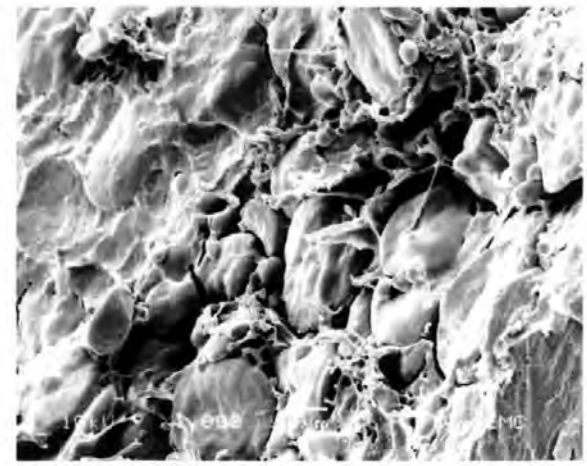
b) Glucagel™ level 1 cooked



d) Glucagel™ level 3 cooked

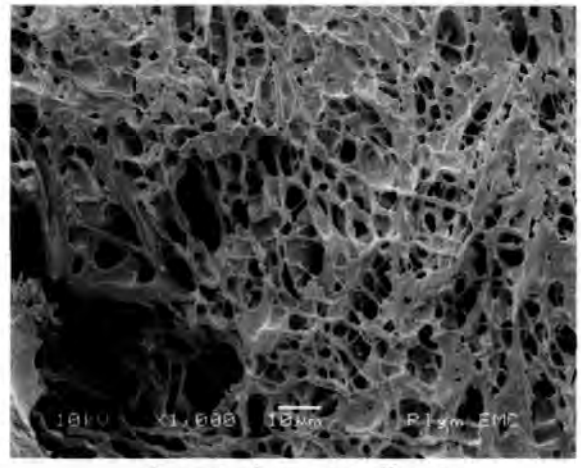


c) Glucagel™ level 2 cooked

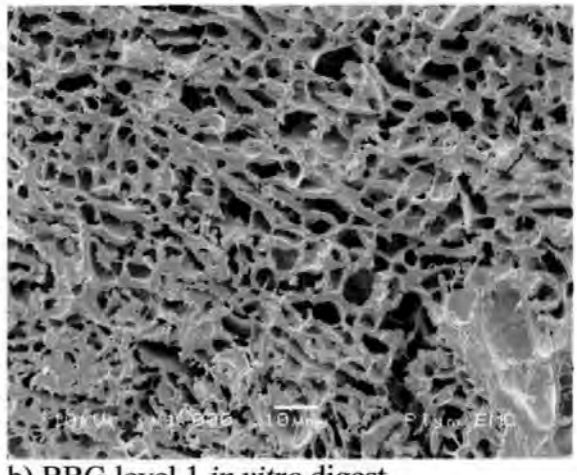


e) Glucagel™ level 4 cooked

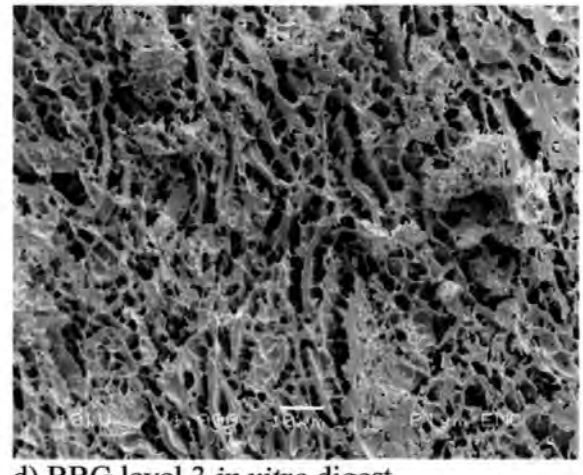
Figure AXI.4 SEMs[1] (x 1000) of cooked control and Glucagel™ pastas.



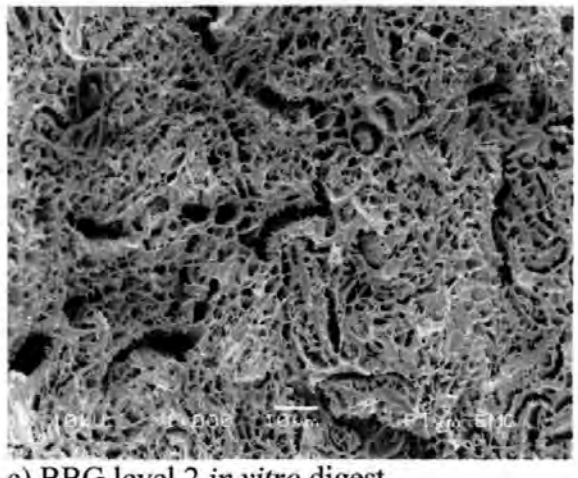
a) control *in vitro* digest



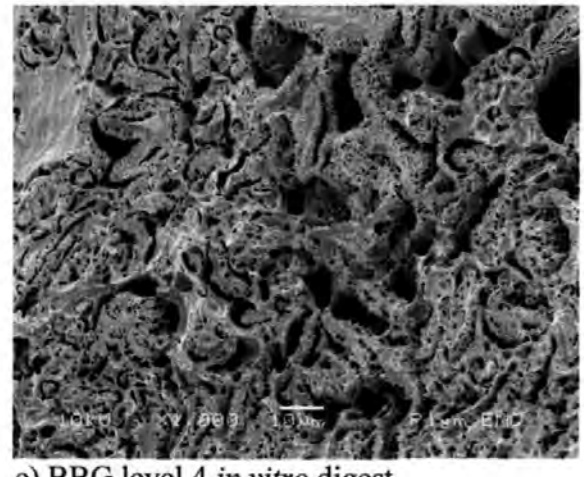
b) BBG level 1 *in vitro* digest



d) BBG level 3 *in vitro* digest

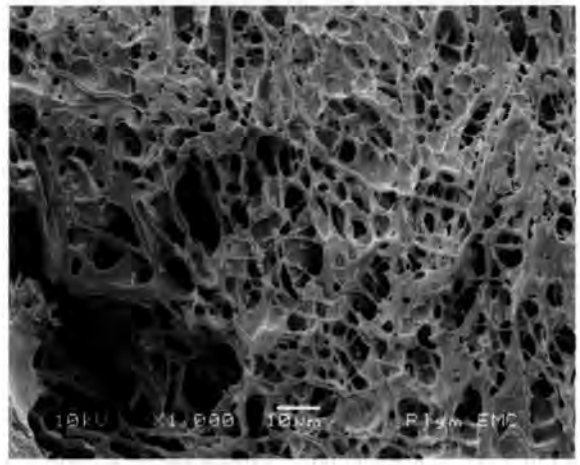


c) BBG level 2 *in vitro* digest

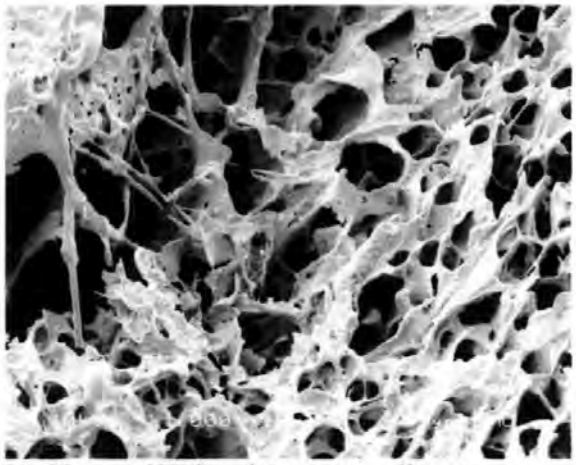


e) BBG level 4 *in vitro* digest

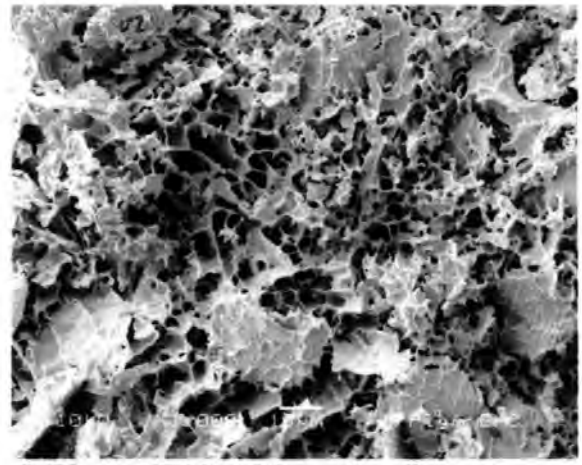
Figure AXI.5 SEMs[1] (x 1000) of *in vitro* digested control and BBG fibre fraction pastas.



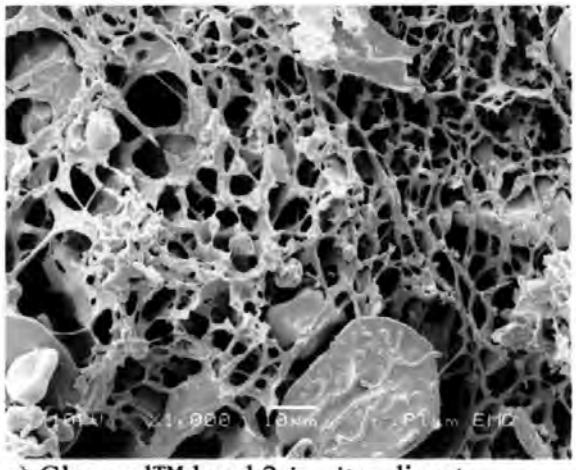
a) control *in vitro* digest



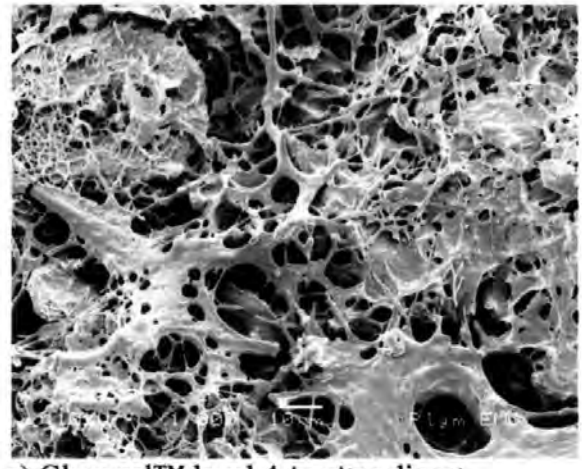
b) Glucagel™ level 1 *in vitro* digest



d) Glucagel™ level 3 *in vitro* digest



c) Glucagel™ level 2 *in vitro* digest



e) Glucagel™ level 4 *in vitro* digest

Figure AXI.6 SEMs[1] (x 1000) of *in vitro* digested control and Glucagel™ pastas.

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Review

The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients

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Abstract

The health-related importance of dietary fibre, as part of a balanced diet, has been recognised for decades. More recently, soluble fibre such as (1→3,1→4)-β-D-glucan (referred to as β-glucan), has been shown to have effects on the glycaemic, insulin, and cholesterol responses to foods. Cereals (such as barley and oats) are good sources for these functional ingredients, with studies clearly demonstrating their potential nutritional benefits. At the same time research has indicated that the efficacy of β-glucans may be related to extraction procedures, and factors such as dose, molecular weight and fine structure, and rheological characteristics of extracted and native β-glucans. Concurrently, research has focussed on the inclusion of β-glucans into both cereal and dairy-based food systems, illustrating their potential as ingredients to manipulate food structure and texture. Thus, β-glucans (from barley, oat, and other cereals) should be regarded as important functional ingredients for the cereal foods industry.

Keywords: Barley; Oats; Bread; Pasta; Dietary fibre; Glycaemic response

1. Introduction

Cereals are an important economic commodity worldwide. In the UK, the cereal harvest is predominated by wheat (15 million tonnes), with barley (7.8 million tonnes) representing the second most important cereal crop, and oats (0.6 million tonnes) being a relatively minor crop (HGCA, 1999). The (1→3, 1→4)-β-D-glucan, commonly referred to as β-glucan, content of cereals ranges from 1% in wheat grains, to 3–7% in oats, and 5–11% in barley (Skendi et al., 2003). Thus, barley grains are a rich source of β-glucans.

Barley belongs to the genus *Hordeum* and can be considered as one of the most ancient crop plants, with its cultivation being mentioned in the Bible. Archaeological studies have revealed two-rowed barley cultivation by about 8000 BC in Iran, with six-rowed barley appearing at approximately 6000 BC (Bothmer and Jacobsen, 1985). World production of barley in 2000/2003 was approximately 134 million metric tonnes. By far the leading barley producer is the EU (51.659 million tonnes) followed by the Russian Federation (25.013 million tonnes), and Canada (13.172 million tonnes).

The principal uses of barley are as feed for animals, in the form of barley meal, and as grain for malting and brewing in the manufacture of beer and whisky. Much research has focussed on the role of endosperm components in determining the malting potential of barley (Bamforth et al., 1979; Bathgate et al., 1974; Brennan et al., 1996a, 1997; Edney and Mather, 2004; Henry and Blakeney, 1986; Molina-Cano et al., 2002; Palmer, 1987). However, the barley crop may be considered relatively under-utilised with regard to its potential use as an ingredient in processed human foods. Recent attention has focussed on the potential use of β-glucan from barley and other cereals as a functional food ingredient (Malkki, 2004; Trepel, 2004).

Oats (genus *Avena*), are generally regarded as a minor cereal crop when considered in terms of grain produced

Abbreviations: DP, degree of polymerisation; FDA, US Food and Drug Administration; GI, glycaemic index; β-glucan, (1→3,1→4)-β-D-glucan; HDL, high density lipoprotein; HWM, high molecular weight; LDL, low density lipoprotein; LWM, low molecular weight; MW, molecular weight.

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annually, or areas sown for production. Traditionally, most of the crop has been used as animal feed. However, UK figures on the usage of oats (HGCA, 1999) sees slightly more of the crop (44%) going towards human and industrial uses, compared to the animal feed sector (38%). Oats have been linked to the health claims attributed to the use of β-glucans (Weightman et al., 2002, 2004) and are a valuable source of β-glucans.

The purpose of this review is to explore some of the applications, and potential nutritional advantages, of using cereal β-glucans (predominately those in barley grain) as functional food ingredients.

2. Occurrence of β-glucans in barley and oat grain

β-Glucans ((1→3,1→4)-β-D-glucans) are the predominant components of cell walls of cereal grains such as barley and oats (Bacic and Stone, 1981a,b; Beresford and Stone, 1983; Buckeridge et al., 2004; Wood et al., 1983; Wood, 1993). Traditionally there have been concerns with the use of barley in animal feeds due to the negative effect that β-glucans, in conjunction with other non-starch polysaccharides, have on nutrient uptake and body weight gain. Work conducted on poultry has clearly illustrated the effect these components have on reducing feed digestibility, metabolisable energy (Annison, 1991; Bergh et al., 1999; Classen, 1996; Jeroch and Danicke, 1995), and the occurrence of other negative consequences such as sticky droppings (Choct et al., 1999). However, most of these problems can be alleviated by the use of (1→3,1→4)-β-D-glucan hydrolysing enzymes in poultry feed (Almirall et al., 1995; Fuente et al., 1998; Von Wettstein et al., 2003). Similar observations have been made in relation to the digestibility of cereal feeds in the pig (Baidoo and Liu, 1998; Knudsen and Canibe, 2000; Leterme et al., 2000; Morel et al., 2003). Thus the perceived anti-nutritional aspects of β-glucans in feed material can be minimised by the addition of specific enzymes to diet formulations.

Additionally, levels of β-glucan have 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 (Bacic and Stone, 1980, 1981a,b; Bamforth and Martin, 1983; Bourne et al., 1982; Brennan et al., 1998; Edney and Mather, 2004) and ultimately the viscosity of wort during brewing (Bourne and Pierce, 1970). Levels of β-glucan can vary dramatically between varieties, but usually range from 2 to 6% dry weight (Bamforth, 1981; Zhang et al., 2002). 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.

Genetic and environmental factors impact on β-glucan content of barley grain (Knuckles et al., 1992; Savin et al., 1997; 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; Henry and Blakeney, 1986; Morgan et al., 1983; Stuart et al., 1988). For instance, Lehtonen and Ailasalo (1987) reported that two-row barley genotypes had higher β-glucan content than six-row barley. Studies have also indicated that waxy barley cultivars, with up to 100% amylopectin, have higher levels of β-glucans in their endosperm than non-waxy varieties (Ulrich et al., 1986; Yoon et al., 1995).

One of the major environmental factors that influence β-glucan levels appears to be the availability of water during grain maturation. Dry conditions (heat stress) before harvest have been shown to result in high β-glucan levels (Bendelow, 1975), with a positive relationship between β-glucan content and final grain weight (Savin and Molina-Cano, 2001). However, other experiments show a reduction in grain β-glucans related to heat stress within the plant during grain fill (Savin et al., 1997; Savin and Nicolas, 1996). This observation agrees with field studies on the effect of drought conditions on β-glucan content of the grain (Coles et al., 1991; Stuart et al., 1988). Conversely, moist conditions have been reported to cause a decrease in β-glucan levels (Aman et al., 1989; Stuart et al., 1988), so that increased levels of irrigation reduce β-glucan content of the grain (Guler, 2003). This may either be related to the fact that final grain fill is adversely affected in drought conditions through impairment of starch synthesis and deposition, or because β-glucan synthesis may be enhanced in dry conditions (Munck et al., 2004).

More recent research by Weightman et al. (2004) concentrated on the effect of nitrogen fertiliser treatments on the levels of β-glucan in oats. A positive correlation between protein and β-glucan content of the grain suggested that β-glucan deposition was associated with protein accumulation. Doehlert et al. (2001) examined the genotypic and environmental effects of grain yield, and composition, of oat lines grown in North Dakota over a 3 year period. In this case the authors found that although a positive correlation was established between starch content and β-glucan levels within the grain, correlations between β-glucan and protein content of the grain were not homogenous across genotypes. A negative interaction was found between β-glucan levels in oats and both crop yield and test weight of the grain. Peterson et al. (1995), examining the agronomic quality of a number of oat lines, found that many of the correlations between β-glucan content and agronomic quality characteristics of the grain, were inconsistent between the different oat lines. As such, the relationship between β-glucan levels in cereal grains and grain quality, or yield parameters, appear to vary greatly depending upon genetic background of the cereal line being examined.

3. Characteristics of cereal (1→3, 1→4)-β-D-glucans

The non-starch polysaccharides found in mature barley grain include the cell wall (1→4)-β-D-glucan (cellulose) (1→3, 1→4)-β-D-glucan, arabinoxylan and glucomannan and the cytoplasmic fructans (Izydorczyk et al., 2003). The endosperm cell wall β-glucan components are linear molecules with approximately 30% (1→3)- and 70% (1→4)-linkages (Bacic and Stone, 1981a; Fincher, 1975; Forrest and Wainwright, 1977). The composition of the walls of the starchy endosperm and the aleurone, both endosperm tissues, are qualitatively similar, but quantitatively different. Thus, walls of the starchy endosperm of barley consist of about 70% β-glucan and 20% arabinoxylan whereas the aleurone walls contain 26% β-glucan and 67% arabinoxylan, both have similar amounts of glucomannan and cellulose (Bacic and Stone, 1981a,b; Wood et al., 1983; Woodward et al., 1983, 1988).

In the linear β-glucan chain the (1→3)-linkages occur singly whereas the (1→4)-linkages are found mostly in sequences of 2 or 3 (Skendi et al., 2003), but sequences of up to 1→4 have been reported (Cui et al., 2000). Hence the molecules may be regarded as being composed of (13)-β-linked cellotriosyl and cellotetraosyl units (Wood, 2001). The application of high performance anion-exchange chromatography to the separation of the oligosaccharide release by specific hydrolysis has aided the analysis of the fine structure of β-glucans from different botanical sources. Such differences have been seen between isolated β-glucan oligosaccharides from oats, barley and other cereals. In particular, the ratio between cellotriosyl and cellotetraosyl units is higher in barley than oats (Cui et al., 2000; Tosh et al., 2004a; Wood et al., 1994b). Molecular weight (MW) ranges reported for β-glucans also show variability between cereals, with oat β-glucans generally having a higher upper MW ($0.065–3\times10^6$ g/mol) compared to barley ($0.15–2.5\times10^6$ g/mol) (Beer et al., 1997a,b; Irakli et al., 2004; Lazaridou et al., 2003, 2004; Wood et al., 1991). The rheological properties of appear to depend on a number of factors including the ability of β-glucan chains to associate, determined by the proportion of cellotriosyl/cellotetraosyl units and their arrangement (Cui and Wood, 2000; Izawa et al., 1993; Tosh et al., 2004a,b) and by the degree of polymerisation (DP) and hence the MW of the β-glucan (Tosh et al., 2004b; Wood, 2001).

Factors which affect the rheological characteristics of β-glucans have obvious links to their viscosity behaviour (either as native forms or as extracts in formulated foods), and their potential effects on food structure, texture and nutritional properties. For instance, Tosh et al. (2004a) showed that differences in the ratio of cellotriosyl/cellotetraosyl units affected the gelation characteristics and elasticity of β-glucan systems. Indeed, reduced solubility of β-glucans has been attributed higher ratios of cellotriosyl/cellotetraosyl units (Skendi et al., 2003).

Lazaridou and Biliaderis (2004) demonstrated that the storage modulus (G') of β-glucan cryogels increased with decreasing MW, and hence a reduced gelation time and increased gelation rate were observed (Lazaridou et al., 2003 and 2004a). Similarly, Vaikousi et al. (2004) investigated the solution flow behaviour and gelling properties of barley β-glucans. Their work on low molecular weight (LWM) β-glucans illustrated that gelation time was decreased for β-glucan gels from LMW sources, and that gels made from high molecular weight (HMW) β-glucan sources exhibited increased yield stress and reduced storage modulus (G'_{max}) values. Similar findings have been reported for water-extractable β-glucans from Greek barley cultivars (Irakli et al., 2004). Thus, because the viscoelastic characteristics of β-glucan gels are related to the MW of the isolated fractions, differences in MW observed between barley and oat β-glucans, and among β-glucans extracted from different cultivars of barley (Izydorczyk et al., 1998a,b) and oats (Autio et al., 1992; Skendi et al., 2003) need to be considered in relation to their potential behaviour in food systems.

4. Extraction procedures

Barley and oat β-glucans, together with other non-starch polysaccharides, occur in the walls of the endosperm cells which enclose starch, matrix protein and lipid reserves of the grain. Thus their recovery is not straightforward. The study of the physicochemical properties of isolated β-glucan fractions requires extraction procedures which optimise yield, purity and retained integrity of the β-glucan molecule. In extraction procedures to obtain a potential food ingredients these considerations have to be balanced and a compromise reached. For instance, the MW profile of β-glucans can be influenced by the method of extraction used (Tosh et al., 2003; Wang et al., 2003).

Much research has focussed on the effects of isolation and purification techniques on the structural, physiochemical and physiological properties of barley β-glucan (Bhatty, 1993, 1995; Burkus and Temelli, 1998; Fincher, 1975; Klopfenstein and Hoseney, 1987; Temelli, 1997; Woodward et al., 1983, 1988).

The extraction of β-glucans from cereal grains generally involves three basic steps: (1) inactivation of endogenous enzymes, (2) extraction of the β-glucans, (3) precipitation of the β-glucans.

Endogenous β-glucanases, need to be inactivated since they are responsible for β-glucan degradation leading to a decrease in the molecular weight, and thereby the functional properties of the extracted β-glucan (Irakli et al., 2004). Inactivation is usually achieved by refluxing the barley with aqueous ethanol or treating the barley flour with dilute aqueous ethanol at temperatures above 60 °C. Additionally, as starch polymers can be co-extracted with the β-glucan when the temperature of extraction rises above 60 °C

(gelatinisation temperature), care must therefore be taken to remove starch components from the extracts.

Key extraction methodologies for barley and oat β-glucans were developed by Wood et al. (1977). The researchers assessed the effects of particle size, temperature, pH and ionic strength on β-glucan yield on the laboratory scale, and prepared an oat gum fraction (from oat bran) on a pilot plant scale by extracting hot 75% ethanol-inactivated oat bran (outer starchy endosperm and overlying aleurone and pericarp-seed coat) with a sodium carbonate solution at pH 10 to give a preparation containing 78% β-glucan (Wood et al., 1989). Although this simple extraction process was successful in generating β-glucan material from cereals, McCleary (1988) showed that sequential water extractions at 40, 65, and 95 °C, increased the extraction rate of barley β-glucans to 90%, thus enabling an increase in overall yield. Different extractants were investigated by Bhatty (1993) who showed that optimum recovery barley and oat gums with retention of viscosity characteristics could be obtained using 1 M NaOH. However the extract was contaminated with considerable amounts of starch and protein, resulting in an impure product. To counteract this, Saulnier et al. (1994) used a hot water extraction procedure in the presence of thermostable *alpha*-amylase to minimise the contamination from starch, and to optimise the purification of the β-glucan material.

One of the major limiting factors to the industrial utilisation of these extraction techniques by the food industry is their cost. Thus, pure preparations of β-glucans have often been ignored as potential functional food ingredients, mainly due to the relatively inexpensive use of barley or oat flour fractions. This in turn has meant that the actual characteristics of these products in food systems are often variable due to fluctuations in protein or starch composition of the flour fractions. Hence subsequent viscosity, structural and nutritional effects on foods have to be considered in relation to the nature of the β-glucan extract, or the composition of the flour material used.

Investigation of different organic solvents as precipitants of β-glucans (Beer et al., 1996; Morgan and Ofman, 1998) has shown that the structural conformation, MW, and hence solubility, of the precipitated β-glucan is affected by the extraction solvent. To offset these potential negative factors, whilst endeavouring to produce a more cost effective extraction process, Morgan and Ofman (1998) developed a hot water extraction procedure with recovery of the β-glucan by freezing and thawing of the extract. The resulting product ('Glucagel™') contained between 89 and 94% β-glucan, depending on the duration of the initial extraction and is one β-glucan preparation commercially available as a food ingredient.

The temperature and pH of the extraction process also affects the recovery of β-glucans. Temelli (1997) demonstrated that β-glucan extraction increased with temperature. A further evaluation of the effect of extraction conditions on yield, composition and viscosity stability of barley gum was conducted by Burkus and Temelli (1998) using regular barley (Condor) and a waxy cultivar blend. Extraction conditions were evaluated including an extraction with no additional treatment, boiling of the extract prior refluxing of flour with 70% ethanol, and treatment of extract with thermostable *alpha*-amylase. The highest β-glucan purity was achieved with a boiled Condor extract at pH 7 (81.3% yield), closely followed by refluxed waxy barley extracted at pH 8 and amylase treated (79.3% yield). Refluxed gums followed by purification at pH 7, had the most stable viscosity. Symons and Brennan (2004a) also compared extraction procedures showing that extraction with thermostable *alpha*-amylase yielded the purest β-glucan fraction.

As previously mentioned, the nature of the extraction procedure can have a profound effect on the molecular weight, which in turn affects its functional behaviour. Carr et al. (1990) observed that the use of NaOH for complete extraction resulted in partial depolymerisation of the β-glucan. Although Knuckles et al. (1997a) included sodium borohydride in NaOH extraction at 65 °C to prevent alkaline depolymerisation, the MW of the extracted β-glucan was lower than with water at 100 °C. Beer et al. (1997a) also observed that the MW of β-glucan extracted from oats and barley with NaOH was lower than that extracted with hot water. Knuckles et al. (1997a) also demonstrated that sequential extractions resulted in a decrease in the molecular weight of the β-glucan in the extract. However, the temperature used for sequential water extractions has also been shown to affect MW, the ratio of $(1\rightarrow4)$ to $(1\rightarrow3)$ linkages, and the amount of cellulosic regions on the β-glucan chain (Storsley et al., 2003). Care must therefore be taken to optimise the yield and rheological characteristics and avoid depolymersiation during extraction of β-glucan components.

5. The role of β-glucans as components of dietary fibre

Much of the more recent interest in the use of β-glucans in food systems has stemmed from their 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 the Dietary Fibre Technical Committee of the American Association of Cereal Chemists AACC (2000, 2001, 2003).

Potential health benefits of dietary fibre include, reduction of bowel transit time (Feldheim and Wisker, 2000), prevention of constipation, reduction in risk of colorectal cancer (Bingham, 1990; Faivre and Bonithon-Kopp, 1999; Hill, 1997), lowering of blood cholesterol and regulation of blood glucose levels for diabetes management (Bornet et al., 1987; Frost et al., 1999; Gallagher et al., 1993; German et al., 1996), production of short chain fatty acids (Karppinen et al., 2000; Velasquez et al., 2000; Wisker et al., 2000), promotion of the growth of beneficial

gut microflora (i.e. as a prebiotic) (Crittenden et al., 2002; Tungland, 2003).

Research into dietary fibre has broadly examined the effects of soluble and insoluble fractions as purified fibre, or in naturally fibre-rich whole foods. High fibre foods have been related to the modulation of glycaemic response, on the basis of studies by Jenkins et al. (1976–1978) using both purified fibre, and naturally fibre-rich foods (Jenkins et al., 1980; Truswell, 2002; Tudorica et al., 2002). In particular, foods high in soluble dietary fibre have been shown to have a positive effect on reducing hyperglycaemia and hyperinsulinaemia, in relation to the control of diabetes (Brennan and Tudorica, 2003; Li et al., 2003a) and the reduction of risk factors for degenerative diseases, such as obesity (Burley et al., 1987), hyperlipidaemia (Jenkins et al., 1985; Maki et al., 2003;Yang et al., 2003), cardiovascular disease (Keogh et al., 2003), cancer (Sier et al., 2004) and hypertension (Anderson, 1983, 1990).

Many attempts have been made to clarify the mechanisms by which dietary fibre and β-glucans have these effects. The potential reduction of glycaemic response following ingestion of dietary fibre has led to proposals which implicate: the amount and quality of fibre (Nishimune et al., 1991; Wolever, 1990); increased intrinsic viscosity of the food in combination with fluids (Mourot et al., 1988) and hence the gastrointestinal environment; maintenance of physical integrity of the food material (O'Dea et al., 1980) and incomplete starch gelatinisation (Brennan et al., 1996b; Ross et al., 1987; Tudorica et al., 2002). The cholesterol-lowering potential of cereal fibre is considered to result from effects manifest in the upper gastrointestinal tract. These in turn may be related to the ability of cereal fibre to form a gel-like network and alter gastrointestinal viscosity (Reimer et al., 2000; Thorburn et al., 1983).

6. Physiological effects of β-glucan enrichment in cereal food

A small number of studies have indicated that β-glucans may have a preventative role in the aetiology of colorectal cancer. Part of this response may be due to effects of β-glucans in increasing caecal and colon mass through increasing the resistance of starch to digestion, and hence altering the amount of fermentable material reaching the cecum. Higher levels of fermentable material in the caecum will in turn lead to increased short chain fatty acid (SCFA) levels in the caecal contents (Dongowski et al., 2002). This 'bulking' effect of dietary fibre may be a consequence of increased water holding capacity of fibre rich foods. The effect of β-glucans, in the form of oat bran and gum, in promoting gastric emptying due to the rheological characteristics of the β-glucans (Johansen et al., 1996, 1997) may help explain some of these events. Other notable, but less well documented effects of β-glucans include the diminished absorption of nutrients (Edwards et al., 1988; Lund et al., 1989), prolonged postprandial satiety (Anderson, 1990; Bourdon et al., 1999) and increased stool bulk and relief of constipation (Hojgaard et al., 1980; Odes et al., 1993; Valle-Jones, 1985).

The most widely documented nutritional benefit of β-glucan in foods is the flattening of the postprandial blood glucose and insulin rises. Both barley (Hallfrisch et al., 2003; Li et al., 2003a,b) and oat β-glucans (Poyhonen, 2004; Jenkins et al., 2002; Wood et al., 1990, 1994a) produce this response. Likewise, both barley (Delaney et al., 2003; Li et al., 2003a; Smith et al., 2004; Yang et al., 2003), and oat (Beer et al., 1995; Braaten et al., 1994; Kang et al., 2003; Kerckhoffs et al., 2003) β-glucans have been shown to reduce serum cholesterol levels. Attempts have been made to ascertain if the botanical source of the β-glucan affects its cholesterol lowering capacity. In particular, the study of Delaney et al. (2003) compared the cholesterol lowering effect of β-glucans from barley and oats using a hamster model system. Although the diets rich in oat or barley β-glucan significantly reduced the cholesterol levels of the hamsters, no significant difference was observed between the two experimental diets, leading to a conclusion that the cholesterol-lowering potency of β-glucan is not dependent on botanical source. Similar observations were recorded by Hallfrisch et al. (2003) in a comparison of the effect of barley and oat β-glucan diets on glucose and insulin responses in humans.

In part, these physiological properties appear to be related to the rheological characteristics of β-glucan. Wood et al. (1994a), investigating the effect of varying dose and MW of oat β-glucan administered in a beverage, demonstrated an inverse relationship between the viscosity of the beverage and the magnitude of both blood glucose or blood insulin levels. Variations in viscosity accounted for 79–96% of the modifications in glucose and insulin responses. Thus, physiological responses—probably through effects on gut content viscosity—appear to be affected by solution concentration and the molecular weight of β-glucans. Further studies by Wood et al. (1994b) indicated that the glycaemic response of fibre-rich foods was inversely related to viscosity (dependant on concentration and molecular weight). Tappy et al. (1996) also found that inclusion of oat β-glucan into breakfast cereals could reduce the postprandial glycaemic response by up to 50%, and that at low levels (below 5%) this appeared to be dose responsive. Levels above 5% did not show large reductions in the glycaemic response, possibly indicating a saturation point. This may be an important when considering the appropriate levels of β-glucan inclusion in food systems.

However, the effect of β-glucans on the structure of the food should not be overlooked. It is possible that the β-glucans can modify the structure of foods in a similar way to other soluble dietary fibre (Brennan et al., 1996b; Tudorica et al., 2002). This in turn could affect the rate of food degradation and the susceptibility of the starch component of the food to amylolysis. Thus it is likely that a combination of viscosity altering and structure altering

properties of β-glucans are involved in the neutraceutical effects of β-glucan rich foods.

There has been considerable interest on the level of β-glucan supplement needed to affect a significant nutritional benefit. Most studies have been on the effectiveness of dietary fibre or oat β-glucan in relation to food labelling claims through the US Food and Drug Administration (FDA). Accordingly, the FDA has acknowledged nutritional claims that the use of dietary fibres (including oat β-glucan material) reduces the glycaemic and cholesterol responses of individuals. Current recommendations suggest an intake of 20–40 g of dietary fibre per day (DeVries, 2001). More specifically, Behall et al. (1997) reported that 2.1 g of β-glucan per day reduced total cholesterol levels by 9.5%, whilst Jenkins et al. (2002) indicate that 1 g of β-glucan per 50 g of ingested carbohydrates can reduce the glycaemic index of food by 4 units. The FDA have adopted a recommendation of 3 g per day of β-glucan as having a nutritional affect, this as a component of the recommended 30–35 g of dietary fibre per day as advised by the American Association of Dieticians (FDA, 1997).

7. Potential use of β-glucans in cereal food products

The potential nutritional benefits of β-glucans in food systems have been illustrated by studies using a number of cereal food commodities. Hallfrisch and Behall (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 barley ('NutrimX™') and oats, and their corresponding effects upon plasma glucose and insulin responses in non-diabetic adults, concluding that barley β-glucans were more effective in the regulation of glucose and insulin responses compared to oat β-glucans.

Pasta is one food product to which β-glucans have been successfully included as a functional ingredient. Yokoyama et al. (1997) compared blood glucose and insulin responses of healthy individuals following the ingestion of a control durum wheat pasta (100 g of available carbohydrate and 5 g of total dietary fibre) to that of a pasta sample with added barley β-glucan (100 g available carbohydrate, 30 g of dietary fibre and 12 g of β-glucan). Postprandial blood glucose and insulin responses were significantly reduced following ingestion of the pasta enriched with barley flour added to durum wheat flour. The authors attributed this reduction in the glycaemic response to the incorporation of β-glucan. Similar findings have been reported by Knuckles et al. (1997b). It is probable that the reduction in glycaemic response is associated with both the higher β-glucan, and increased total dietary fibre content of the experimental pasta samples. However, the exact role of β-glucan in pasta may need further investigation.

β-Glucans have also been used in other cereal based food systems such as bread. Cavallero et al. (2002) incorporated barley β-glucan rich fractions into wheat bread. Four breads were produced with 100% bread wheat (total β-glucan 0.1: soluble β-glucan 0.1), 50% bread wheat flour and 50% barley flour (total β-glucan 2.4: soluble β-glucan 2.0), 50% bread wheat flour and 50% sieved barley fraction (total β-glucan 4.2: soluble β-glucan 2.8), and 50% bread wheat flour and 50% water-soluble barley fraction (total β-glucan 6.3: soluble β-glucan 5.7). Eight adults were fed test meals of each of the four breads and glycaemic indexes calculated from finger prick capillary tests. A linear decrease in glycaemic index was associated with increasing β-glucan concentrations. The addition of the 50% barley flour in the bread showed a reduction of glycaemic index from the control bread (GIs = 85.42 and 89.49, respectively). However, only the bread containing the water soluble fraction produced a significantly reduced glycaemic index (GI = 69.67) compared to the control breads flour (GI = 89.49). The authors concluded that it was the β-glucan level in the bread (notably the increased soluble β-glucan level) that were responsible for the reduction in glycaemic index, and that this did not a result from impaired food degradation and amylolysis, but through the effect of β-glucan on digesta viscosity and glucose absorption.

More recently, Symons and Brennan (2004b) enriched breads using purified barley β-glucan fractions (at 2.5 and 5% replacement levels), and subjected these to an in vitro digestion process. Significant reductions in starch degradation and sugar release were demonstrated proportional to the amount of β-glucan incorporated into the breads. Since this procedure was not reliant on glucose absorption, it would appear that the glycaemic reducing effect of β-glucan may also be related to the way the β-glucan is incorporated into the structure of the bread and may impede starch swelling and subsequent susceptibility to enzymic degradation.

The ability of β-glucans to influence the rate of starch degradation and hence the glycaemic index of foods has obvious benefits with regard to obesity and diabetes. Jenkins et al. (2002) 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 50 g portions of white bread, commercial oat bran breakfast cereal (4.4% β-glucans) a prototype β-glucan enriched breakfast cereal and a β-glucan breakfast bar (8.1 and 6.5% β-glucan, respectively). The glycaemic indices of those fed the prototype β-glucan enriched cereal (GI = 52) and bar (GI = 43) were significantly lower than the indices of those fed commercial oat bran breakfast cereal (GI = 80) and white bread (GI = 100). Thus, blood glucose levels of diabetic and pre-diabetic individuals can be moderated by using β-glucan rich foods.

8. Hypocholesterolemic properties of β-glucans

In addition to the documented effect of β-glucans on the glycaemic index of foods, considerable research effort has focussed on the potential benefit of β-glucan as a cholesterol reducing agent. Kerckhoffs et al. (2003) investigated the effects of β-glucans from oat bran added to bread and cookies and orange juice, consumed by mildly hypercholesterolaemic subjects. Despite consumption of the β-glucan enriched bread and cookies (daily intake β-glucan 5.9 g) there was no significant change in LDL cholesterol. In contrast, consumption of the orange juice containing 5 g resulted in decreases in LDL cholesterol by 6.7% and in the ratio of total to HDL cholesterol by 5.4% compared with other drinks. In contrast to the previously discussed studies (Beer et al., 1995; Braaten et al., 1994; Delaney et al., 2003; Kang et al., 2003; Kerckhoffs et al., 2003; Li et al., 2003a; Smith et al., 2004; Yang et al., 2003) and an investigation by Keogh et al. (2003) failed to show a significant serum cholesterol lowering response to a high β-glucan barley supplement. Such variability in response may in part be due to a reduction in the efficacy of the β-glucan following processing. Thus, the mechanisms by which β-glucans lower cholesterol are still not clearly defined (Kritchevsky, 1997) although the role of viscosity alteration in digesta is important (Jensen et al., 1993) in that increases in intestinal viscosity may decrease the absorption of cholesterol and the reabsorption of bile acids.

As mentioned previously, the studies investigating the hypocholesterolaemic effects of barley β-glucans have tended to be conducted in animal models. For example, studies of the effects of waxy hull-less barley in chicks by Fadel et al. (1987), Martinez et al. (1991), Newman et al. (1991, 1992) and Wang et al. (1992), all reported reductions in HDL or LDL cholesterol. Similarly, Ranhotra et al. (1991) found significantly lower serum cholesterol in rats fed diets containing bran or flour from hull-less waxy barley with the magnitude of the reductions being related to the amount of soluble fibre in each fraction. More recently Yang et al. (2003) suggested a molecular basis for the hypocholesterolaemic effects of β-glucans. Using a rat model, refined β-glucan and waxy barley were incorporated into a diet for a 2 week period. Both total cholesterol and LDL-cholesterol were reduced in the β-glucan diets compared to a control group, which was associated with up-regulation in the activity of cholesterol 7 alpha-hydroxylase (CYP7A1), an enzyme associated with the regulation of the pathway through which cholesterol is converted into bile acids. More research is required to elucidate the effect of β-glucan on enzyme and immuno-regulation.

9. Potential use of β-glucans in dairy food products

The increased interest in the use of in foods is not solely related to beneficial nutritional properties, but also to optimisation of processing of foods containing added β-glucan. A good example of the latter is the use of β-glucans in the dairy industry. Recent research has focussed on the use of soluble dietary fibre, and in particular β-glucans, in the manufacture of low-fat ice creams and yoghurts (Brennan et al., 2002). Incorporation of β-glucans, with other soluble dietary fibre, into low fat dairy products can make their mouthfeel, scoopability and sensory properties resemble those of full-fat products. Similarly, β-glucan incorporation into low fat cheese curds has beneficial effects on their gelation and rheological characteristics (Tudorica et al., 2004). The addition of β-glucan solutions to milk modifies curd formation, including reducing curd cutting time and increasing curd yields (Tudorica et al., 2004). These effects appear to be related to the gelling capacity of β-glucans and their ability to form a highly structured and elastic casein–protein–glucan matrix.

However, when β-glucans are incorporated into a manufactured cheese system, the texture and mouthfeel of the cheese may be altered deleteriously. Konuklar et al. (2004) demonstrated that the incorporation of the β-glucan-starch-rich material (in the form of 'Nutrim™') significantly reduced the firmness of cheddar cheeses, resulting in a starchy and paste-like product. This reduction in cheese hardness may be related to the decreased melt time observed for the 'Nutrim™' rich cheeses, and may in part be related to negative effects on the casein matrix of the cheese. Whether this is related to the starch or the β-glucan component of the ingredient is unclear. A similar observation was made for soft brined cheeses (Volikakis et al., 2004). The incorporation of β-glucan also altered the appearance and flavour of low-fat white-brined cheese when compared to a full fat control sample. However, in this case the oat β-glucans concentrate used (22.5% β-glucan in the ingredient) reduced the hardness of a low fat cheese when added at 0.7 and 1.4% ingredient levels. This had the effect of making the low fat cheese more closely resemble the full fat cheese control, similar to the observation of Tudorica et al. (2004) on low fat curd rheology. Thus, the choice of dairy system and the purity of β-glucan additive is of great importance.

10. Effect of processing on nutritional and rheological characteristics of β-glucans

Relatively little work has been reported that the effects of food processing on the rheological or nutritional characteristics of β-glucans. Processing may affect the molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding capacity and solubility) which, in turn, could affect the sensory, physiological and ultimately the health benefits of β-glucans. Changes in the properties of β-glucans 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 (Wursch and Pi-Sunyer, 1997), resulting in decreased molecular weight and reduced viscosity. Furthermore, mild extraction conditions (50–60 °C) may not deactivate endogenous β-glucanases, which in turn may lead to increased depolymerisation of the β-glucans (Fastnaught, 2001; McCleary, 2001).

Inclusion of barley β-glucans into breads showed that increased mixing and fermentation times resulted in a decrease in their MW, although the cellotriosyl/cellotetraosyl ratio was unaffected by these processes (Andersson et al., 2004). No significant difference was observed between dough samples and baked breads, indicating that the baking process did not affect the MW of the β-glucan in the dough. There is a need to understand and manipulate processing in order to ensure the possible alterations to the structure of β-glucans do not compromise the nutritional, or sensory quality of foods to which they are added.

11. Conclusions

Although there is little doubt that β-glucans offer many nutritional and rheological advantages to the food industry, it is reasonable to say that when unpurified extracts are used there is still a lack of clarity as to the specific components responsible for such effects. For instance the work conducted on barley or oat flour, and/or β-glucan enriched cereal flours, may not be directly comparable to the use of purified β-glucan fractions. This also leads to potential problems when incorporating these agents into food systems, in terms of predicting exact processing parameters.

The challenge now exists to optimise extraction procedures so as to produce consistent raw material. Investigations are also needed on the potential effects of incorporating β-glucan into both dairy and cereal-based food systems. Specifically investigations are required to determine the effect of process parameters on the rheological characteristics and MW profiles of β-glucan extracts, and determine if processing affects the efficacy of incorporated β-glucan. Such research would broaden our understanding of how β-glucans can affect the nutritional characteristics of foods by altering their structure, texture and viscosity.

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