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Evaluation of the role of soluble and insoluble dietary fibres in affecting the physico-chemical and nutritional characteristics of cereal and dairy based food products

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AN EVALUATION OF THE ROLE OF SOLUBLE AND INSOLUBLE DIETARY FIBRES IN AFFECTING THE PHYSICO-CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF CEREAL AND DAIRY BASED FOOD PRODUCTS

by

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An evaluation of the role of soluble and insoluble dietary fibres in affecting the physico-chemical and nutritional characteristics of cereal and dairy based food products

Current dietary guidelines recommend high dietary fibre (DF), low fat and low glycaemic index (GI) diets as means to prevent or manage diet related chronic diseases which have become increasingly frequent amongst younger individuals. However there is a shortage of such products on the market.

This study investigates the behaviour of DF in cereal and dairy systems with the aim to screen for DF ingredients that may lead not only to nutritionally valuable, but also to good quality, palatable functional foods. A range of insoluble and/or soluble DF was used in cereal (bread and pasta) and milk (fresh cheese and yoghurt) formulations and their influences on the structural, textural, rheological and overall quality of the products were assessed.

The results indicate that DFs can be successfully used to design good quality functional cereal or dairy products, with acceptable or improved attributes. However, it was shown that there is no generic behaviour, which can be applied for each product. Instead, a careful selection of suitable DFs and levels needs to be carried out in relation to the type of product to be made in order to ensure that such products meet consumer expectation with regards to overall quality and sensory characteristics.

In vitro and *in vivo* studies carried out strongly demonstrate that certain types of DFs significantly lowered the GI of the cereal products studied in comparison to the controls. The reduced rate of starch digestibility in products containing these DFs was found to be the result of a combination of factors: reduced starch swelling, changes in the internal structure by formation of a layer coating starch granules, lengthened path between starch granules and α-amylase, and potential inhibition of α-amylase. DFs used were ranked according to their effect on the GI of cereal foods studied.

In the dairy products, interactions between DFs and milk proteins were found to promote significant changes in product structure, with direct influence on their rheological properties; in several cases improved sensory attributes of the final products were obtained.

Scanning electron microscopy strongly indicated that various different microstructures can be obtained in milk products depending on the type of DF, level used and also on the fat content of milk. This enables the production of low fat dairy products, with non-compromised quality characteristics and which also bring the added health benefits of DFs.

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LIST OF ABBREVIATIONS

CVD	- cardiovascular disease
db	- dry basis
DF	- dietary fibre
DM	- dry matter
dmb	- dry matter basis
dwb	- dry weight basis
DP	- degree of polymerisation
FOS	- fructooligosaccarides
G'	- storage modulus
GI	- glycaemic index
HI	- hydrolysis index
IAUC	- incremental area under the blood glucose response curve
II	- insulinaemic index
LBG	- locust bean gum
min	- minutes
mm	- millimetres
NIDDM	- non insulin dependent diabetes mellitus
PHGG	- partially hydrolysed guar gum
pps	- points per second
SCFA	- short chain fatty acids
SDS	- slowly digestible starch
sec	- seconds
SEM[1]	- scanning electron microscopy
SEM	- standard error of the mean
SI	- satiety index
SMP	- skimmed milk powder
tanδ	- tangent of the phase angle (=loss modulus/storage modulus)
tan(delta)	- tanδ
WHC	- water holding capacity
WWB	- white wheat bread

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Chapter 1. Literature review

1.1 Food products and health. Functional foods.

Recent growing acceptance of the role diet may play in prevention and management of various diseases has lead to an increase in consumer demand for healthier food products. Although this trend appears to be relatively new, the causal relationship between humans' diet and their health status has been acknowledged for generations in some parts of the world. For example in the Far East, food and medicine were traditionally considered equally important in preventing and curing diseases (Xu, 2001).

The present interest of Western consumers on the diet - health relationship is mainly a consequence of their increasing concerns on the risk of developing conditions such as obesity, diabetes, coronary heart diseases, intestinal diseases, etc (Jones, 2002). These are in most cases associated with an imbalance in the nutritional intake due to a change in lifestyle which is dominated by a lack of regular physical exercise, and at the same time by an increased consumption of highly processed foods (Kwak and Jukes, 2001).

Ironically the risk of developing such diseases appears to be higher now when life expectancy in most parts of the world is higher today than ever before. A recent FAO/WHO report (WHO, 2003) states that, in 2001, chronic diseases contributed to approximately 60% of the 56.5 million of total reported deaths in the world, and the proportion of non-communicable chronic diseases is expected to increase to 75% by 2020, and to affect a large part of the population earlier in life. Due to increased concern, health authorities (WHO, 2003) and governments are trying to control the situation by raising consumer awareness on the importance of a balanced diet.

In line with these efforts we have witnessed a rapid development of a new class of food products to confer health benefits, which seem to represent a great opportunity for the food

industry's future (Hilliam, 1998; Jones, 2002). This new class of foods is generally referred to as 'functional foods', but they are also known as 'designer foods', 'nutraceuticals', 'foodiceuticals', 'medical foods', 'superfoods', 'prescriptive foods' 'medifoods' or 'better for you foods' (Finley, 1996).

1.1.1 Regulation and definition of functional foods

The term "functional food" originated in Japan in 1984 but the concept evolved internationally, first to China and Korea, and later stimulating interest in Europe and North America (Arai, 1996). This was not without attracting controversies, most often due to questionable claims used to market various products.

Originally, foods were known to have two functions (Arai, 1996):

1. *Primary*: Nutritional function, i.e. to provide sufficient nutrients and energy to the body to meet the metabolic requirements of an individual.
2. *Secondary*: Give the consumer a feeling of satisfaction and well being through the hedonistic attributes: good taste, texture and flavour.

Therefore for a long time, food products have been developed for taste, appearance, value, and convenience for the consumer.

A 'functional' food is also characterised by a tertiary function:

3. *Tertiary*: Modulation of physiological and psychological systems in the body in a positive direction, besides the basic nutrition. In this way diet plays a role not only in achieving optimal health and development, but it might also play a role in reducing the risk of disease (Arai, 1996; Xu, 2001).

Functional foods are thus defined as foods which have a tertiary function and provide 'positive nutrition' (Kwak and Jukes, 2001), affecting beneficially an individual's health, physical performance or state of mind. They are foods that contain ingredients that serve therapeutic roles (e.g. antioxidants, dietary fibres, folic acid, omega(ω) - 3 fatty acids, etc), with potential effects on the immune, nervous, endocrine, circulation or digestive systems, and may prevent or ameliorate diseases.

The legislation regarding functional foods is less well defined across Europe than in Japan and the USA, with no official, universally accepted definition in existence (Blades, 2000). A working definition was proposed by the International Life Science Institute - Europe (ILSI - Europe) according to whom a food can be regarded as 'functional', "if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well being and/or reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet; they are not pills or capsules, but part of a normal food pattern" (Diplock et al., 1999).

While there may be still discussions concerning the definition and permissible claims, the concept is now accepted worldwide, and functional foods have been identified as the most important consumer trend impacting new food product development (Kevin, 1997). The European market for functional foods is relatively small in comparison to the North American and Japanese market, and it is represented mainly by dairy products (65%) due to the success of probiotic yoghurts; however it is expected to continuously grow and to encompass products such as breakfast cereals, biscuits, bread and drinks (Anon., 2001d).

It has been suggested that the top ten opportunities for the functional foods market are: meal replacements, weight loss/appetite satiety, cholesterol/heart health, cancer, digestion/gut health, hormone replacement/menopause, skeletal strength, diabetes and emotion commotion (Sloan, 1999). The majority of them could be potentially addressed by including dietary fibre (DF) in the diet.

1.2 Dietary fibre as an ingredient for functional foods

1.2.1 What is dietary fibre?

For thousands of years, as far as is known, the human diet was rich in plant fibre due to high intake levels of cereals and seeds. However in modern times human nutrition has changed (sugar and meat consumption have increased and bread consumption have decreased) (Endress and Fisher, 2001) resulting in a continuous decline in fibre intake, a 20% reduction being recorded only in the last 50 years (Ahmad, 1995). This is somehow surprising taking into account that the health benefits of a high fibre diet (especially the laxative effects) have been recognised since the time of Hippocrates (Johnson and Southgate, 1994b).

Although modern interest in fibre rich diets began in the 19th century with the pioneering views of Graham and Kellog (USA) and Allinson (UK), it was not until 1935 when the first definition of DF was proposed by Williams and Olmstead (Jones, 2000). The term was then extended by Hipsley in 1953 (Asp, 2001) to cover the non-digestible components of the plant cell wall, and to include cellulose, hemicellulose and lignin.

However, the DF 'era' started with the "DF hypothesis" launched by the British scientists Burkitt, Painter, Trowell and Walker, during the 1960's and 1970's. They postulated an

inverse relationship between DF consumption and the incidence of certain conditions such as diabetes, obesity, heart diseases, large bowel disease, colon cancer, haemorrhoids, and gall stones (Jones, 2000; DeVries and Prosky, 1999). They described the term as "the remnants of plant cells resistant to hydrolysis by alimentary enzymes of man" and included cellulose, hemicellulose, lignin and associated minor substances such as waxes, cutin and suberin (Trowell, 1972).

1.2.2 Dietary fibre: definition

Although the DF definition proposed by Trowell and co-workers is still widely accepted, the knowledge of fibre chemistry, structure and function has grown substantially since and with it, the DF definition has evolved. For instance, some food components (e.g. oligosaccharides) were proven to possess physiological benefits and to have similar effects as DF. Concurrently, scientists started to be concerned about DF methods and definitions that do not include all fibre sources. Thus, for a long time there has been no general consent on the definition, partially because of the lack of agreement on what is to be included, how it is to be measured and what most closely approximates what actually happens in the human gut (Jones, 2001). An ILSI North America - AACC workshop held in June 1999 proposed an updated definition of DF: 'DF consists of the remnants of edible plants and associated substances, (poly)saccharides and carbohydrate analogues, and lignin, resistant to digestion and absorption in the small intestine (DeVries and Faubion, 1999). This was reworded in the year 2000, when a Committee of Members of the American Association of Cereal Chemists (AACC) agreed on a definition that included other various compounds that are not absorbed, and exert a physiological effect: "DF is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human intestine with complete or partial fermentation in the large

intestine. DF includes polysaccharides, oligosaccharides, lignin, and associated plant substances. DF promotes beneficial physiological effects, such as laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (Anon., 2001a).

Figure 1.1. Constituents of dietary fibre (adapted from (Anon., 2001a))

DIETARY FIBRE

Non-starch polysaccharides and resistant oligosaccharides

- Cellulose
- Hemicellulose
- Arabinoxylans
- Arabinogalactans
- Polyfructose
- Inulin
- Oligofructans
- Galactooligosaccharides
- Gums
- Mucilages
- Pectins

Analogous carbohydrates

- Indigestible dextrins
- Resistant maltodextrins
- Resistant potato dextrins
- Synthesized carbohydrate compounds
- Polydextrose
- Methyl cellulose
- Hydroxypropylmethyl cellulose
- Indigestible (resistant) starches

Lignin

Substances associated with the non-starch-polysaccharides and lignin complex in plants

- Waxes
- Phytates
- Cutin
- Saponins
- Suberin
- Tannins

This latest definition includes all non-starch polysaccharides resistant to digestion in the small intestine and fermentable in the large intestine (celluloses, hemicelluloses, pectins, modified celluloses, oligosaccharides, and polyfructans such as inulin, gums, and

mucilages). It also includes oligosaccharides with various degrees of polymerisation (DP) and non-polysaccharide material bound to the plant cell wall (lignin, waxes, cutin, and suberin). Materials with analogous characteristics to DF are included in the new definition under the term "analogous carbohydrates". The constituents of DF according to this latest definition are listed in the Figure 1.1. It is important to point out that although this definition includes generally the same food constituents as the historical working definition used for almost 30 years, the emphasis made this time is on the chemical and physiological component rather than on the laboratory analysis.

More recently, in 2002, the Food and Nutrition Board (FNB) developed the following definitions (Asp, 2003):

- Dietary fibre - consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants
- Functional fibre - consists of isolated, non-digestible carbohydrates and lignin that have beneficial physiological effects in humans
- Total fibre - is the sum of 'DF' and 'functional fibre'.

Although these new definitions of the FNB include oligosaccharides, resistant starch and lignin, they do not seem to clarify the concept of DF. The definitions differentiate 'dietary' from 'functional fibre', and they create confusion by leading to the idea that DF as part of the plant cell walls have no physiological properties. In a recent issue of Cereal Foods World, the AACC Technical Committee stated that "because the 'intrinsic' and 'intact' terms used in the FNB definition are not measurable quantities, the definition creates analytical concerns and would be not acceptable to food manufacturers and regulatory personnel, would have a negative impact on nutrition research and education, and will also mislead consumers" (AACC, 2003). Therefore the AACC Board of Directors continues to support the definition of DF as adopted in May 2000 (Anon., 2001a). The DF definition

continues to be the subject of controversies, and it will probably have to be modified, as new research becomes available.

1.2.3 Dietary fibre: methods of analysis

The methods for quantifying DFs in foods are strongly related to the DF definition and over the years they have been greatly debated with some scientists wanting to describe DF by its physiological attributes, and some by its chemical composition (Prosky, 2001). Initially the only method available of measuring DF was as crude fibre, which was inappropriate to quantify the total DF in the diet since it gave only an estimate of the cellulose and lignin in the foods. In the following years, researchers such as Southgate, Asp and Prosky have developed methods based on the DF indigestibility and hence more closely approximated conditions in the human gut. Once these methods were adopted and used, DF began to be defined by methodology (Jones, 2000).

Two main types of methods have been developed:

- Methods that use gravimetric assay techniques based on the procedures initially developed by Asp et al. (1983), and
- Methods based on the modification of the original colorimetric procedure developed by Southgate which measure the individual DF components. The subsequent developments have used gas chromatography (GC) and high-performance liquid chromatography (HPLC) to measure the β-glucans.

The gravimetric procedures further developed by Prosky et al. (1985) are extensively used in the USA and in some European countries as methods for determining total DF (TDF), because it is believed they are simpler, more rapid, require less investment in capital equipment, and are therefore more suitable for regulatory control of labelling (Johnson and

Southgate, 1994c). The method was adopted by the Association of Official Analytical Chemists as AOAC Official Method 985.29 (AOAC, 1995) and also by AACC as AACC Approved Method 32-05 (AACC, 1985). The method has been modified since to allow the measurement of soluble and insoluble components, and also the use of alternative buffers (AOAC method 991.43; Lee et al., 1992).

The other methods that describe the fraction measured un-equivocally have been called the 'non starch polysaccharide' (NSP) methods (Englyst and Cummings, 1988). The fraction measured does not include lignin and resistant starch and hence it is not identical to DF as originally defined (Johnson and Southgate, 1994c). Thus, the gravimetric TDF values are slightly higher than the NSP values. For most fruits and vegetables the differences are not significant (Johnson and Southgate, 1994c), but they might be of the order of 1g/100g for unprocessed cereal foods (accounting for lignin), or 2-3g/100g higher for heat-processed cereals and potatoes (because of resistant starch). Nevertheless, NSP values provides a good index of DF as originally defined, and in the UK the procedure was adopted for a long time as the official method for nutritional labelling of foods (Anon., 1995), whilst the term NSP was preferred to DF by the British Nutrition Foundation (Anon., 1990)

However, since most countries of the European Union were using the term of DF and the AOAC Official Method 985.29 to quantify the DF, the MAFF Joint Food Safety and Standard Group (JFSSG) proposed in 1999 to adopt the same methodology - AOAC Official Method 985.29, and AOAC Official Method 991.43 - as the UK's preferred methods for fibre analysis, and labelling purposes (Prosky, 2001; DeVries, 2001). This was a necessary step in order to harmonise DF labelling across the EU, and to ensure that consumers receive consistent information.

It is known that some highly soluble components (such as fructans with nearly all DP) are not quantified as DF by using the present approved AOAC method for TDF analysis. Methods have been developed for quantification of such components in foods. Thus, fructans can be determined using either AOAC Official Method of Analysis 997.08 - Fructans in Food Products, Ion Exchange Chromatographic Method (AACC Method of Analysis 32-21), or AOAC Official Method of Analysis 999.03 - Measurement of Total Fructan in Foods, Enzymatic/Spectrophotometric Method (AACC Method of Analysis 32-32) (AOAC, 1998).

In the continuous search for methods of analysis, another procedure was recently proposed that quantifies the major non-digestible components in plant foods: DF (including oligosaccharides), non digestible proteins, some polyphenolic compounds, etc., thought to be fermented by colonic microflora, and to have physiological effects similar to those of DF (Saura-Calixto et al., 2000). This would extend the DF concept to include all food constituents reaching the colon, and would be quantified as an 'indigestible fraction'. The future will probably bring the development of new edible carbohydrate-based polymers characterised by physiological behaviours similar to DF; these developments will need new appropriate analytical methods in order to quantify these materials and possible adjustments of the present definition.

The 'DF hypothesis' of Burkitt and Trowell had proven extremely productive scientifically. Current evidence suggests that high fibre diets do seem to have protective effects against a range of diseases such as colon cancer, atherosclerosis, associated hypercholesteremia, diabetes, hypertension, and obesity (Lo et al., 1991). Hence, DFs are considered as ingredients for functional foods. Nevertheless, it is not clear yet if these benefits are due to

DF alone or due to DF and associated substances, and the presence of DF in the diet could be a 'marker' for diets that are protective (Johnson and Southgate, 1994c).

1.2.4 Characterisation of dietary fibre

1.2.4.1 Physicochemical properties of DF

As a result of the research effort of the past years, DF is no longer regarded as an inert carbohydrate fraction with little nutritional value, but an essential component of our diet. Consumption of foods rich in DF have been associated with decreased risks of developing diet related chronic diseases (WHO, 2003) and their physiological effects are usually compared with the intakes or contents of TDF, forgetting that DF refers to a large number of substances, and encompasses very diverse macromolecules, exhibiting a large variety of physico-chemical properties.

Different sources of DF can have different metabolic and physiological effects determined by the chemical and physical properties and also by their fate during gut transit and fermentation (Guillon and Champ, 2000). An understanding of these characteristics is useful in predicting the physiological response to a source of fibre (Schneeman, 1999b).

In an attempt to clarify the concept of DF, scientists proposed over the years various classifications. Some were based on their chemical structure, which was further related to their role in the plant, some on their physical characteristics, or more recently on their chemical structure in relation with the degree of polymerisation (DP).

1.2.4.2 Structural aspects of dietary fibre

DF includes primarily polysaccharides, but also oligosaccharides, and substances from plant cell walls associated with the non-starch polysaccharides (Figure 1.1). Their common

characteristic is that they escape digestion in the small intestine and reach the large intestine where they undergo fermentation; hence their effects on metabolism and disease risk are likely to be mediated through their physico-chemical properties as they pass through the gastrointestinal tract.

The large majority of DF constituents are represented by carbohydrates: poly and oligosaccharides. Like the oligosaccharides, polysaccharide molecules are composed of glycosyl units in linear or branched arrangements, but larger than 20, which is the limit of oligosaccharides (BeMiller and Whistler, 1996). The degree of polymerisation (DP), varies from less than 100 (only a few of them) to 10,000-15,000 (e.g. cellulose). The majority of them have DPs ranging between 200 and 3000. Each type of polysaccharide is characterised by its monosaccharide unit, and the nature of the linkages between them.

The simplest structure is that of homoglycans, where all the glycosyl (monosaccharide) units are the same; for example, both cellulose and β-glucan have as monosaccharide residue glucose. In cellulose the linkages are β (1-4) and the structure is linear, whereas in β-glucan, β (1-4) linkages are interspersed with 1-3 linkages. Heteroglycans are composed of two or more different monosaccharide units, larger repeating sequences being common in polysaccharides of bacterial origin (Morris, 2001). Examples of heteroglycans are: hemicelluloses having a monosaccharide backbone consisting of xylan, galactan or mannan with side chains of arabinose or galactose (e.g. arabinoxylans), or pectins with a galacturonic acid core esterified to a varying extent with methoxyl groups on the uronic acid residues (Davidson and McDonald, 1998).

The physical properties of polysaccharides are dominated by the conformation of the individual chains (ordered or disordered 'random coil' chain geometry) and the way they

interact with one another. The position of linkage between adjacent sugars and the axial or equatorial orientation of the bonds to the glycosidic oxygen, determine overall chain geometry, leading to a disordered coil form or an ordered structure.

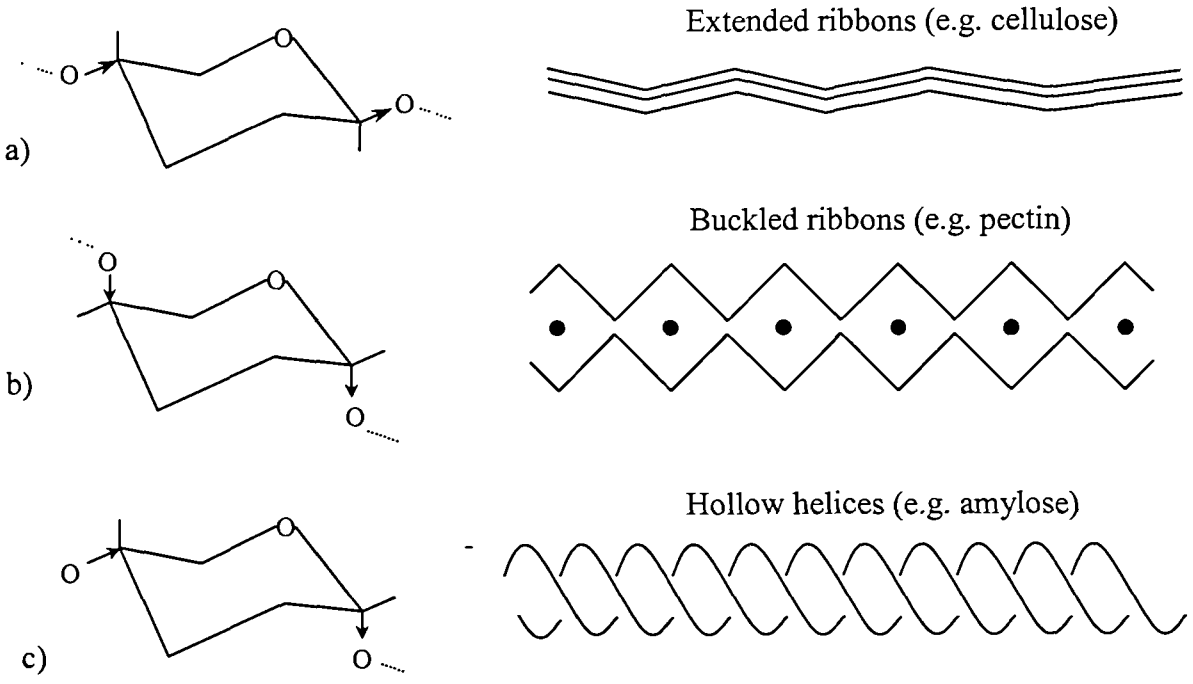
Morris (2001) presented three patterns of linkage geometry relevant to DFs (Figure 1.2), which are related to the orientation of the bonds between the monosaccharide residues. The first conformation is typical for cellulose (Figure 1.2a), which is a linear polymer of D-glucose linked β (1-4). In this case the bonds to and from each sugar residue in the cellulose chain are diagonally opposite one another across the sugar ring and both are equatorial. Thus they are parallel and only slightly offset from each other. The geometry of this linkage leads to flat, ribbon-like structures of individual chains which can undergo hydrogen bonding with each other, and pack together to form crystallites, typical for cellulose. The same type of linkage pattern occurs in mannan (i.e. poly-β-D-mannose), the backbone of plant galactomannans such as guar and locust bean gum.

The second characteristic arrangement for DF occurs in the negatively charged poly-α-D-galacturonate sequences of pectin (Figure 1.2b). The linkage between the glycosyl residues is still β (1-4), but through the axial bonds of each position. The bonds to and from each sugar residue are parallel but are offset from each other by the full width of the sugar ring, resulting in a zig-zag chain geometry with large cavities which can accommodate metal cations (e.g. Ca^{+2}). By neutralising the negative charges and suppressing the electrostatic repulsion between the constituent polysaccharide chains, the cations could promote the formation of compact, ordered assemblies.

The third class of linkage arrangement is the most common for polysaccharides, leading to a helical conformation (Figure 1.3). The bonds to and from each residue are no longer

parallel to one another and this results in systematic twist in the direction of the chain. These ordered structures are hollow helices, which stabilise one another by packing together coaxially.

Figure 1.2. Relationship between the linkage geometries encountered within polysaccharides chains and their conformations adopted in the solid state: a) extended ribbons; b) buckled ribbons; c) hollow helices (from Morris, 2001)



The chemical structures and chain conformations of DFs dictate their physical characteristics, which may have profound effects on their physiological role as constituents of digest, and may introduce both local and systemic responses. Some of the most important physical characteristics of DF are: hydration properties, solubility dispersability in water, rheological properties, bulk due to nondigestibility, the ability to adsorb or bind bile acids, fermentability by gut microflora and surface area characteristics (Schneeman, 1999b; Schneeman, 2001; Malkki, 2001; Guillon and Champ, 2000).

1.2.4.2.1 Hydration properties

Polysaccharides contain glycosyl residues, that have hydroxyl groups, each hydroxyl group having the capacity to hydrogen bind to one or more water molecules. Moreover, the ring oxygen atom and the glycosidic oxygen atom can also form hydrogen bonds with water molecules. Consequently, glycans posses a strong affinity for water and readily hydrate when water is available (BeMiller and Whistler, 1996); they are hydrophilic molecules. In aqueous systems, polysaccharide particles can take up water, swell, and undergo partial or complete dissolution.

Hydration characteristics of DF have been extensively studied in relation to both physiological effects (original DF hypothesis) and also to various technological aspects related to their presence into foods. It is well known that water is bound to polysaccharides with differing strengths and in different amounts; water binding properties of DF may be determined by filtration (water holding capacity), centrifugation (water binding capacity) or freeze drying (Chaplin, 2003). Bound water is often divided into two types (freezable and non-freezable) with the non-freezable water being more tightly or specifically bound.

Measurement of hydration properties of DFs has proven to be problematic although the parameters appear to be simple (Oakenfull, 2001). A recent European collaborative study - Profibre, gave special attention to the definitions and standard measurements of hydration properties of DF. According to Robertson et al. (2000), the definitions related to hydration properties of DF as arising from Profibre are:

- Swelling: 'the volume occupied by a known weight of fibre under the condition used' is measured as settled bed volume.

- Water retention capacity (WRC) or water binding capacity: 'the amount of water retained by a known weight of fibre under the condition used' is measured by centrifugation and is preferred to either water holding capacity or water binding capacity.
- Water absorption or water holding capacity: 'the kinetics of water movement under defined conditions' is measured using a Baumann apparatus or using osmotic/dialysis techniques.

Different hydration characteristic of DFs are related to their chemical structure: as examples swelling values range from 5.65 ml/g for resistant starch (Novelose) to 10.45 ml/g for citrus fibre, and water retention capacity range from 2.95 g/g for Novelose to 10.66 g/g for citrus fibre (Robertson et al., 2000). It has also been suggested that some values were lower than expected and this may be due to processing condition and consequent matrix structure breakdown. Processes such as grinding, drying, heating and extrusion can modify the physical properties of the fibre matrix, and consequently affect their hydration properties (Guillon and Champ, 2000). Several examples of hydration characteristics for various DFs are presented in Table 1.1.

Swelling and water retention capacities provide a general view on DF hydration and will provide useful information for designing DF supplemented foods. High water holding capacity suggests that these materials can be used not only as DF enrichment, but also as functional ingredients to reduce energy, avoid syneresis and modify the viscosity and texture of the final product in various fields (bakery products, snacks, meat products and dietetic beverages).

Water absorption is thought to provide more information on the DF, in particular its substrate pore volume, and may be useful in understanding DF behaviour during the transit of the gastrointestinal tract (Guillon and Champ, 2000). It is thought that faecal bulking

capacity of DF is related to both their water absorption/retention characteristics and their impact on microbial proliferation (Davidson and McDonald, 1998). However this is not entirely true. For instance, DFs such as pectin have high water capacity when compared with wheat bran, but the latter has a more pronounced effect on faecal bulking since it is poorly fermentable and therefore retains its structure in the colon.

Table 1.1. Hydration characteristics of some DFs (adapted from (Guillon and Champ, 2000; Robertson et al., 2000; Grigelmo-Miguel and Martin-Belloso, 1999))

Source of fibre	**Treatment**	**Particle size** (μm)	**Swelling** (mgl^{-1})	**Water retention** (g water * g^{-1} dry pellet)	**Water absorption** (ml water*g^{-1}dry DF)
Sugar beet fibre	-	385	21.4	22.6	8.8
	-	205	15.9	19.2	7.3
	native	-	10.8	6.1	-
	depectinated-drastic drying	27.6	14.0	-	-
Citrus fibre	-	540	15.7	10.4	7.0
	-	139	10.4	10.7	4.6
Apple fibre	-	540	9.6	6.9	3.8
	-	80	5.6	7.1	2.7
Pea hull	-	67	7.5	3.94	
	native	-	6.2	4.2	
	depectinated and freeze dried	-	11.8	7.2	
Wheat bran	-	900	11.9	6.8	1.0
	-	320	5.9	3.0	0.9
	native	-	7.0	7.0	
	delignified	-	11.0	10.4	
	extruded	-	9.0	4.4	
Oat bran	-	-	5.5	3.5	
Resistant starch					
Novelose	-	40	5.6	2.9	3.0
Eridania	-	84	7.4	3.1	3.9

1.2.4.2.2 Solubility

Generally speaking, solubility is considered as a major factor in functional and nutritional properties of DF.

The solubility of polysaccharides is a consequence of their internal structure and relative stability of the ordered and disordered forms. Most of the polysaccharides exist in some sort of helical shape (BeMiller and Whistler, 1996). Certain polysaccharides have a structure in which the chains may adopt regular, ordered conformations (Figure 1.2) and pack together into crystalline assemblies; the polymer is likely to be energetically more stable in the solid state than in solution (Guillon and Champ, 2000). The chemical regularity of a chain increases the strength of the links, confers insolubility, and resistance to enzymatic attack. Thus linear structures such as cellulose with its flat ribbon-like conformation (Figure 1.2 a) may undergo only limited degradation during colonic fermentation, because the crystalline regions are nearly inaccessible to enzyme penetration. The highly ordered polysaccharides with orientation and crystallinity represent the exception rather than the rule (BeMiller and Whistler, 1996). If a chemical irregularity or a branch is present on the linear structure, the links will be weaker. Most of the polysaccharides have some irregularities in their structure (in the backbone or as side chains) and hence tend to readily hydrate and to be soluble. The majority of unbranched heteropolysaccharides containing non-uniform blocks of glycosyl residues and most branched polysaccharides can not pack into a crystalline order because chain segments are prevented from becoming closely packed over lengths necessary to form strong intermolecular bonding; this will promote solubility. An example is guar gum, which is a soluble polysaccharide and with a structure formed by a backbone similar to cellulose to which sugar side chains are attached irregularly. In general, the polysaccharides become more soluble as the degree of irregularity of the molecular chains increases (or the ease with which molecules pack together decreases).

Another factor that prevents ordered packing and confers solubility is charge. Neutral polysaccharides such as cellulose and starch have a strong tendency for self-association; the existence of charged groups within the molecule (as in pectins) promotes solubility due to electrostatic repulsion, which inhibits the formation of ordered arrangements. However, the negatively charged polysaccharide may associate in the presence of metal ions that can bind to the chain and balance their charge (Morris, 2001). The resulting structure could be the 'egg - box' previously shown in Figure 1.2b.

Temperature also plays an important role in the stability of the ordered assemblies; some materials insoluble in cold water will dissolve as the temperature increases because it promotes conversion to disordered form (Morris, 2001).

The understanding of DF structure in relation to their solubility may provide an insight into DF behaviour in food products, and interactions with other food components on one hand, and on the other hand may explain some of their nutritional benefits. Based on their solubility, one widely used classification is that of water-soluble and gel-forming viscous DF and water insoluble DF. This distinction was for a long time convenient, since many of the physiological effects of fibre seemed to be based on this property: soluble viscous DFs are associated with carbohydrate and lipid metabolism and are highly fermentable, while insoluble DF generally contributes mainly to faecal bulk improving bowel habits (Kritchevsky, 2001; Jenkins et al., 2001)

The concept of soluble and insoluble DFs has been developed through the fractional extraction of polysaccharides from foods by controlling pH as in the case of the human alimentary system. However, this classification may not be entirely useful in understanding the relationship between the gastrointestinal effects of fibre and metabolic consequences (Schneeman, 1999a), since other variables such as fermentability, viscosity, and binding

capacity appear to be as important in understanding the physiological response to consumption of DF. Consequently a FAO/WHO report (FAO, 1998) on dietary carbohydrates recommended that the terms 'soluble' and 'insoluble' fibre should not be used anymore. They recommended a chemical division based on their DP.

Furthermore, some researchers consider that introducing a 'viscosity index' as a criteria for classification of DF based on their viscosity, would represent a step forward in differentiation of DF (Fisher, 2003). However, this approach will lead to a problem related to what we call today 'insoluble fibre' since the viscosity of such fibre can not be measured.

Nevertheless, the terms of soluble insoluble DF are still widely used by both the academic community and food industry; therefore I will use it myself in the following chapters.

1.2.4.2.3 Polysaccharide solution viscosity (rheological properties) and stability

Water-soluble DFs are frequently used in the food industry primarily to modify/control the flow properties of liquid food products and the deformation properties (textural properties) of semisolid foods. They are commonly known as gums or hydrocolloids and because they have the capacity to produce viscosity and form gels, they are often used in very small concentrations (0.25-0.5%). Developing viscosity is one of the most important physical properties of soluble DF not only from the perspective of food application but also regarding several physiological effects.

The viscosity of solutions or suspensions of certain types of polysaccharides is dependent on the intrinsic characteristics of the polysaccharides (mean molecular weight, shape of the molecules, the presence and magnitude of charges of these hydrated molecules, and the conformations they adopt in the solvent), on their concentration, on the type of solvent, and also on the temperature.

For most polysaccharides, intrinsic viscosity [η], a characteristic of the polymer is proportional to molecular weight. However, the shape and flexibility of polysaccharide molecules in solution are as important. Linear polysaccharides gyrate and flex in solution, sweeping out a large space and frequently collide with each other, in comparison with a highly branched polysaccharide molecule with the same molecular weight. Therefore, at the same concentration, highly branched polysaccharides will produce a much lower viscosity than linear molecules with the same DP (BeMiller and Whistler, 1996). Similar to linear polysaccharides, chains wearing electrical charge result in an extended configuration due to electric repulsion, increasing the end-to-end chain length. Consequently the volume swept by the polymers increases and with it the viscosity of the solutions.

Polysaccharide concentration in solution also influences its flow behaviour. At low concentrations when the molecules are away from each other and free to move, the logarithm of the viscosity of a polysaccharide solution is directly proportional to the logarithm of the multiple of the concentration and intrinsic viscosity (Malkki, 2001). Above a critical value, the effect of concentration becomes very pronounced and the viscosity becomes more shear rate dependent (Guillon and Champ, 2000). At this point the molecules become sufficiently crowded to interpenetrate one another (Oakenfull, 2001).

Under certain conditions, some polysaccharides can form gels through association of their ordered regions, forming a continuous three-dimensional network of connected molecules, and entrapping a large volume of a continuous liquid phase. The polymer molecules are joined in junction zones by hydrogen bonding, hydrophobic associations (van der Walls attractions), ionic cross bridges, entanglements, or covalent bonds (BeMiller and Whistler, 1996). The fluid-like solution changes into a material that has a sponge-like structure,

which can retain its shape. Gels have rheological characteristics of both fluids and solids; they are known as viscoelastic semisolids.

Viscosity, directly related to hydration properties and solubility is one of the most important physical properties of DF from both physiological and technological points of view. DFs, which have the ability to form viscous solutions/gels, can change the rheology of the intestinal contents, and are known to produce local responses along the gastrointestinal tract, which are associated with several systemic effects discussed later in this chapter.

Apart from the nutritional aspect, DFs can be used for economical and technological purposes. When added to foods, DF can change their rheological behaviour, texture and consequently their sensory characteristics of the endproducts; they can also stabilise suspensions, emulsions and foams and can improve products' freeze-thaw stability and control syneresis. Nevertheless, not all the DF can be incorporated in the same way (levels and form) and in the same type of products. Their uses have to be considered in connection with their functional properties which are related to the processing conditions and food structure (Guillon and Champ, 2000).

1.2.4.2.4 Fermentability

Once they reached the large intestine, almost all of the DF can be fermented totally or partially by the microorganisms present in the colon, with the production of flatus gases (carbon dioxide, hydrogen, and methane - responsible for unpleasant symptoms) and short chain fatty acids (SCFA): acetate, propionate, and butyrate produced in roughly a 60:25:15 ratio (Bourquin et al., 1993a). Uptake of these fatty acids by colonocytes has been associated with increased cell proliferation rates, enhanced sodium and fluid uptake and

inhibition of hepatic cholesterol synthesis (Davidson and McDonald, 1998). In addition, the fermentation leads to an increase in bacterial mass in the large bowel, contributing to the faecal bulk. It is also presumed that chemical properties of the polysaccharides will influence the types of microbial activity in the large intestine (Cummings et al., 2001). Based on the amount of SCFA produced, fibre sources contribute up to approximately 2-2.5kcal/g (Livesey et al., 1995; Meyer and Tungland, 2001).

The degree to which DFs are fermented depends on the chemical, internal structure and physical properties (especially solubility), with ordered conformations being broken down to a smaller extent than polysaccharides with some irregularities in their molecules. Fermentability depends also on the degree of purification (DF fermentability was found to be higher when consumed in a purified form, than as constituent of food (Bourquin et al., 1993b)), and particle size (smaller particles offer an increased available contact area to gut microflora, resulting in a more complete fermentation). These factors mentioned above could probably explain some inconsistencies in findings between studies, which used DF, conducted either clinically or *in vitro*. Taking into account their fermentability in addition to solubility, DF have been classified as soluble-viscous-fermentable, insoluble-nonviscous-unfermentable and mixed type (Roberfroid, 1993)

1.2.4.2.5 Bulk

DF are not digested by the enzymes of the human small intestine, therefore they increase the dry weight of the intestinal contents and subsequently the mass of the material passed into the large intestine. The ability of DF to influence faecal bulk depends on their physicochemical properties and the bacterial population in the colon. For example, both wheat bran (source of insoluble fibre) and oat bran (source of soluble fibre) increase stool

weight of human subjects but in different ways (Chen et al., 1998). For the wheat bran diet, the bulking effect was due mainly to undigested plant residue, whereas when oat bran was fed, the increased bacterial mass affected the stool weight. The faecal bulking capacity of DF has also been reported as being related to the amount of pentose within a particular fibre (Oakenfull, 2001; Davidson and McDonald, 1998).

1.2.4.2.6 Binding / adsorption capacity of ions and organic molecules

Certain DF are thought to bind organic and inorganic molecules, and this ability has been long associated with impaired mineral absorption, and it was seen as a contraindication to the general advice to increase the DF intake.

In vitro studies showed that the highest affinity for binding mineral cations (Ca, Fe, Zn, Mg) is found among DFs with functional groups (free carboxyl, hydroxyl or amino groups), with pectin appearing to have the strongest binding potential. However, it has also been suggested that mineral absorption is not affected by DF components *per se,* but is mainly due to associated substances such as phytates (Sebastia et al., 2001; Gudiel-Urbano and Goni, 2002), but once the phytates are removed the effects were eliminated (Frolich, 2001). In addition, many foods rich in fibre are also rich sources of minerals, thus the mineral balance would not be adversely influenced by ingestion of these foods (Frolich, 2001; Johnson and Southgate, 1994a).

Although some findings are contradictory, a general conclusion emerges: providing that DF intake is at a reasonable nutritional level, that dietary minerals are adequate and interference with proteins and phytates are cleared out, DFs have no significant effect on the bioavailability of the major cations.

Numerous studies have shown that certain DFs contribute to the adsorption in the colon of Ca^{2+} (e.g. fructoolisaccharides, guar gum hydrolysate) (Gudiel-Urbano and Goni, 2002; Hara et al., 2000; Hara et al., 1999b), zinc and iron (beet fibre) (Fairweather-Tail and Write, 1990), or calcium and magnesium (synergistic effect of inulin and resistant starch) (Younes et al., 2001). Additionally, *in vitro* and *in vivo* studies illustrate that certain DFs also have the capacity to bind bile acids and phospholipids (Vahouny et al., 1980; Story and Furumoto, 1990) and this is believed to significantly impact on lipid absorption and cholesterol metabolism (Eastwood and Morris, 1992). Examples of such fibres are ispaghula, pectin and sugar beet fibre (Blackwood et al., 2000; Nishina et al., 1991; Hara et al., 1999a).

All these characteristics of DF are essential in understanding the physiological responses they promote when part of the regular diet.

1.2.4.3 Physiological effects of DF. Effects in the gastrointestinal tract

1.2.4.3.1 Effects of DF in the mouth and stomach

When part of foods, DFs (especially insoluble DF) are likely to prolong chewing time and consequently to extend the time of consumption, with direct influence on the amount of food consumed (Endress and Fisher, 2001), and possibly on the size of food particles that are passing through the oesophagus to the stomach. These alter the rate of food ingestion (Johnson and Southgate, 1994b) and may result in a reduced intake of calories which can have positive effects on weight control/loss. Once in the stomach food particles are retained until their size is reduced to about 1-2 mm, to ensure that the duodenal contents have a optimal texture for digestion (Johnson and Southgate, 1994b). This is the case for hard, fibrous, insoluble DF when ingested in intact form of plant cell wall - as whole

grains, breakfast cereals. Soluble DFs however behave differently. Some of them develop viscosity when they reach the stomach and this is believed to retard gastric emptying to some extent. The result is not only a delay in the rate at which liquids enter the small intestine (with immediate effects on carbohydrate metabolism), but also a prolonged satiety, and therefore reduced further consumption of food (Johnson and Southgate, 1994b; Pereira and Ludwig, 2001; Howarth et al., 2001).

In an attempt to quantify the satiety effects of different foods, a parameter called 'satiety index' (SI) has been introduced (Holt et al., 1995; Holt, 1999). The SI compares how a group of volunteers feel hunger/satiety after eating isoenergetic meals with constant carbohydrate / fat / protein ratio, the results being reported against the satiety for white bread. Both the extent and duration of satiety are evaluated usually over 2-3 hours, using a scoring system from -10 (representing extreme hunger) to +10 (representing extreme satiety) (Haber et al., 1977; Skabanja et al., 2001). Delargy et al. (1997) showed that soluble and insoluble DF behave differently, with soluble DF reducing the appetite for a longer time after eating than insoluble DF, but the subjects consumed less energy at the subsequent meal after high insoluble than after high soluble DF intake. Another study found that psyllium (reasonable doses) reduces hunger feelings and energy intake in normal volunteers (Rigaud et al., 1998). In this case the mechanism was not related to delayed gastric emptying, but to prolonged time allowed for intestinal absorption (as suggested by the flattening the postprandial serum glucose, insulin, and triglycerides curves).

1.2.4.3.2 Effects of dietary fibre in the small intestine

The small intestine is the principal site of nutrient digestion and absorption. Hydrolysis of the digestible polymers in food occurs within the first 2 m of duodenum and jejunum; with the first fermentable residues from a meal leaving the small intestine about 4½ hours after ingestion (Johnson and Southgate, 1994b). Although DFs are resistant to hydrolysis both in the stomach and small intestine, their presence in the small intestine might affect the physical characteristics of the gut contents. Their water holding capacity and/or viscosity DFs may affect the volume and bulk of small intestine contents, and hence influence transit times. While insoluble DFs reduce transit time (Harris et al., 2000; Key and Mathers, 1993) by increasing the contraction frequency, certain soluble DF (e.g. guar gum, pectin) have been shown to modify intestinal motility and to prolong transit time through increased viscosity.

Table 1.2. Characteristics of DF in relation to small intestine function (from (Schneeman, 1998; Schneeman, 1999b))

Characteristic	Effect on small intestine	Physiological implication
Dispersability in water (water holding capacity)	Increases volume in the intestinal contents	- Slows digestion and absorption of carbohydrate and lipid
	Dilution of compounds	- Promotes nutrient absorption more distal in the intestine
Bulk	Expands bulk material phase of contents	- Associated with reduction of plasma cholesterol and blunting of glucose and insulin response
	Alters mixing of contents	
Viscosity	Slows gastric emptying	
	Alters mixing and diffusion	
Absorb/bind compounds	Increased excretion of bile acids and other bound compounds	- Associated with plasma cholesterol reduction

Metabolic effects are also observed in response to ingestion of DF and they are thought to be again related to their physico-chemical characteristics: viscosity, water holding capacity, bulk and binding capacity. When in the small intestine DF are thought to increase viscosity, to strengthen the so-called unstirred water layer in the gut, which leads to a

higher diffusion barrier, and also to bind enzymes non-specifically and thus reduce their activity, both with direct influence on the rate of digestion and effectiveness of nutrient absorption (Endress and Fisher, 2001; Schneeman, 2001). The effects include moderation of postprandial blood glucose and insulin concentrations, reduction in total and LDL cholesterol and regulation of appetite (Davidson and McDonald, 1998). Some of these effects are summarised in Table 1.2.

1.2.4.3.2.1 Metabolic effects

1.2.4.3.2.1.1 Effect on carbohydrate metabolism

It is widely accepted that through their presence in the alimentary bolus, certain DFs affects digestion and absorption of macronutrients. The scientific community acknowledges that in the small intestine one of the major effects of DF is on starch hydrolysis and carbohydrate absorption resulting to a reduced and flattened glycaemic and thus insulinaemic response. The influence of certain DFs on plasma glucose and insulin levels has been demonstrated in both healthy and diabetic individuals (Jenkins et al., 2000; Anderson, 1989), and they are extremely important both in reducing the risk of developing type 2 diabetes, and also for the glucose control in diabetic patients (Anon., 2002a).

It is important to emphasise that besides the presence of DF, there are many other factors that may influence the rate of digestion and thus the rate of glucose delivery by the small intestine to the peripheral tissues *via* the portal vein (Bjorck et al., 1994). Both *in vitro* and *in vivo* studies have attempted to identify the factors involved in the modulation of the glycaemic response and they lead to the followings:

- Botanical source and genotype. The evolution of postprandial glycaemia following the ingestion of starchy foods was demonstrated to be strongly affected by

the starch botanical source (Skrabanja et al., 1999; Liljeberg et al., 1992). The explanation was related to the amylose/amylopectin ratio of starch (Skrabanja et al., 1999; Granfeldt et al., 1994b; Akerberg et al., 1998; Slaughter et al., 2001), since amylopectin is hydrolysed much faster by pancreatic α-amylase (Kabir et al., 1998). Studies on the glycaemic response of high amylose (e.g. 70%, 63.1%) *vs.* low amylose (e.g. 25%, 23.3%) content starches carried out *in vivo* (Granfeldt et al., 1994b; Granfeldt et al., 1995a) and *in vitro* (Skrabanja et al., 1999) showed a decrease in the glycaemic response (with 57% and 20% respectively).

- Presence of resistant starches. Some starches were found to escape digestion during passage through the stomach and small intestine, to arrive in the large intestine and to be fermented by colonic microflora. They are described as resistant starches (Muir et al., 1995; Topping and Clifton, 2001), and may occur in various forms: physically entrapped, inaccessible starch (RS1); native starch consisting of ungelatinised granules (RS2); retrograded starch (RS3), and chemically modified starch (RS4) (Niba, 2002). When present in foods they were found to lower glycaemic responses (Garcia-Alonso and Goni, 2000; Garcia-Alonso et al., 1998; Bravo et al., 1998), and also to exert a 'second meal' effect (Axelsen et al., 1999).

- Food structure and particle size (physical form of the food ingested). Studies in healthy subjects have found that postprandial blood glucose and insulin responses are greatly affected by food structure and any process that disrupts the physical or botanical structure of food ingredients will increase the plasma glucose and insulin responses. (Bjorck et al., 1994; Juntunen et al., 2002; Granfeldt et al., 1994b; Liljeberg et al., 1992).

- Starch interaction with other food components such as lipids (Fardet et al., 1999b), proteins (Fardet et al., 1999a; Fardet et al., 1998; Wolever et al., 1986; Jenkins et al., 1987) and DF (Brennan et al., 1996).

Several epidemiological studies arrived at the conclusion that there is a link between consumption of DF-rich foods and reduced risk of type 2 diabetes and CHD (Liu, 2002). An inverse relationship between the intake of whole grain or cereal fibre and the risk of type 2 diabetes has been reported from the 'Nurses Health Study' and the Health Professionals Follow-up Study (Liu et al., 1999; Salmeron et al., 1997b; Salmeron et al., 1997a). No significant reduction in the risk of diabetes was found for diets containing DF from fruits or vegetables (Salmeron et al., 1997b) or with increased intake of TDF (Salmeron et al., 1997a). Although this would suggest a direct effect of DF, we should not ignore the fact that fibre-rich foods contain different types of DF as well as other potentially beneficial compounds, and many foods naturally high in fibre have low GI and insulinaemic index (II), possibly due to the food form (Granfeldt et al., 1995b).

In comparison, in dose-response studies, the effects of DFs on the glycaemic response were found to be mainly associated with water soluble, gel forming DF and not to the insoluble DF. Soluble DF that form viscous solutions, may slow the rate of starch digestion and alter the rise of postprandial glycaemia' (Wood et al., 1994; Vuksan et al., 2000; Bjorck et al., 1994). These effects are relatively well established for guar gum (Wolever et al., 1979; Ellis et al., 1988; Brennan et al., 1996; Ellis et al., 1991a) and oat gum (Malkki and Virtanen, 2001; Wood, 1994; Wood et al., 1994; Braaten et al., 1991; Braaten et al., 1988). There are various proposed theories aiming to explain the effects of these viscous DFs on the glycaemic and insulinemic responses:

- Delayed gastric emptying. It was suggested that viscous DF due to their water holding capacity can cause delayed gastric emptying and slower transit through the small bowel, resulting in a reduced rate of nutrient digestion and absorption (Blackwood et al., 2000; Cherbut, 1995). A study conducted on healthy volunteers fed high (20g/100kcal) *vs* low (4g/1000kcal) TDF diet, showed a decrease in gastric emptying for the meals containing high fibre (Benini et al., 1995). Diez et al. (1998) followed the gastric emptying rate of dogs on a diet supplemented with cellulose, pectin, or guar gum (at 3.4% on dry matter basis), and concluded that only guar gum lowered the gastric emptying rate.

  Not all the data support a delayed gastric emptying promoted by the ingestion of soluble, viscous DF. For instance although it provides viscosity in the stomach, the intake of psyllium failed to result in a delayed emptying of the stomach in a study performed on healthy volunteers (Rigaud et al., 1998). A study on pigs fed an oat based diet also found no relationship between the DF content and gastric emptying (Johansen et al., 1996).

- Reduced activity of hydrolytic enzymes within the small intestine is another proposed hypothesis (Dunaif and Schneeman, 1981) and it was related to inhibition of mixing effects generated by peristalsis, formation of a physical barrier during starch digestion, or inhibitory effects on pancreatic α-amylase.

  ✓ The increased viscosity in the small intestine following the ingestion of certain DFs is thought to affect propulsive and mixing effects generated by peristalsis (Ellis et al., 2001). A study on the effects of DFs with different physicochemical properties (wheat bran, sugar beet and ispaghula) on small intestinal motility and postprandial

glycaemic response in healthy humans showed that only water soluble DFs influenced duodeno-jejunal activity (Cherbut et al., 1994). They inhibited stationary contractile activity, increased the propagation length and velocity of propagated activity, and at the same time decreased the blood glucose response, whereas the insoluble DF had no effect. Based on these observations the authors suggested that the contractile activity induced by DF in the small intestine probably plays a major role in delayed glucose absorption (Cherbut et al., 1994). Under inhibited mixing activities within the small intestine, the interactions between the digestive enzymes and their substrates are likely to be less frequent. As a result, two simultaneous effects may occur with direct influence on the postprandial level of blood glucose: a decrease in the starch digestion rate by pancreatic α-amylase and a slower diffusion of the products of amylolysis (e.g. maltose, dextrins) to the intestinal mucosa.

✓ Formation of a physical barrier by the DF during starch digestion also appeared as a possible mechanism (Brennan et al., 1996). In a study on guar galactomannan enriched bread, Brennan et al. (1996) related the decreased rate of starch hydrolysis *in vitro* to the formation of a layer of guar gum surrounding the starch granules. The observation was based on the examination of wheat bread containing guar gum by scanning electron microscopy (SEM) and by fluorescence microscopy, which revealed that the starch granules were coated with a layer of galactomannan mucilage. The authors concluded that guar gum may influence food structure and act as a physical barrier between α-amylase and starch interactions and/or subsequent release of hydrolysed products (e.g. maltose). This, in addition to the effect of guar gum on digesta viscosity, may have immediate results: a decreased

rate of starch digestion and glucose supplied to the blood. In a follow-up study on model systems investigating the effects of guar galactomannan on the kinetics of amylolysis, the authors concluded however that the existence of a significant physical barrier to the enzyme resulting from galactomannan-starch interaction seemed unlikely under the conditions the experiment was conducted (Slaughter et al., 2002). Nevertheless, the hypothesis on the formation of a layer of soluble DF surrounding the starch granules is supported not only by microscopy investigations, but also by the theory on 'thermodynamic incompatibility' of biopolymers (Tolstoguzov, 2003b; Tolstoguzov, 2003a). Thermodynamic incompatibility of biopolymers implies that macromolecules 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 macromolecules such as guar gum. This theory on incompatibility, was used to explain why the addition of guar gum increases the amount of starch which resists digestion (Tolstoguzov, 2003b). According to Tolstoguzov (2003) amylopectin is incompatible with guar gum, 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 can stop the leaching of amylose. This phase separation is thought to increase the concentration of macromolecules inside the starch granules, with consequences in an increase rate and degree of starch retrogradation (decrease in starch digestibility/formation of resistant starch) (Tolstoguzov, 2003b).

✓ Inhibitory effect of certain DFs on α-amylase was suggested as another possible mechanism involved in lowering the glycaemic response (Endress and Fisher, 2001). Recently Slaughter et al. (2002) investigated the effects of guar

galactomannan on the kinetics of starch amylolysis to arrive at the conclusion that guar galactomannan has a direct non-competitive inhibitory effect on α-amylase (through direct binding of α-amylase to galactomannan). The mechanism suggested was direct binding of the enzyme to galactomannan resulting in the formation of an inactive galactomannan-amylase complex. The authors concluded that although the affinity of guar galactomannan for α-amylase is relatively low in comparison with starch affinity for α-amylase, guar galactomannan acts as an inhibitor and may contribute to lowering postprandial glycaemic response by a direct inhibition of amylolysis. The conclusions of another *in vitro* study also referred to a possible direct inhibition effect DF might have on α-amylase (Ou et al., 2001).

- Increase in the thickness of the unstirred water layer. A theoretical concept of an unstirred layer has been used to understand the absorption rate of water soluble nutrients from the small intestine. They must penetrate this unstirred layer at the cell surface to reach the transport mechanisms on the brush border of enterocytes (Schneeman, 2001). Thickening the unstirred layer would present a greater barrier to absorption and therefore would delay absorption (Schneeman, 1998). Viscous polysaccharides are thought to reduce mixing within the small intestine and propulsion movements, therefore to increase the thickness of the unstirred layer, with consequences in slowing the diffusion of glucose to the intestinal membrane, and therefore reducing its absorption rate (Johnson and Gee, 1981 cited by Mathers and Daly, 2001; Wursch and PiSunyer, 1997). As the nutrient absorption is slowed, the process of absorption is extended in time and displaced physically along the length of

the small intestine. This reduces the rate at which nutrients appear in circulation and increases the exposure of the small intestine surface to nutrients.

- The capacity of DF to absorb glucose was proposed as a hypothesis by a group of researchers following an *in vitro* study on possible mechanisms DF how might affect postprandial serum glucose (Ou et al., 2001). Investigating the effect of DF (both soluble and insoluble) on glucose diffusion through dialysis tubings, they found out that all the DFs reduced glucose diffusion rates; glucose diffused was decreased 20-30% and 10-20% respectively when soluble and insoluble DF fibres were present in the system. Given that the insoluble DF do not contribute to the viscosity formation, the authors concluded that it is difficult to explain their results based only on the effect the increased viscosity might have on diffusion rates, and that another possibility might be binding of glucose to DF. They explored that possibility to find out that all samples of DF were capable of binding glucose, with the highest capacity of glucose binding being shown by the water insoluble DF, followed by the guar gum, and the lowest capacity was shown by carboximethyl cellulose and resistant starch (Ou et al., 2001). However, under the conditions of the experiment, the results for binding capacity of soluble DFs would be affected by the increased viscosity as well. Therefore the values presented in this case should be treated with caution.

As a means of comparing some of these physiological effects of food carbohydrates in terms of postprandial glycaemia, the concept of glycaemic index (GI) has been introduced (Jenkins et al., 1981b). The concept was developed based on the glucose tolerance test - a clinical technique for evaluating glucose metabolism in humans. In healthy subjects the concentration of blood glucose is maintained within the normal range by the opposing actions of the pancreatic hormones insulin and glucagon. After a meal containing

carbohydrates, glucose enters the blood circulation, resulting in a rapid rise in serum glucose levels, reaching a peak level 30-60 min after ingestion. This concentration rise stimulates the production of insulin, which stimulates the uptake and utilisation of glucose by the liver and peripheral tissues. The concentration of glucose then rapidly falls (within the next 30-60 min) to the initial fasting level of 3.5 - 5mmol/l approximately. The glucose tolerance test is based on monitoring the change in blood glucose concentration of an individual after the consumption of a standard carbohydrate test meal.

Table 1.3. Factors influencing glycaemic response to food (adapted from (McLaren, 2000) and (FAO, 1998))

Factors influencing glycaemic response to food
Rate of ingestion
Food form
Nature of the monosaccharide components
Glucose
Fructose
Galactose
Nature of starch
Ratio amylose/amylopectin
Resistant starch
Methods of cooking and processing food
Food form/structure
Degree of starch gelatinisation
Particle size
Food components
Carbohydrate content
Fat and protein content
Dietary fibre content
Antinutrients
Organic acids
Physiologic effects
Pregastric hydrolysis, gastric hydrolysis
Gastric emptying rate,
Intestinal hydrolysis and absorption
Pancreatic and gut hormone responses
Colonic effects

For the GI test, a group of healthy volunteers who fasted overnight are given a test meal of the experimental food containing a standardised amount of carbohydrate. The concentration of blood glucose is then monitored for 3 hours. The GI for the experimental food is calculated as the ratio between the area under the blood glucose curve following the test meal and that produced by ingesting an equal amount of carbohydrates as a reference food (e.g. glucose or white bread) (Johnson and Southgate, 1994b). This simple method provides an effective means to compare the rate of glucose assimilation following the ingestion of various foods, and has proven to be a relatively useful tool in the dietary management of diabetes mellitus, and also in guiding food choices. It also demonstrated that the effect of a carbohydrate source on blood glucose level is determined by much more than just its chemical composition. Some factors that influence glycaemic response to foods are presented in Table 1.3.

A considerable amount of work has been put into ranking various foods based on their GI, since there is increasing evidence that there are health benefits to be obtained from a diet rich in low GI foods: decrease in the postprandial blood glucose, thus improved control of blood glucose in both non-insulin dependent (NIDDM) and insulin dependent diabetes mellitus (IDDM), reduced insulin secretion, reduced blood lipids in hyper-triacylglyceridemic subjects. In addition, low GI diets or diets with low glycaemic load (dietary glycaemic index x dietary carbohydrate) were found to be negatively related to the development of type 2 diabetes (Salmeron et al., 1997a; Salmeron et al., 1997b). It is also believed that they enhance satiety and increase athletic performance (McLaren, 2000).

Based on the values of the GI foods have been classified as:

- low GI foods (GI <55)
- intermediate GI foods (GI between 55-70)
- high GI foods (GI>70).

The highest GI is commonly associated with potatoes and breads (around 70-80%). Lower values are related to fruits (around 50%), pasta (45-50%), dairy products (around 35%), dried legumes (around 30%). Some examples of foods and their corresponding GIs are presented in Table 1.4.

Table 1.4. Glycaemic index values for a range of foods (values from Jenkins et al., 1981a; Anon., 2001b; Anon., 2001c; Bjorck et al., 2000)

Food	**Glycaemic index (GI)** (glucose as reference)	**Glycaemic index (GI)** (white bread as reference)
Cereal products		
Bread (white)	69	100
Bread (wholemeal)	72	-
Bread	-	89-131
Sourdough bread	-	83
Kernel based bread	-	35-75
Rice (white)	72	60-80, 120
Rice (brown)	66	-
Spaghetti (white), pasta	40-50	40-70
Spaghetti (wholemeal)	40-49	-
Spaghetti (protein enriched)	28	-
Digestive biscuits	50-59	-
Breakfast cereals	-	74, 96-131
Porridge oats	49	-
Vegetables		
Potatoes	70-90	80, 98-120
Beans (tinned baked)	40	-
Beans (kidney)	29	-
Beans (soya)	15	-
Fruits		45-74
Apples	39	-
Oranges	40	-
Banana	62	-
Banana (unripe)	30	-
Dairy products		
Ice cream	40-50	-
Milk	34	15-60
Yoghurt	38	15-60
Sugars		
Glucose	100	138
Fructose	20	27
Sucrose	59	92
Honey	87	-

Among the possible mechanisms governing the glycaemic response, the rate of starch digestion was suggested as playing a leading role (O'Dea et al., 1981; Bornet et al., 1989). Consequently, research studies have proposed and used several *in vitro* methods of starch digestion to estimate the extent of starch digestion over time in the small intestine and thus to predict the glycaemic response. Incubation of starchy food with enzymes was performed using either salivary (Granfeldt and Bjorck, 1991; Jenkins et al., 1982) or pancreatic α-amylase (Bornet et al., 1989) alone, or in combination with proteolytic enzymes such as pepsin (Casiraghi et al., 1992; Brighenti et al., 1995; Granfeldt et al., 1994b; Granfeldt and Bjorck, 1991). Incubations with the enzymes were either unrestricted (Brennan et al., 1996; Bornet et al., 1989; Schweizer et al., 1988; Holm and Bjorck, 1988; Tufvesson et al., 2001) or restricted by dialysis tubings (Granfeldt and Bjorck, 1991; Granfeldt et al., 1994b; Jenkins et al., 1982; Casiraghi et al., 1992; Brighenti et al., 1995). Enzyme preparations (enzymatic activities or concentrations) varied greatly. For instance Englyst et al. (1999) used 10 ml pepsin solution (5g pepsin/l) and 5 ml of enzyme mixture (3g pancreatin to 20ml water to which 4 ml amyloglucosidase and 6ml invertase were added) to digest foods containing less than 0.6g carbohydrate). Brighenti et al. (1995), Giacco et al. (2001) and Casiraghi et al. (1993) used 25U salivary α-amylase, 575U hog pepsin, and 50 U hog pancreatin *per* gram of starch. Holm and Bjorck (1988) used 200 units of α-amylase *per* gram of starch, while Fardet et al. (1998) used 32 units of crude α-amylase from human saliva *per* mg of dry product, 1325 units Anson of pepsin from porcine stomach *per* mg dry product, and 200 units of pancreatic α-amylase from pig *per* mg of dry product. Skabanja et al. (2001) used 50mg pepsin/ml buffer, and 110 units of α-amylase *per* ml buffer. Bornet et al. (1989) used 166 units *per* g starch hog pancreatic α-amylase, whilst Beer et al. (1997) used 250µl human salivary α-amylase solution (5mg/ml), 625µl porcine

pepsin solution (0.5mg/ml) and 1.25ml pancreatin solution (0.5mg/ml) for the digestion of 5 g food samples. Sample preparation involved disruption of the food structure to different extents, using various methods such as grinding and milling, or chewing (Granfeldt and Bjorck, 1991; Granfeldt et al., 1992) or simulated chewing (Brighenti et al., 1995; Schweizer et al., 1988; Englyst et al., 1999) to investigate the digestion of food as eaten. The results were expressed in different ways:

- as the proportion (maltose equivalents) of starch degraded to maltose: (mg maltose equivalents - mg maltose equivalents in the sample before incubation) x 0.95 x 100/mg starch (Holm and Bjorck, 1988; Granfeldt et al., 1994b; Tufvesson et al., 2001);
- as the proportion of starch degraded to glucose (glucose released x 0.9) (Englyst et al., 1996; Fardet et al., 1998);
- determining the total reducing sugars (Casiraghi et al., 1992);
- as a 'hydrolysis index' (HI) calculated as the area under the hydrolysis curve (0-180 min) with the product as a percentage of the corresponding area with a reference white bread (Granfeldt et al., 1994b; Akerberg et al., 1998);
- as predicted glycaemic index using a predictive equation proposed by Brighenti et al. (1995) based on a multi-enzymatic digestion system confined within dialysis tubings followed by analysis of the reducing sugars in the permeate. The food followed three treatments: the first with active enzymes, the second with deactivated enzymes (blank) and the third with deactivated enzymes plus a known amount of maltose, which allow the measurement of sugar diffusivity through the dialysis tube in the presence of food (diffusion). The amount of reducing sugars in the dialysate was determined every 30min for 5 h and the results were used to calculate a digestion index (digestion results minus blank reported to those obtained for a reference white bread), and a diffusion

index (diffusion results reported to those of a pure maltose solution in the absence of food). These *in vitro* results together with those from chemical analyses were used as variables to calculate a predicted glycaemic index: $GI_{predicted}$= 105.52 fibre:carbohydrate-76.46 protein:carbohydrate+1.23 digestion index at 150min+69.41 sugar diffusion index at 270 min -13.41 index of physical form -70.46. The equation also includes a variable (index of physical form) which takes into account food structure: index = 0 when the original cellular structure is retained (e.g. rice, beans, chick peas etc), and =1 when the foods were submitted to milling at any time before the final preparation (e. g. chestnuts, lentils and corn flours, bread, pasta etc.) (Brighenti et al., 1995). This equation was found to successfully predict the glycaemic response to foods *in vivo* in both healthy volunteers (Brighenti et al., 1995) or subjects with type 2 diabetes (Giacco et al., 2001).

- as predicted glycaemic index using predictive equations:
  - proposed by Granfeldt (1994a) cited by (Akerberg et al., 1998):

    $GI_{predicted}$=0.862HI+8.189; HI=hydrolysis index calculated as previously mentioned.

  - proposed by Urooj and Puttaraj (2000) using multiple linear regression analysis based on nutrient composition and *in vitro* analysis data (degree of gelatinisation, digestibility index, starch digested (%) and 3h sugar value. The multiple linear equations proposed were:

    $GI_{predicted}$=-66.04 - 6.91x1 + 5.23x2 + 0.6x3 - 7.39x4 - 0.06x5, R=0.87, where x1 is protein, x2 is fat, x3 is energy, x4 is fibre and x5 is starch content.

    $GI_{predicted}$=139.47 - 0.6x1 + 0.51x2 + 0.74x3 - 2.40x4, R=0.90, where x1 is the percentage of starch digested, x2 is the 3h sugar value, x3 is degree of gelatinisation, x4 is digestibility index.

- As rapidly available glucose (RAG) and slowly available glucose (SAG), since it has been suggested that RAG is a major determinant of the magnitude of the glycaemic response demonstrated by a significant correlation between *in vivo* glycaemic response and RAG (Englyst et al., 1999). The determination of RAG, SAG, and starch fractions (rapid digestible starch (RDS), slowly digestible starch (SDS), total starch, and resistant starch) is based on an *in vitro* technique which involves the measurement by HPLC of glucose released from a test food during timed incubation with digestive enzymes under standardised conditions (Englyst et al., 1999).

These *in vitro* methods which allow the evaluation of the hydrolysis rate index (HI) or the prediction of the GI are generally closely correlated with the GIs and therefore are recommended as a tool which facilitates ranking of starchy foods (Bornet et al., 1989; Lichtenstein et al., 2002; Granfeldt et al., 1994b).

Among other factors that affect postprandial glycaemic response and the GI values of foods, the DF content has received significant consideration. Wolever (1990) showed that the GI of foods is correlated with the DF content. Similarly, Englyst et al., (1996) found weak but significant inverse correlation between DF and GI for some starchy foods. Many experiments have been carried out using isolated components of fibre administrated (especially guar gum, pectin, β-glucan and wheat fibre) in combination with a liquid test meal containing glucose. Under these conditions only soluble fibres with a high viscosity led to a significant reduction in postprandial glycaemia, whereas wheat bran was ineffective (Jenkins et al., 1978 cited by Johnson and Southgate, 1994b). It is thought that foods containing viscous DF or foods that are resistant to starch gelatinisation show slower rates of digestion and absorption and may be considered low GI foods. Consequently a lot of interest started to be expressed for soluble DFs, and guar gum in particular. Ironically,

following some epidemiological studies it was shown that the insoluble DFs are the ones that offer protection from diabetes (Jacobs et al., 1998; Salmeron et al., 1997a; Salmeron et al., 1997b; Meyer et al., 2000). Some authors consider that insoluble DFs may also slow the absorption of food (Anderson, 1986 cited by Meyer et al., 2000).

Numerous research studies have investigated the effect of guar gum ingestion on glycaemic response. Lafrance et al. (1998) showed that a high fibre diet containing guar gum (40g TDF *per* day, of which 15g were represented by guar gum) improved the glycaemic response in a group of nine patients with type 1 diabetes. Similarly, the postprandial blood glucose and insulin levels in 14 NIDDM patients were improved when fed with bread containing guar gum of different molecular weights (7.6g *per* meal) (Gatenby et al., 1996). The physiological effects seemed to not be influenced by partial depolymerisation of guar gum. In another study, Ellis et al. (1991a) investigated the effect of ingestion of bread containing guar gum (different molecular weights and particle sizes) on postprandial blood glucose and insulin response in healthy subjects. The authors found no significant differences in the postprandial blood glucose responses between the control and guar breads, but a significant decrease in the postprandial rise in plasma insulin. Fairchild et al. (1996) fed a group of 10 healthy volunteers with guar containing wheatflake product (6.3g guar gum *per* serving) to find out that significant reductions in postprandial glucose and insulin responses were seen following the guar wheatflake meal compared with the control meal. Research studies concentrating on the effects of guar gum have found that the quantities needed to be incorporated in the meals ranged from 3 to 20g, in order to result in a positive effect. At these levels the test meals became extremely viscous and not pleasant to ingest. However, several pharmaceutical products containing guar gum were made to assist in the management of diabetes. Guar gum has also been

added to bread but the product it is not available on the market. Very few foods contain viscous DF at high enough levels to make a difference to intraluminal viscosity; oats, rich in β-glucan represent an exception.

Cereal β-glucans have also received considerable attention since their effect on glycaemic response has been shown to be similar to that of guar gum (Braaten et al., 1991) and related to viscosity development (Wood et al., 1994). Experiments with solutions of oat gum revealed a significant relationship between the glucose or insulin response and the logarithm of viscosity (79-96% of the changes in plasma glucose and insulin were attributable to viscosity (Wood et al., 1994), or a combination of logarithm of the concentration and logarithm of molecular weight (Wood et al., 2000). The same correlation was found for guar gum (Braaten et al., 1991; Wood et al., 2000). In a dose-response study, patients with type 2 diabetes that received breakfast cereals containing various amounts of β-glucan (4, 6 and 8g), had the maximum increases in plasma glucose of 67%, 42%, and 38% respectively, compared with the control; a linear inverse relationship was found between the dose of β-glucan and the area under the glucose curve (Tappy et al., 1996). Yokoyama et al. (1997) have demonstrated that by increasing the amount of barley β-glucan in pasta with 5.3% (dwb), a reduction in both glycaemic and insulinaemic responses can be achieved in healthy volunteers. In another study on healthy volunteers fed pasta enriched with barley β-glucan (Bourdon et al., 1999), the glycaemic response was not significantly different from the control, but a reduced insulinaemic response was observed indicating that carbohydrates from the meal with β-glucan enriched pasta were digested and absorbed slower than from the control. A study conducted both *in vivo* and *in vitro* by Granfeldt et al. (1994b) showed decreased values of both GIs and HIs (and a good correlation between them) for barley products when compared to the white bread. The

authors related the results to the viscous properties of β-glucans. However, some other studies suggest that for oat products, the decrease in the glycaemic response was due to the food form rather than to the presence of viscous DF (Granfeldt et al., 1995b).

DFs with high viscosities and ion-exchange capacity such as pectins seems to generate similar effects. For instance, a study on healthy volunteers who consumed a diet enriched with sugar beet fibre, reported a substantial reduction of postprandial glucose response associated with the presence of sugar beet fibre in the diet (Torsdottir et al., 1998). A similar conclusion was drawn by Frape and Jones (1995): both glucose and insulin responses in blood were lower after a diet enriched with sugar beet fibre.

Psyllium seed husk is also known to reduce blood glucose levels, thus being suggested as an adjunct to dietary therapy in patients with type 2 diabetes (Rodriguez-Moran et al., 1998) cited by Guillon and Champ, 2000; (Watters and Blaisdell, 1989; Vuksan et al., 1990). A study where healthy volunteers ingested a test meal composed of glucose and 15 g of psyllium, sugar beat fibre or wheat bran, showed that both psyllium and sugar beet fibre reduced glucose response, whereas wheat bran had no effect (Cherbut et al., 1994).

Yamashita et al. (1984) studied the systemic effects of adding fructooligosaccharides (8 g) to the daily diet of patients with type 2 diabetes, and found a 8% decrease in fasting blood glucose. This effect could not be reproduced by Alles et al. (1999) who used 15g/day fructooligosaccharides (in the diet of patients with type 2 diabetes) for 20 days; they concluded that fructooligosaccharides do not have important effects on blood glucose (). However in a study involving 8 healthy subjects, 10 g supplement of inulin isolated from Jerusalem artichokes reduced the glucose response when fed half way through a meal of bread containing 50 g of wheat starch (Rumessen et al., 1990).

Over the past decades several research studies carried out both *in vitro* and *in vivo* focused on the effect of DF on the glycaemic responses, and some of the outcomes are presented in Table 1.5. It is worth noting that the majority of the studies focused on the effects of wheat bran as insoluble fibre and of guar gum, psyllium, pectin and oat fibre as soluble fibres. Very few investigated the effects of other fibres such as inulin, xanthan gum, locust bean gum etc.

Nevertheless, from the summary presented in Table 1.5 it seems relevant to promote the consumption of low GI, DF enriched foods since their influence on glucose response recommend them as a promising tool for the management and also for the prevention of developing diabetes. The major problem however in implementing a low GI, high DF diet is the lack of palatable products available on the market.

Table 1.5. The effect of DFs on glycaemic and insulinaemic responses - studies *in vivo* and *in vitro* (* = decrease)

Dietary fibre/ level used	Studies *in vivo*		Studies *in vitro*		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
Guar gum (2.5, 7.5, 12.5g)	↓* insulin ↓ glucose absorption	-	-	-	(Torsdottir et al., 1989)
Guar gum 20g/100kcal *vs* 4g/100kcal (on healthy subjects)	↓ plasma glucose	↓ GI (with aprox. 30%)	-	-	(Benini et al., 1995)
Guar gum (various molecular weights and particle sizes) (on healthy volunteers)	no effect on plasma glucose level ↓ plasma insulin level	-	-	-	(Ellis et al., 1991a)
Guar gum at 20 or 40 g/kg (on pigs)	↓ blood glucose level (inverse relationship between blood glucose level and 'zero shear' viscosity of digesta	-	-	-	(Ellis et al., 1995)
Guar gum 3.4% on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
Guar gum, xanthan gum, methilcellulose, wheat bran - 70g (on rats)	↓ blood glucose level for meals containing viscous DF (significantly different only for xanthan gum)	-	-	-	(Cameronsmith et al., 1994)
Guar gum *In vitro* and *in vivo* (on pigs)	↓ blood glucose level	-	↓ amount of glucose produced	-	(Brennan et al., 1996)

Table 1.5. (continued)

Dietary fibre/ level used	Studies *in vivo*		Studies *in vitro*		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
Guar gum, xanthan gum, CMC, water insoluble DF, water soluble DF, resistant starch (dialysis tubings)	-	-	↓ amount of glucose produced ↓ amount of glucose diffused (with 10-30% from insoluble to soluble)	-	(Ou et al., 2001)
Guar gum (various molecular weights) 7.6g *per* serving - NIDDM patients	↓ blood glucose level ↓ blood insulin level	-	-	-	(Gatenby et al., 1996)
Guar gum (60g TDF of which 15g were guar gum *vs* 16g TDF) Patients with type 1 diabetes	↓ blood glucose level	↓ GI	-	-	(Lafrance et al., 1998)
Guar gum 6.3g *per* serving Healthy volunteers	↓ blood glucose level ↓ blood insulin level	-	-	-	(Fairchild et al., 1996)
Inulin 10 g fed half way through the meal	No effect ↓ blood glucose level	-	-	-	(Rumessen et al., 1990)
Inulin (15g/day) (patients with type 2 diabetes)	No effect	-	-	-	(Alles et al., 1999)
Inulin 8g/day (patients with type 2 diabetes)	↓ fasting blood glucose	-	-	-	(Yamashita et al., 1984)
Inulin 10g/day (on healthy volunteers)	↓ fasting insulin level	-	-	-	(Jackson et al., 1999)

Table 1.5. (continued)

Dietary fibre/ level used	Studies *in vivo*		Studies *in vitro*		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
Pectin Liquid diet 2.5%	↓ maltose absorption	-	-	-	(Chun et al., 1989)
Pectin 3.4%on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
Cellulose 10%	↓ blood sugars (-5%)	-	-	-	(Mahapatra et al., 1988)
Cellulose 3.4%on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
I. Psyllium and mixture psyllium-citrus pectin (2.2g) II. Sugar beet fibre (6g) and cellulose (2g) (on healthy subjects)	I. no effect II. ↓ blood glucose level ↓ blood insulin level	-	-	-	(Frape and Jones, 1995)
Sugar beet fibre (on healthy subjects)	↓ blood glucose level	-	-	-	(Torsdottir et al., 1998)
Psyllium 2.5%	reduced fasting glucose levels	-	-	-	(Watters and Blaisdell, 1989)
Psyllium 7.4 g (healthy volunteers)	↓ insulin level ↓ blood glucose level	-	-	-	(Rigaud et al., 1998)
Psyllium 15g (healthy volunteers)	↓ blood glucose level	-	-	-	(Cherbut et al., 1994)
Wheat bran 15g (healthy volunteers)	no effect	-	-	-	(Cherbut et al., 1994)
Wheat bran 12g/day	flatten glucose	-	-	-	(Kritchevsky, 1988 cited by Roberfroid, 1993)

Table 1.5. (continued)

Dietary fibre/ level used	Studies *in vivo*		Studies *in vitro*		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
Wheat bran (6%) In pigs	no significant effect on blood glucose or insulin levels	-	Increased the rate of hydrolysis	-	(Leclere et al., 1993)
Beet fibre (6%) In pigs	no significant effect on blood glucose or insulin levels	-	Decreased initially *in vitro* hydrolysis	-	(Leclere et al., 1993)
Sugar beet fibre 15g (healthy volunteers)	↓ blood glucose level	-	-	-	(Cherbut et al., 1994)
Pea fibre	flatten glucose	-	-	-	(Hamberg et al., 1989a; Hamberg et al., 1989b)
β-glucan from oats (4, 6 and 8g) (NIDDM subjects)	↓ blood glucose level ↓ blood insulin level estimate of 50% decrease in glycaemic response after ingestion of 5g β-glucan	-	-	-	(Tappy et al., 1996)
β-glucan from barley (healthy subjects) 5.3% dwb in pasta (or 17.4%dwb TDF *vs.* 4.1% dwb TDF)	*↓ blood glucose level* ↓ blood insulin level	-	-	-	(Yokoyama et al., 1997)
β-glucan from barley 15.7g TDF (5g of which was β-glucan) and 5g TDF (no β-glucan) (healthy subjects)	no effect on glucose level ↓ blood insulin level	-	-	-	(Bourdon et al., 1999)
β-glucan from barley foods and cellulose from wheat foods 21-38 gTDF/day (hypercholesterolemic men)	no effect on glucose level	-	-	-	(McIntosh et al., 1991)

Table 1.5. (continued)

Dietary fibre/ level used	Studies *in vivo*		Studies *in vitro*		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
β-glucan from barley *in vivo* (10 healthy volunteers) and *in vitro*	↓ blood glucose level ↓ blood insulin level	↓ GI	↓ reducing sugars released	↓ HI	(Granfeldt et al., 1994b)
β-glucan from oats (9 healthy volunteers)	no effect on glucose or insulin levels unless the food structure was intact	no effect on GI or II unless the structure was intact	-	-	(Granfeldt et al., 1995b)
Oat bran - β-glucan *in vivo* (healthy volunteers) and *in vitro*	↓ blood glucose level ↓ blood insulin level		↓ sugars released		(Holm and Bjorck, 1992)
Oat bran - β-glucan			↓ sugars released		(Hudson et al., 1992)
β-glucan : oat and barley products healthy volunteers	↓ blood glucose ↓ blood insulin levels	-	-	-	(Liljeberg et al., 1996)
β-glucan from oats (review paper)	↓ blood glucose level (50% reduction for 10% β-glucan)	-	-	-	(Wursch and PiSunyer, 1997)
β-glucan from oats, barley, and rye *in vivo* (healthy subjects) and *in vitro*	↓ blood glucose level	↓ GI	↓ sugars released	↓ HI	(Liljeberg et al., 1992)
β-glucan (barley) *in vivo* (subjects with type 2 diabetes)	↓ blood glucose level ↓ blood insulin level	-	-	-	(Urooj et al., 1998)
β-glucan and guar gum (14.5g)	↓ blood glucose level ↓ blood insulin level	-	-	-	(Braaten et al., 1991)
Arabinoxylan (7 and 14% on dry weight basis). *In vivo* (18 healthy volunteers)	↓ blood glucose level ↓ blood insulin level	↓ GI	-	-	(Lu et al., 2000)

1.2.4.3.2.1.2 Effect of DF on lipid metabolism

Consumption of DF was shown to affect blood lipids concentration (total plasma cholesterol and low density lipoprotein associated (LDL) cholesterol) and in relation to this, the risk of cardiovascular disease (CVD). A number of epidemiological studies have reported a relation between DF intake and the reduction in the risk of developing CVD, especially for cereal fibres (Rimm et al., 1996; Jacobs et al., 1999; Ludwig et al., 1999; Liu et al., 1999). In addition, several meta-analyses recognise the ability of certain DFs to lower plasma and LDL cholesterol; guar gum, pectin, locust bean gum, β-glucan, psyllium seem to reduce total cholesterol levels with: 0 - 18% in trials with oat products, -3 - 17% for psyllium, 5 - 16% for pectin and -4 - 17% for guar gum (Brown et al., 1999; Anderson et al., 2000). In general they seem to lower both total and LDL cholesterol, with no, or minimal change in HDL cholesterol (Braaten et al., 1994; Hecker et al., 1998; Tredger et al., 1991; Behall et al., 1997; Anderson et al., 2000). For certain soluble DF such as oat β-glucan and psyllium, the consensus of scientific opinion on their hypocholestrolemic effects led to the approval of Federal Department of Agriculture (FDA) of the USA claims. For example FDA permits a health claim related to cholesterol lowering and decreased risk of CVD if a product contains 1g or more of β-glucan *per* serving (Anon., 1996; Anon., 1997a).

1.2.4.3.2.1.3 Effect of DF on satiety and weight reduction

As mentioned earlier in this chapter the intake of soluble and insoluble DF prolong after-meal satiety. It has been suggested that when present in the small intestine they increase the contact time of fat or amino-acids with the receptors, thus promoting an increased

secretion of cholecystokinin (known to regulate gastric emptying, glycaemic response and to enhance satiety), and related to this a prolonged satiety (Malkki, 2001; Koh-Banerjee and Rimm, 2003).

Published studies indicate that an additional 14 g DF/day is associated with a 10% decrease in energy intake and body weight loss of 1.9 kg over 3.8 months (Howarth et al., 2001). The observed changes in energy intake and body weight occur both when the DF is from naturally high-fibre foods and when it is from a fibre supplement, suggesting that an increased intake in DF could be efficient in diets for weight-reduction/control.

1.2.4.3.3 Effects of DF in the large intestine

The functions of the large intestine are to recover by microbial action food residues that escaped digestion and absorption the small intestine, to absorb water and electrolytes and to form and store faeces (Johnson and Southgate, 1994b).

Table 1.6. Characteristics of DF in relation to the large intestine function (Schneeman, 1998; Schneeman, 1999b)

Characteristics	**Effect on large intestine**	**Physiological implication**
Dispersability in water	Provides an aqueous phase for penetration of micro-organisms	Increases microbial breakdown of polysaccharide structure
Bulk	Increases material entering the large intestine Affects mixing of contents	Provides substrate for microflora Aids laxation
Absorb/bind compounds	Increases the amount of bile acids in the large bowel	Excretion is increased Microbial modification of bile acids
Fermentability	Growth of microflora Microbial adaptation to polysaccharide substrates Formation of SCFA	Increased microbial mass and products of metabolism Effects related to SCFA

DFs that remained undigested during the transit through the stomach and the small intestine, reach the colon relatively intact, to exert various effects due to their physico-

chemical characteristics mentioned earlier in this chapter: fermentability, water holding capacity, bulk, and binding capacity. Table 1.6 summarises these characteristics of DF in relation to their effects on the large intestine and physiological implications.

DF has undoubtedly an essential role in ensuring normal laxation and the microbial population in the large bowel.

1.2.4.3.3.1.1 Effects of DFs on gut function - laxation effects

The importance of DF in increasing faecal bulk and stool frequency was one of the original assumptions of the DF hypothesis as formulated by Burkitt et al. (1974) and is still the most widely recognised physiological property of DF. Large intakes of DF have been associated with short transit times and large stool weights, and thus with reduced constipation and reduced risk of large bowel cancer and diverticular disease (Schneeman, 1998). Based on the results from a large number of clinical studies, Cummings et al. (1992) and Staniforth et al. (1991) reported a linear relationship between DF intake and stool weight. However, the ability of different DFs to increase faecal bulk and influence transit times depends on their physicochemical properties. Insoluble DFs, rich in pentoses and fermentable to a very small extent are known to increase faecal bulk and decrease transit time by maintaining their structure during the passage through the colon and resisting the re-absorption of faecal water during transit through the bowel (Johnson and Southgate, 1994b). However, it was suggested that the increment in stool mass is dependent on the particle size of DF (Johnson and Southgate, 1994b). Soluble, viscous DFs and resistant starches are readily fermentable in the large intestine and therefore they reduce their mass and modify their water holding capacity during transit through the large intestine. They are known to generally increase transit times, and to influence the faecal output through their

contribution to bacterial growth (Nyman et al., 1986; Chen et al., 1998; Heijnen et al., 1998; Cummings et al., 1996).

1.2.4.3.3.1.2 Dietary fibre and gut microflora composition

DFs reaching the colon are fermented to greater or lesser extent by the microflora in the large bowel. Some can provide a selective advantage for the proliferation of particular bacterial groups within the intestinal microbiota, with changes to number and types of bacteria, and also to their metabolic activities. Inulin, oligofructosaccharides and resistant starches are reported to be particularly effective in this respect. For instance, consumption of inulin and oligofructosaccharides has been demonstrated to promote the growth of bifidobacteria in the colon (Gibson et al., 1985; Havenaar et al., 1999; Crittenden et al., 2002), whilst resistant starches appear to increase the proportion of both lactobacilli and bifidobacteria, often with decreases in potentially patogenic microorganisms such as clostridia and enterobacteria (Gibson and Roberfroid, 1995; Silvi et al., 1999). Bifidobacteria and lactobacilli are considered beneficial genera within the human intestinal microbiota (probiotics), since they contribute to the maintenance of a favourable intestinal microbial balance (Zimmermann et al., 2001).

1.2.4.3.3.1.3 Dietary fibres and short chain fatty acids

Fermentation of soluble fibre and resistant starches by colonic microflora results not only in an increase of bacterial mass, but also yields short chain fatty acids (SCFA: propionic, acetic and butyric acids), which may be responsible for some of the metabolic effects that occur in response to ingestion of DF. The extent of fermentation and the profile of SCFA depend on the substrate (Salvador et al., 1993; Guillon and Champ, 2000), and different SCFA have been shown to have specific physiological effects. Propionic acid was suggested to inhibit hepatic cholesterol synthesis from acetic acid and the higher the ratio

of propionic to acetic acids, the more beneficial the effects (Jenkins et al., 1998). Animal studies indicated that acetate may influence carbohydrate metabolism; it appears however that such data was not confirmed by human studies (Roberfroid, 1993). Butyric acid as a main energy substrate for the colonic mucosa, is known to protect against some colonic diseases and most importantly, against colonic cancer (Brouns et al., 2002; Bingham, 1996).

1.2.4.3.3.1.4 Dietary fibre and colon cancer

Burkitt et al. (1974) hypothesised that DF provides significant protection against colon cancer risk. This hypothesis has been challenged over years, resulting in controversial reports (Goodlad, 2001; Levi et al., 2001); the general evidence is that insoluble DF may have a protective effect, while soluble, ready fermentable DF may promote tumour development. Nevertheless, data collected through epidemiological studies leads to the conclusion that cereal DFs have a role in the prevention of colorectal cancer (Hill, 1997b; Hill, 1997a). In support of this, the most recent and largest epidemiological study on the relationship between diet and cancer (EPIC - European Prospective Investigation of Cancer) reported that DF was inversely related to incidence of large bowel cancer, and a doubling in the total DF intake would result in a 40% decrease of the risk of colorectal cancer (Bingham et al., 2003).

1.2.4.3.4 Dietary fibre intakes and recommended levels

Despite the large body of evidence on the beneficial effects of high DF diets, the figures showing the consumption of DF are not necessarily encouraging, being usually far below the recommended levels. The median intakes vary from 12g/day in parts of Eastern Europe to 26g/day in Finland, in many countries more than 50% of the population failing to reach

even 20g of DF *per* day (Jones, 2003). Analysis of trends in consumption of DF show that in the UK intakes have continuously declined, except for the periods during the 1914-1918 and 1939-1945 wars (Johnson and Southgate, 1994c).

Disease risk, particularly of developing diabetes or obesity is a grave concern. One strategy of dealing with those risks involves increased DF intake, reduced digestible carbohydrates and proper exercise (AACC, 2003). An adequate daily intake of DF of 38 and 25 g *per* day for men and women respectively (19-50 years of age) has been recommended (AACC, 2003). A recent joint FAO/WHO report (WHO, 2003) made dietary recommendations for the prevention of diet related chronic diseases; amongst them were a decrease of intake in free sugars below 10% of the daily energy, an increase in consumption of fruits and vegetables (more that 400 g /day), and an increase of TDF intake (minimum intake 25g /day) were suggested. However, the same report states that the percentage of British adults complying with the national dietary guidelines is very small. For example, only 2-4% of the population is consuming the recommended level of saturated fat, and only 5-25% of the population is consuming the recommended level for DF (WHO, 2003). While men eat more DF daily, women have more dense DF diets. The figures for DF intakes in the UK are similar to those of other developed countries, the exception being the Scandinavian countries where the intakes are higher due to the use of rye and mixtures of wheat and rye flours in breadmaking.

In spite of nutrition education efforts in many countries and a raising of the DF recommended levels for adults to as high as 37g of DF *per* day, DF intake in most countries is decreasing rather than increasing. Part of the problem is related to the availability of such food products. Apart from breakfast cereals, and wholemeal or granary bread, there are relatively few other high DF products on the market, especially those

which are palatable, and can be easily chewed. More needs to be done in order to produce healthier and palatable high DF foods. It is essential that such foods are well accepted by the consumers, since a healthy food that is not eaten has no health benefit.

1.2.4.4 Technological properties

The vast majority of the research studies have been carried out on DF enriched foods mainly for nutritional reasons. Food product's characteristics that are of relevance for the consumers and for the food industry have been considered only to a limited extent, although they provide the key to the success of such products (both economic and nutritional).

It is known that DF can change the rheological behaviour, texture, and sensory characteristics of the final product, sometimes with a negative impact on food preferences. For instance the inclusion of high molecular weight guar gum into breads has been reported to result into a product which was not acceptable for the consumers (Ellis et al., 1991a). Moreover, some DF enriched foods require more effort put into chewing. It has been suggested that for breakfast cereals, the amount of energy required to break the product into small pieces increases as the amount of DF in the product increases, and as a result, the popularity of these products decreases (Oakenfull, 2001). Hence, the use of DF especially at high levels has often been limited. However, the emergence of novel sources of DF together with improvements of fibre functionality (for example, partially hydrolysed guar gum) opens new opportunities for their use in food formulations.

Positive effects of DF on food preferences were also noticed through their ability to give texture, mouthfeel and other sensory characteristics. These technological characteristics are derived from their physico-chemical properties, mentioned at the beginning of this chapter,

which make them suitable for use as water-binding agents, thickeners, suspending agents, gelling agents, film formers, emulsifying agents and stabilisers. Applications may be directed towards low fat and low energy foods, aiming to improve their textural characteristics and thus to enhance consumers' appeal for products which would otherwise be unattractive.

Some studies report on the use of DF as fat replacers (Mendoza et al., 2001; El-Nagar et al., 2002; Warner and Inglett, 1997; Inglett, 1997; Ward, 1997; O'Brien et al., 2003), or gluten substitutes in gluten free breads and pasta (Arendt et al., 2004). It has also been reported that certain DF are able to modify starch gelatinisation (Tolstoguzov, 2003b), to impart freeze/thaw stability (Yoon and Lee, 1990), to limit the formation of ice crystals (Kindel et al., 1989; Schaller-Povolny and Smith, 2001), or to control syneresis (Niederauer, 1998). DFs used as texture-modifying agents are generally soluble and they are added into foods at relatively low levels; nevertheless, even at low levels they can contribute towards the daily recommended intake of 25-37 g DF. Insoluble DF can also be used as texturising and bulking agents (Femenia et al., 1997) due to their hydration properties, but may create a gritty texture and reduction of functional properties of the food due to various particle sizes.

In developing new high DF food products, the functionality of DF within the food matrix is of paramount importance. Equally important are the concentrations used, processing parameters (i.e. temperature, pH, ionic concentrations etc), and also synergistic effects between the components leading to emphasised or suppressed functional characteristics of DF (Meuser, 2001). The complex interactions of these factors shape the characteristics of DF products, determining finally their quality, and also their role for gastrointestinal functions. Therefore, a better understanding of functional behaviour of DF within the food

matrix is required before an acceptable food product can be developed.

It is thought that two main types of food products can be prepared by incorporating DF (Endress and Fisher, 2001):

- Products that are rich in DF but do not differ significantly in terms of sensory properties from traditional products. This can be a useful way to increase the DF intake by the population.
- Products in which additional properties of certain DF such as colour or texture forming are used to design new products that differ from the traditional ones.

1.2.4.4.1 Application fields of dietary fibre in food products

Surprisingly studies offering a comprehensive approach to DF behaviour in food systems are still limited. Research studies that have been conducted on the physical and chemical characteristics relevant to physiological properties of DF additions to food, have been mainly focused on few DFs such as guar gum and oat β-glucan. The behaviour of other DFs in the food matrix remains to be investigated from both the technological and physiological point of view, especially at the present when a wide range of high DF isolates are available.

1.2.4.4.1.1 Dietary fibre in cereal products.

Cereal products already make a major contribution to the DF intake; they also offer an opportunity for further DF enrichment as the acceptance threshold is likely to be more easy to move than for highly refined foods. Work has been conducted on increasing the DF content of a wide range of cereal based products, from bakery products, biscuits, pasta, cereal bars, breakfast cereals, to cakes and muffins by the addition of cereal fibres (wheat bran, oat, barley), fruit fibres (citrus, apple), vegetable fibres (pea, sugar beet) and cellulose powders. The DF substituted part of the flour or fat and they were mainly added

for nutritional benefits (increased DF content, reduce calorie values), but in some cases also for flavour.

Some studies have exploited their water retention capacity in relation to textural properties, effects on volumes of cakes and also regarding anti-staling aspects. In cookies, the substitution of flour with wheat bran gave a firmer texture. Similarly, when added to cake and biscuit formulations, DF increased the firmness, but improved the storage properties in terms of texture. Grigelmo-Miguel et al. (1999) reported that the firmness of muffins increased with increasing level of peach fibre, and that for low levels of addition the products were as acceptable as the controls. Another study on DF enriched muffins reported that oat bran, high barley β-glucan fraction or rice bran, lead to products acceptable to the consumers, and, more importantly, their glycaemic response (determined *in vitro*) was low, with values comparable with whole cooked grains or pasta products (Hudson et al., 1992). DF have been also used in tortilla formulation where they were found to weaken the gluten matrix, thus modifying the microstructure and quality of both dough and wheat flour tortillas (Seetharaman et al., 1997).

1.2.4.4.1.1.1 Dietary fibre enriched pasta

Pasta is a very popular product all over the world due to its ease of cooking, convenience in terms of price, and diverse ways it can be prepared. Moreover, pasta products are well accepted among all categories of population regardless of age or health status.

In recent years pasta has become even more popular due to its nutritional properties. The low fat content of pasta is in accordance with the current dietary recommendations. More importantly though, pasta is among the low GI food products (Bjorck et al., 2000; Jenkins et al., 1988), despite the fact that it was considered for a long time as being a "fattening"

food which has to be consumed with moderation. Research has shown that pasta (or pasta like products) progressively release sugars that the body needs, and hence leads to low postprandial blood glucose and insulin responses (Jenkins et al., 1983; Bornet et al., 1989; Juntunen et al., 2002). Therefore pasta products are to be preferred to white bread, in the diet of both healthy and diabetic subjects.

Being such a popular product, the World Health Organisation (WHO) and the U.S. Food and Drug Administration consider pasta a good vehicle for incorporating ingredients with added health benefits (Marconi and Carcea, 2001). Thus, it seems relevant to enrich pasta with DF as a means of improving the DF intake amongst a wider range of population. However, in order to achieve a successful DF enrichment of pasta, it is important that there are not side effects on either taste, colour, texture or cooking properties.

Several studies have focused on the nutritional and physiological aspects of DF inclusion in pasta formulations. Yokoyama et al. (1997) incorporated barley β-glucan in pasta used to feed healthy volunteers; the postprandial glycaemic and insulinaemic responses determined were reported to be lower in comparison to a control without DF. These results were confirmed by another study in which postprandial lipid, glucose, and insulin responses were monitored in healthy volunteers fed pasta enriched with barley β-glucan at high and low levels of addition, providing 15.7g (of which 5g was β-glucan) and 5g TDF (0.3g β-glucan) respectively (Bourdon et al., 1999). Pasta products enriched with β-glucan were found to have acceptable quality in terms of texture, cooking and sensory attributes (Knuckles et al., 1997; Marconi et al., 2000).

Pasta with added guar gum (20%) has been shown to reduce the glycaemic response in subjects with diabetes; however, the acceptability of the end product was poor (Gatti et al., 1984; Briani et al., 1987). Another study investigating guar gum enriched pasta (20%)

found no effect of guar on the glycaemic response in subjects with type 2 diabetes and therefore the conclusion was that addition of DF to a complex meal is not useful in the dietary management of NIDDM (Sels et al., 1992). The effects of using xanthan gum and locust bean gum in the sensory attributes of non gluten pasta were also investigated (Huang et al., 2001).

Following a study involving rye breads and dark durum wheat pasta it was concluded that postprandial insulin responses to grain products are determined by the form of food rather than by the amount of DF or the type of cereal in the food (Juntunen et al., 2002). Addition of fibre rich durum bran to semolina for pasta production was also investigated; the resulting product was assessed as being tasty, but with a decreased firmness and poorer cooking quality (Kordonowy and Youngs, 1985; Manthey and Schorno, 2002).

Nevertheless, there are a limited number of studies investigating the effects of including DF (other than barley or oat β-glucan) into pasta formulation on the quality of the end product (texture, structure, cooking characteristics) and also on their glycaemic response.

1.2.4.4.1.1.2 Dietary fibre enriched bread

Bread is a staple foodstuff and today there are only a few countries in the world where bread is not eaten. Traditionally bread is based on flour derived from wheat. However, bread products have evolved to incorporate some other types of cereal flours (e.g. rye) and to take many forms. In most European countries and North America, consumers prefer white bread, despite its known high GI. The DF content of the most commonly used wheat flour is about 4g/100g, which leads to a final content of below 3g/100g bread (Endress and Fisher, 2001). This fibre content can be improved by using high extraction flour

(wholemeal flour), whole grains, or added DFs. However, only a minor part of the consumers choose to eat wholemeal and wholegrain products.

The increase of DF content in bread has been investigated by several researchers in relation to the glycaemic response. Following an *in vitro* and *in vivo* study, Bjorck et al. (1994) concluded that the glycaemic responses to bread products were reduced by the use of ingredients with an intact botanical or physical structure or by enrichment with viscous DFs. A similar outcome was reached by Liljeberg et al. (1992) who investigated the potential of developing bread with 'lente' characteristics by incorporating intact kernels from various cereals (wheat, rye, oats or barley). Following *in vivo* and *in vitro* determinations they reported that for the majority of breads (except those made with oat kernels and wholemeal barley) the GIs were significantly lower than for white wheat bread, while the insulinemic indexes and the rates of starch digestion were constantly lower than the control bread (Liljeberg et al., 1992).

The effects of guar gum on the postprandial blood glucose and insulin responses of both healthy and diabetic subjects have been extensively investigated, especially using bread as a vehicle. Very small differences in glucose response have been found when healthy volunteers were fed guar enriched white wheat bread and white wheat bread (control), while the values of postprandial blood insulin were significantly different (Apling and Ellis, 1983). The same study reported that greater feelings of satiety followed meals containing guar enriched bread. The effectiveness of guar gum in reducing postprandial blood glucose and plasma insulin levels in human subjects was also investigated in relation to its molecular weight and thus its ability to increase the viscosity of digesta (Ellis et al., 1991a). Following the ingestion of breads made with guar gum of various molecular weights by healthy volunteers, blood glucose and insulin responses were monitored. The

results showed no significant differences in the postprandial blood glucose responses between the control and guar breads, but significant decreases in the postprandial plasma insulin. The effects did not appear to be related to differences in the molecular weights of guar gum, but the sensory qualities of the breads were improved when low molecular weight guar gum was used. Microstructural characteristics of guar enriched bread before and after ingestion by pigs were examined by bright field, epifluorescence and scanning electron microscopy and used to interpret the results obtained from an *in vitro* digestibility experiment (Brennan et al., 1996). The lower rates of starch hydrolysis in breads containing guar gum were attributed to the capacity of the this polymer to act as a physical barrier to starch digestion in addition to increasing the viscosity of digesta (Brennan et al., 1996).

Partially depolymerised guar gum when consumed within bread was shown to lower blood cholesterol levels with a similar magnitude to that of high-molecular-weight guar gum (Blake et al., 1997), and to significantly reduce the postprandial rise in blood glucose and plasma insulin in people with type 2 diabetes (Gatenby et al., 1996).

Pick et al. (1998) used barley β-glucan (waxy hulless barley) in bread making and evaluated its effect on the glycaemic response in subjects with type 2 diabetes. Their results indicate that barley bread products (5g/d β-glucan) were well accepted and when part of the diet led to improved glycaemic response. Similar results were obtained by Urooj et al. (1998) who fed diabetic (NIDDM) subjects with bread containing barley (both pearled and whole barley at 5 to 25% levels). The postprandial blood glucose was significantly lower, while the satiety scores were higher in comparison to standard white bread (Urooj et al., 1998).

Although physiological benefits may be achieved by incorporating certain DF into breads, it has also been reported that the increase in DF content could cause several changes in dough and bread characteristics. Dough yield tends to increase and the dough becomes shorter and moister, thus affecting kneading, handling, and proofing, while bread volume may decrease, as well as crumb elasticity (Laurikainen et al., 1998). These effects of DF addition on dough and bread structure are thought to be due to the 'damage' of the gluten network, which leads to reduced gas retention within the structure (Pomeranz et al., 1977). For instance, the use of bran or whole wheat affects the quality of the end product; they reduced loaf volume, increased crumb density (Ozboy and Koksel, 1997; Gan et al., 1992), and reduced crumb softness. Crust and crumb colours were obviously darker with wheat bran than without. The reduction in loaf volume was attributed to a decrease in gas retention by dough containing wheat bran, due to the disruption caused to the starch-gluten matrix (Gan et al., 1992). Fine particles of wheat bran were also reported to result in decreased dough mixing tolerance and a stronger dough when compared to dough containing coarse bran; overall, by increasing the wheat bran addition level, the dough strength is decreased (Zhang and Moore, 1997) with possible consequences for bread quality.

Fructooligosaccharides (inulin) extracted from Jerusalem artichokes were used for bread making, and their performance was compared against that of commercially available products (Raftiline and Raftilose). Organoleptic evaluation showed a high quality of wheat/rye bread enriched with inulin from Jerusalem artichoke in comparison to the control bread or breads made with Raftiline/Raftilose (Praznik et al., 2002). In another study, inulin was used as a fat replacer in breadmaking; the results suggest that breads containing inulin gel at 2.5% are comparable in terms of dough rheological properties, loaf volume and crumb texture with the breads containing fat (O'Brien et al., 2003).

Rice bran fibre was also used in bread formulations (at 5 and 10% addition) and it was found to significantly reduce loaf volume and increase the firmness of the breads. However, sensory evaluations revealed that breads containing rice bran fibre were comparable to high-fibre bread commercially available (Abdul-Hamid and Luan, 2000). Sensory evaluation of bread containing various levels of barley flour fractions enriched with β-glucan led to positive results; the bread was found acceptable, although the loaf volume was lower than the control bread, and also the colour was darker (Knuckles et al., 1997). Rosell et al. (2001) used k-carrageenan, xanthan gum and hydroxy-propylmethylcellulose (at 0.5% addition) in bread formulation and found out that the dough stability during fermentation was improved, and in terms of bread characteristics, the specific volume was increased, while crumb firmness was reduced (except for xanthan gum addition).

Bread is a very popular food product and the market offers high fibre varieties such as wholemeal and wholegrain bread. However, the contribution of bread towards the daily-recommended intake of DF is still minor, since consumers prefer white bread. An opportunity exists therefore to use DFs other than bran from wheat, barley or rye for producing white bread of good quality and which also represent a main source of DF. By adding only 5-6% (on flour basis) DF isolate, it could be possible to obtain a reasonably good quality bread which could be labelled as rich in DF and physiologically comparable with whole meal or whole grain breads (Endress and Fisher, 2001). Research studies have investigated mainly the performance of guar gum and barley β-glucan on bread sensory attributes and on the glycaemic and insulinaemic response in humans. Nevertheless, there are many other types of DF, which could be used, but information regarding their performance from both functional and technological point of view is lacking.

1.2.4.4.1.2 Dietary fibre in dairy products.

The idea of using DF in dairy products might seem unusual, but considering the physico-chemical characteristics of DF (such as water binding capacity, solubility in relation with developing viscosity *etc*) *vs.* some of the major technological challenges related to milk products (e.g. prevention of syneresis, producing low fat products with acceptable sensory characteristics *etc*) a potential in developing such products can be identified.

In recent years, dietary recommendations and consumer demand for low fat foods have encouraged research on such products (i.e. low fat yoghurt, low fat cheese, low fat ice cream et.). However, the removal of fat from dairy based systems comes with a series of problems associated with inferior organoleptic and physical properties of such products.

In the case of yoghurt made from low fat milk or skimmed milk, an increase in syneresis is usually observed, the resulting product being unappealing and therefore perceived as having poor quality. Gelatine or modified starches have been used in yoghurt formulation as stabilisers, but they can be replaced by DF which exhibit good solubility and a high water binding capacity.

Low fat cheeses are also known for their depreciated quality; they are likely to be hard, rubbery, and have poor flavour development. For certain cheeses such as Mozzarella, low fat content is related also with poor functional properties (meltability and performance when cooked in pizza) (Merrill et al., 1994; Mistry and Anderson, 1993). These properties could be improved/ameliorated if the removed fat is largely replaced by water, and thus the ratio moisture in fat free substance is similar to that of a full fat cheese. There are several options for achieving this (Merrill et al., 1994), and amongst them adding ingredients (stabilisers, fat replacers) that can bind moisture or impart fat like characteristics when

included into the food matrix represents an option (Mitchell, 1993). Such ingredients may be represented by DFs, but unfortunately there are only a few reports investigating their potential to be used as fat replacers in low fat cheeses, and generally information on the effect of DF on the quality of dairy products is scarce.

The possibility of development of a sweetened youghurt enriched with several insoluble fibres was studied by Fernández-Garcia and McGregor (1997). The majority of fibre sources used (soy fibre, sugar beet fibre, rice fibre, corn fibre and oat fibre) led to an acceleration in the acidification rate, a tendency for increased apparent viscosity, but more importantly the resulting products had lower overall flavour and texture scores. The exception was made by the use of oat fibre, which permitted the development of a good quality product, with flavour and texture characteristics similar to the control.

Yoghurt rheological properties and its syneresis have been evaluated by Keogh and O'Kennedy (1998) in relation to the addition of various components into the formulation. Amongst these, a 50:50 blend of locust bean gum and xanthan gum was shown to result in a product with increased consistency and reduced syneresis.

A study conducted by McMahon et al. (1996) investigated the effects of various fat replacers on the functional properties and microstructure of low fat Mozzarella cheese. Stellar® (a blend of modified corn starch and xanthan gum) and Novagel® (a blend of mycrocrystalline cellulose and guar gum) were used in low fat formulations and their effects compared with a low fat control and those of two protein based fat replacers (Simplesse® and Dairy-Lo®). They found that the blend containing guar gum modified cheese structure increasing its 'openness' in comparison to that of the control cheese, while all the other ingredients had no apparent effect on cheese microstructure but they affected the melting characteristics. Use of soluble DF such as gellan gum or carrageenan in cheese

production may present another advantage in their ability to retain whey protein into cheese structure, leading to increased yield and improved nutritional value (Konombirira and Kailasapathy, 1995; Kailasapathy, 1998) without affecting the texture.

In low fat ice cream inulin increased the apparent viscosity of the ice cream mix, and improved its melting characteristics (Schaller-Povolny and Smith, 2001; El-Nagar et al., 2002), while xanthan gum was effective in retarding ice recrystallisation (Regand and Goff, 2003). Yanes et al. (2002) investigated the use of kappa-carrageenan in the formulation of low calorie flavoured milk beverages; the evaluations done were related to product viscosity and sensory acceptability. Other soluble DFs such as inulin (Raftiline - with 10 - 60 fructose units) and oligofructose (Fibrex and Ultracel) were also used in a study to optimise the texture of low fat content spreads (Chronakis, 1997).

Generally, very few published studies have described specific casein/casein fractions - carrageenan mixtures or micellar casein - guar gum mixtures in terms of flow behaviour, viscoelastic properties, phase diagrams, microstructure etc.; this was also noticed by Bourriot et al. (1999). There is a lack of available information on the behaviour of other non starch polysaccharides (e.g. β-glucan, partially hydrolysed guar gum, inulin etc.) within milk systems, in relation to possible applications for designing functional foods.

1.2.5 Dietary fibre - examples

1.2.5.1 Cellulose

Cellulose is the most abundant organic chemical on earth, and one of the major components of the fraction of plant food, cereals in particular, that used to be commonly

known as 'roughage' (BeMiller and Whisler, 1996). Cellulose is a high molecular weight, linear, insoluble homopolymer formed by repeating units of β-D-glucopyranose joined by (1-4) glycosidic linkages. The molecular weight of cellulose ranges between 500,000 and 1 million Daltons. The linkage leads to a flat-ribbon arrangement maintained by intramolecular hydrogen bonding, as shown in Figure 1.2a, with the formation of microfibrils - chains packed together in a highly ordered manner maintained by intermolecular hydrogen bonding. The stability of this ordered structure gives cellulose both its insolubility in virtually all reagents and also great strength of the microfibrils (Coultate, 2002). Through substitution, cellulose can be converted in water dispersible forms, which can be used as bulking agents in foods.

Cellulose and its modified forms are classified as DFs since they do not contribute significantly to nourishment or calories as they pass undigested through the human digestive system, but they do have functional properties common to the DF group. The most acknowledged is the bulking properties of cellulose; it increases the bulk of stool and softens its consistency, therefore it is widely used in the management of constipation (Lennardjones, 1993). It is also thought to be inversely related to the risk of developing colorectal cancer (Levi et al., 2001).

Cellulose is rather inert; it will only absorb modest amounts of water, but chemical reactions only occur if the material has been swollen in acids or solvents, which disrupt the hydrogen bonding. Cellulose fibres can absorb small molecules on their surfaces because the external hydroxyl groups have weak acidic properties, and can also hydrogen bond.

Purified cellulose powder with various origins is available as a food ingredient, and it usually has negligible flavour, colour and microbial contamination. It is most often used in

baked goods not only for the increase in their DF content, but also to prolong their freshness (Gomez et al., 2003).

1.2.5.2 Guar gum and locust bean gum

Guar gum and locust bean gums are important thickening polysaccharides used in the food industry, and their use has been mentioned earlier in this chapter. Guar gum produces the highest viscosity of any natural, commercial gum (BeMiller and Whisler, 1996). Both gums are the ground endosperm of seeds (*Cyamopsis tetragonoloba (L.) Taub* for guar and *Ceratonia siliqua (L.)* for locust bean) (Bayerlein, 1992). The main component of both endosperms is a galactomannan - a polysaccharide consisting mainly of the monosaccharides mannose and galactose. Galactomannans consist of a main chain of β-D-mannopyranosyl units joined by 1-4 bonds with single unit α-D-galactopyranosyl branches attached at O-6.

The specific polysaccharide component of guar gum is guaran, in which about one half of the D-mannopyranosyl main chain units containing a D-galactopyranosyl side chain. The galactomannan of locust bean gum (LBG) has fewer branch units in comparison to guaran and a more irregular structure (BeMiller and Whisler, 1996). Both components have long, rigid chains (with conformations similar to that presented in Figure 1.2, that provide high viscosity solutions. However, their different chemical structures result in some different physical properties as well. Guar gum has its galactosyl units placed evenly along the chain, therefore there are few locations on the chains suitable for formation of junction zones. In comparison, LBG has long chains of D-mannosyl units non-derivatized which can easily form junction zones (BeMiller and Whisler, 1996).

Guar gum was isolated and used in increasing amounts from the 1950s, to meet the

demands of the modern food industry, and, alongside psyllium, it represents the source of water soluble DF most extensively evaluated in clinical studies for its effects on lowering blood cholesterol and glucose concentrations (Lo et al., 1991). Research studies used guar gum as a model dietary NSP, either isolated or in mixture with other food components (e.g. other polysaccharides, proteins) to model its flow behaviour, and thus to predict its behaviour in food systems (Ellis et al., 1991b; Rayment et al., 1995; Rayment et al., 2000; Wang et al., 2003). The relationships between guar gum molecular weight, particle size distribution and hydration properties were also investigated in relation to the beneficial effects (on postprandial blood glucose and insulin levels) on both humans and animals (Ellis et al., 1991a; Ellis and Morris, 1991; Ellis et al., 1995).

Although guar gum is of particular interest to the food industry for its water-binding properties, viscosity increasing, and gel forming capacity, the high viscosity obtained for low amounts of guar gum (1%) was found to limit its use in various food applications. An alternative is using partially hydrolysed guar gum (PHGG) which provides the same health benefits as guar gum (Gatenby et al., 1996; Blake et al., 1997), but it produces low viscosity solutions at 5% concentration which makes it a excellent source of DF in various applications (Juneja et al., 2001). Moreover, studies on rats suggest that highly fermentable, low viscosity fibre, namely PHGG improved Ca absorption (Hara et al., 1999b).

Locust bean galactomannans (LBG) appear to have been used prior to other galactomannans; it was mentioned that it had served to thicken the mucilage, which was used to glue textile windings around mummies in ancient Egypt (Bayerlein, 1992). LBG is now widely known for its strong viscosity increasing effect and it is used typically in frozen dessert products, either alone or in combination with other polysaccharides (CMC,

carrageenan, xanthan and guar gum) at levels of addition of 0.05-0.25% (BeMiller and Whisler, 1996). The use of LBG in bakery products has also been reported. Wang *et al.* (2002a) investigated the effect of LBG on viscoelastic properties of dough and quality attributes of bread. Their results indicated that the rheological properties of the dough and overall quality of the bread were not negatively affected by the addition of 3% LBG (Wang et al., 2002a), thus suggesting a potential to use LBG in bakery products. However, LBG it is also known for its health benefits in relation to cholesterol attenuation and antioxidative properties. It was suggested that when part of the daily diet (15 g *per* day), LBG could lower total and LDL cholesterol (Haber, 2002).

1.2.5.3 Xanthan gum

Xanthan gum is produced by the bacterium *Xanthomonas campestris* (Coultate, 2002), commonly found on leaves of plants of the cabbage family. Xanthan gum has a backbone identical to that of cellulose: β-D-glucopyranosyl units linked (1-4), and the molecule contains between 10000 and 250000 sugar units (Coultate, 2002). However, in xanthan gum, every fifth glucose unit carries a trisaccharide side chain, which includes one, and sometimes two, carboxyl groups. These charged groups are responsible for its high affinity for water.

Xanthan is widely used in the food industry due to its solubility in both hot and cold water, high viscosity at low concentrations (typical levels of usage are 0.1-0.3%), no change in viscosity within a temperature range from 0°C to 100°C, good stability and solubility over a wide range of pH (2-12), good freeze-thawing stability. Its constant viscosity over a wide range of temperatures dictated its largest utilisation in salad dressing, gravies, and chocolate syrups (BeMiller and Whisler, 1996). The potential use of xanthan gum in combination with LBG, was investigated in yoghurt (Keogh and O'Kennedy, 1998). Due to

its freeze-thaw stability it is also used in frozen doughs and it is thought to improve water binding in bakery products (Ward and Andon, 2002).

1.2.5.4 Inulin

Inulin belongs to the wider group of fructooligosaccharides (FOS) (Roberfroid, 1993) and it has a degree of polymerisation (DP) ranging from 2 to 60 or more. It consists of linear chains of fructosyl units linked by β (1-2) bonds and often ended by a glucosyl unit. It is usually found in chicory, onion, bananas, wheat etc. The estimated intake of inulin in European countries is 2-12 g/day (Jackson et al., 1999; Niness, 1999). Due to their structure, they can not be hydrolysed by human digestive enzymes, and escape the passage through the stomach and small intestine to be largely fermented by specific colonic microflora, thus presenting one of the major characteristics of DF. They have been relatively recently included amongst the components of DF (Anon., 2001a).

Inulin selectively stimulates the *in vivo* growth of bacteria such as Bifidobacterium, Lactobacillus, and Bacteroides at the expense of potential pathogenic microorganisms (Roberfroid, 2001; Delzenne, 2003); hence it is considered as a prebiotic (Van Loo et al., 1999; Coussement and Franck, 1998). In addition, when part of the diet inulin has been found to reduce serum triglicerides and possibly cholesterol levels (Causey et al., 2000; Jackson et al., 1999), and to possibly influence carbohydrate metabolism shown by the decrease in fasting blood glucose (Yamashita et al., 1984). The later effect has been also mentioned by Roberfroid (1993), but the hypothesis was not proven since there are also contradictory reports (Rumessen et al., 1990; Alles et al., 1999). It has also been reported that inulin promotes mineral absorption (Ca, Mg, Zn and Fe) in the large intestine (Roberfroid, 1998; Younes et al., 2001), and it also has a potential immune stimulating effect (Meyer et al., 2001b).

In food products inulin is used due to its physiological functionality as prebiotic DF, as well as its technological characteristics (sugar and fat replacers, texturisers and gelling agents) (Meyer and Tungland, 2001a). Inulin is white in colour, odourless and has neutral to slightly sweet taste. It is heat stable (even at sterilisation temperatures) and at pH above 3.7 it is water soluble (which increases with temperature) and has gelling (at high concentrations) and water binding capacity (Sensus, 2001). Inulin is thought to have the ability to form microcrystals when sheared in water or milk; these crystals are not detectable in the mouth, but may provide fat-like mouthfeel (Sensus, 2001).

The texturising properties of inulin, together with its neutral taste are exploited especially in dairy products. In yoghurt, beside 'balancing the gut microflora' inulin was suggested to improve texture (Meyer and Tungland, 2001a), while in low-fat spread inulin is considered to help reduce the fat content below 5%, with no significant alteration of the textural attributes of the product. Studies were also carried out on the use of inulin in bread formulations in relation to dough and bread quality (Wang et al., 2002a), and also to organoleptic properties (Praznik et al., 2002).

1.2.5.5 Beta glucan

Generally cereal β-glucans are linear polysaccharides which consist of long chains of 4-O-linked β-D-glucopyranosyl units (70%) interrupted by 3-O-linked β-D-glucopyranosyl units (30%). The distribution of the two glucosidic linkages is not random; most of the β-(1-4) linkages occur in group of two or three (dominating the chain), while β-(1-3) linkages occur singly (Wood, 2001). These β-(1-3) linkages disrupt the ordered structure associated with cellulose and impart solubility in water (Coultate, 2002). Moreover, a decreased structural irregularity was related to a decrease tendency to gel (Wood, 2001).

The main sources of β-glucans are oats, barley, wheat; the proportion, structure and molecular weight of these polysaccharides vary between the cereal crops.

β-glucans started to attract considerable attention since it was shown that oat β-glucans are beneficial in relation to heart disease through its effect on reducing the level of serum cholesterol. As a result, the US Food and Drug Administration (FDA) approved the health claims for oat β-glucan (0.75g *per* serving) on the assumption that 4 servings *per* day would reduce the risk of cardiovascular disease (Anon., 1997b; Anon., 2002b). Thus, oat β-glucan became a commercial food ingredient. Beneficial health effects are thought to be associated with barley β-glucans as well, and several research studies started to explore this area. Soluble β-glucans have been reported to lower postprandial serum glucose levels in humans and animals (Bhatty, 1993; Wood et al., 1994; Yokoyama et al., 1997; Pick et al., 1998), and the effects have been found to be significantly related to the logarithm of β-glucans concentration and the logarithm of their molecular weight (Wood et al., 2000). Other studies however, have suggested no effect on blood glucose levels associated with the consumption of β-glucans (McIntosh et al., 1991).

Although numerous reports have been published on nutritional benefits of β-glucan including hypocholesterolemic effects and influence on blood glucose levels, the consumption of β-glucan containing products did not increase accordingly; this is especially true for barley products. Food uses of barley are quite limited and restricted mainly to animal feeding and the brewing industry. Utilisation of barley β-glucan as a source of DF as well as a food hydrocolloid has not been fully explored. The results presented in the literature, are sometimes contradictory but indicate that the desired physiological effect of β-glucan depends on many factors, some of which are only poorly

understood. Viscosity appears to be important and accordingly, factors controlling viscosity (i.e. the amount of β-glucan, solubility, molecular weight and structure) are important (Wood and Beer, 1999) (Wood, 2001). The effects of pH, temperature and presence of salt or sucrose on viscosity of oat β-glucan (Dawkins and Nnanna, 1995) as well as the interaction of oat β-glucan in binary mixtures with LBG, xanthan and guar gum (Nnanna and Dawkins, 1996) were reported. Due to its viscous behaviour, the potential use of β-glucan as a thickener in ice cream, sauces and salad dressings has been suggested (Carr et al., 1990).

In recent years progress has been made in the characterisation of cereal β-glucan, but there are still many gaps in knowledge. Further studies are needed to study their rheological behaviour in different food systems, to understand how β-glucan interacts with the other food components and how these interactions influence its potential physiological effects, as well as overall product quality.

1.3 Rationale of the study

The importance of DF intake is increasing due to its beneficial effects. Although there are still controversial opinions, results from numerous research studies indicate that a diet high in DF provides some protection from 'Western' diseases (e.g. diabetes, CVD, obesity, cancer). Based on the existent evidence health authorities world-wide recommend a decrease in the consumption of animal fats and proteins and an increase in DF rich foods. Although it sounds simple, to accomplish these recommendations is not always straightforward in a complex food system; often there are reported problems of palatability

and consumer acceptability associated with DF enriched foods (related with texture, colour, taste etc).

The majority of the research studies related to DF products have focused on nutritional aspects whilst their physical attributes relevant in food technology have only been considered to a limited extent. DF characteristics such as swelling capacity, water-binding and water-retaining capacity and gel formation have not been explored deeply in relation to the interactions with the other food components. However, these complex interactions determine the final quality characteristics of DF enriched products, and may also play an important role for gastrointestinal functions by regulating the rate and the site of digestion and absorption. This is important for understanding the role of DF in disease prevention and health promotion. A better understanding of the structure and rheological properties of foods containing DF, will help to predict physiological and metabolic responses to consumption of fibre, and to develop new functional products. This issue is important since there is a need to develop food products that are not only health beneficial, but are likely to be selected by consumers. Bearing in mind that DF can not have health benefits unless it is eaten (becomes part of a diet), the development of high quality DF enriched staple foods is desirable. This would have a big impact on the TDF intake across a large range of populations.

In terms of nutritional benefits, the literature seems to be generous in providing information on certain DF (such as wheat bran, oat bran, guar gum, psyllium). However, the range of DF products available on the market has expanded significantly over the past years, even though the evidence in the literature related to their nutritional benefits is scarce.

This study investigates the inclusion of a range of DFs into cereal and dairy food systems with the aim to screen for potential DF ingredients that may lead not only to nutritionally valuable, but also to good quality, palatable food products.

Objectives:

A range of soluble (guar gum, inulin, β-glucan, locust bean gum, xanthan gum) and insoluble DF (pea fibre, cellulose) were used into cereal (bread and pasta) and dairy systems (yoghurt and fresh cheese). The food systems were selected in order to cover a range of technological processes from extrusion, baking, milk coagulation, and fermentation. The main objectives were to:

1. Assess the structural and biochemical effects of DF addition into functional foods - cereal and dairy food systems

2. Evaluate the effect DF enrichment has on textural, rheological attributes and overall quality and acceptability of cereal and dairy based products.

3. Establish the relationship between the type of DF, level of addition, quality of the product and its microstructural organisation.

4. For the cereal based product, to establish the role of DF inclusion on the *in vitro* rate of starch digestibility and consequently on the glycaemic index of the products. Where appropriate, results obtained *in vitro* will be compared against *in vivo* findings.

5. For the dairy products to assess the potential use of DF in low fat formulations.

Chapter 2. Dietary fibre and cereal products. I - The effect of dietary fibre on the nutritional, textural and structural attributes of pasta products

2.1 Introduction

The increased consumption of high-fat, high-calorie foods has been linked to an unprecedented growth in the risk for developing CVD, some forms of diabetes and cancers. In an attempt to tackle the situation, a recent joint FAO/WHO report recommended a decrease in intake of sugars, fats and alcohol, and an increase in consumption of fruits, vegetables and cereal products, with an aim to increase the TDF intake (to minimum intake 25g /day) (WHO, 2003). Cereal products are a diverse group ranging from whole grains to ready-to-eat or -cook convenience foods. Pasta and bread are the most common processed cereal products available, and although they are low in fat and represent a good source of complex carbohydrates they are usually not a good source of DF since consumers prefer white pasta and white bread made from high extraction flour. However there exists the opportunity to enrich these products with DFs for nutritional benefits (increased DF content, reduced calorie values).

Pasta is a traditional food product with origins dating back to the first century BC (Agnesi, 1996), and is favoured by consumers for its ease of transportation, handling, cooking and storage properties. Among cereal products, pasta appears to posses unique nutritional features in that the starch is slowly digested and absorbed in the small intestine (Bornet et al., 1987). Thus, pasta has become even more popular due to its nutritional properties, being regarded as a product 'with low GI' (Jenkins et al., 1988; Bjorck et al., 2000) or with 'lente' carbohydrates. Research has shown that pasta progressively liberates sugars that the body needs, and as a consequence leads to low postprandial blood glucose and insulin responses in humans (Jenkins et al., 1983; Bornet et al., 1989; Granfeldt and Bjorck, 1991), with potential benefits for both healthy and diabetic consumers.

The slower release of starch degradation products from pasta in comparison with other cereal products has been mainly attributed to the compact structure of pasta resulting from the extrusion process, and characterised by a very close protein network which entraps starch granules and delays α-amylase activity (Fardet et al., 1998; Fardet et al., 1999; Pagani et al., 1986). The interactions of starch with other components such as certain DFs have also been suggested to further reduce the rate of starch digestion and thus to lower the glycaemic response (Gatti et al., 1984; Yokoyama et al., 1997). Thus it appears that by combining the benefits of pasta (low GI) with the benefits of the DFs, novel functional food products associated with prevention and treatment of diseases such as diabetes and coronary heart diseases may be developed. This area of research has yet to be explored since there is a paucity of publications investigating the effects of DFs from various sources on pasta quality, cooking characteristics, structure, texture, and starch degradability.

The present study investigates the possibility of using various DFs (both soluble and insoluble) in pasta products, in relation to their cooking properties, textural attributes and starch digestibility, aiming to obtain functional foods of acceptable quality by the inclusion of different types of DF.

The experiment was carried out in two stages:

1. The first of these was used to target the appropriate levels of addition for DF of various origins (guar gum, pea fibre and inulin) in the pasta. The quality of these products was compared against a control product (with no DF added), and the rate of glucose released was estimated using an one step, non restricted *in vitro* digestion method. The results from this exercise were then used to decide on appropriate formulations for the main experiment.

2. The main experiment compared the pasta made with an extended range of DFs (at various levels of addition) against a control product. At this stage the digestibility of starch was assessed using a multi step *in vitro* digestion method based on restricted hydrolysis, and a predicted GI was calculated for each product. In addition the quality of the products obtained (texture attributes and cooking properties as well as product internal structure) was assessed.

2.2 Materials and methods

2.2.1 Stage 1

2.2.1.1 Pasta making

Durum wheat pasta was made using as raw materials: commercial durum wheat semolina (Allied Mills Ltd., UK), water and different types of DF: inulin (Frutafit HD, Calleva Ltd., UK), guar gum (E412) (Calleblend GUA, Calleva Ltd., UK) and pea fibre (Exafine 250, Cosucra, Belgium). Durum wheat semolina had the following specifications as provided by the supplier: protein content - 13.7%; moisture content 14%. DF characteristics as provided by the suppliers are presented in Table 2.1. DFs were incorporated into recipes at replacement levels for durum wheat flour at the following proportions (w/w): pea fibre: 7.5%, 10%, 12.5% and 15%; inulin: 7.5%, 10%, 12.5% and 15%; guar gum: 3%, 5%, 7% and 10%; an additional sample with no DF included was also prepared as a control. The mixture was extruded as spaghetti (1.5 mm diameter) using a Fresco M-P15 domestic pasta maker and following a standard formula (500g semolina flour and 160 g water - recommended in the machine handbook). For the formulations containing guar gum and pea fibre, the amount of water added needed to be increased since the extrusion was not

possible under the standard ratio flour: water; the formulations used are presented in Table 2.2. Samples were wrapped in cling film and stored in air-tight containers and frozen at -40°C until needed.

Table 2.1. Dietary fibres characteristics as provided by the suppliers

	Pea fibre (Exafine 250)	**Inulin (Frutafit HD)**	**Guar gum (Calleblend GUA)**
Chemical composition			
Dry matter content, %	min. 90.0	95.0	min. 90.0
Carbohydrates, % db	min. 89.0	99.0	99.0
of which:			
▪ Total DF, %db	min. 88.0 (of which: cellulose 50%, hemicellulose 20%, pectin 10%, lignin 5%)	95.0 (inulin)	99.0 (guar gum)
Protein, %	<4.0	0.0	0.0
Ash, %	2.0	<0.1	<0.1
Physico-chemical aspects			
Particle size	<250μm	10<80%<85μm	-
Dispersability	-	Good	-
pH	-	Neutral	5.0-7.0
Colour	Light beige	White	Light cream
Taste	Neutral	Neutral, slightly sweet	Neutral

2.2.1.2 Cooking procedure

Optimum cooking time (the time necessary to obtain complete gelatinisation of starch showed by the disappearance of the white central core of the spaghetti strand) was determined as 7 minutes following standard guidelines (Anon., 1989a). 50 grams of each pasta sample were than cooked for 7 minutes in 500 ml of boiling distilled water.

After cooking and draining, pasta samples were analysed for swelling index, dry matter, starch content, textural properties and microstructure. Aliquots of cooking water were used further for determination of cooking losses.

Table 2.2. Formulations used for pasta making

DF addition (%)		Durum wheat semolina (g)	DF (g)	Water (g)
Control	**0.0**	500.00	0.00	160.00
Pea fibre	**7.5**	462.50	37.50	200.00
	10.0	450.00	50.00	213.00
	12.5	437.50	62.50	227.00
	15.0	425.00	75.00	240.00
Inulin	**7.5**	462.50	37.50	160.00
	10.0	450.00	50.00	160.00
	12.5	437.50	62.50	160.00
	15.0	425.00	75.00	160.00
Guar gum	**3.0**	485.00	15.00	181.00
	5.0	475.00	25.00	195.00
	7.0	465.00	35.00	209.00
	10.0	450.00	50.00	230.00

2.2.1.3 Cooking and compositional characteristics of pasta

Swelling index of cooked pasta (SI, g water/g dry pasta) was evaluated by drying pasta samples to constant weight (Fardet et al., 1999) at 105^0C, and expressed as: (weight of cooked product, W_1 - weight after drying, W_2)/(weight after drying, W_2) (Mestres et al., 1988). Three measurements were conducted for each pasta type.

Dry matter of raw and cooked pasta was determined according to standard methods (AACC, 1996-926.07B) (AACC, 1995). Three measurements were conducted for each pasta type.

Cooking loss in the cooking water collected from each sample was determined by evaporating to the constant weight in an air oven at 105°C. The residue was weighed and

reported as percentage of the original pasta sample (Debbouz and Doetkott, 1996). Three measurements were conducted for each type of pasta.

Starch content (total starch) was determined in both raw and cooked pasta using the Megazyme kit (Megazyme International Ireland Ltd., Ireland) - according to AOAC method 996.11, AACC method 76.13 (AOAC, 1998; AACC, 1995). Prior to starch analysis pasta samples were freeze dried and milled using a laboratory scale hammer mill (0.5mm screen size). Starch measurements were performed in triplicate.

2.2.1.4 Texture characteristics of pasta

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

- *Elasticity* (or *'tensile strength'*) was determined by tension test, using the A/SPR- Spaghetti/Noodle Rig (settings: pre-test speed: 3mm/sec., test speed: 3mm/sec., post test speed: 5mm/sec., distance: 120mm at a rate for data acquisition of: 200pps). Maximum force recorded when the elastic limit is exceeded and the pasta strand snaps gives an indication of pasta elasticity; the test was performed on 15 replicates per sample and a typical curve is presented in Appendix 2.1a).

- *Adhesiveness and stickiness* were determined by the adhesive test using P35 - 35mm diameter cylinder probe (settings: pre-test speed: 1mm/sec., test speed: 0.5mm/sec., post test speed: 10mm/sec., distance: 100mm, time: 2sec., trigger type: auto 20g, and a rate for data acquisition of: 500pps). A typical curve recorded during this type of test is presented in Appendix 2.1b) and gives information about product adhesiveness (related to negative area between the x

axis and the graph) and stickiness (related to peak negative force); the test was again performed using 15 replicates per sample.

- *Firmness* of pasta samples was evaluated according to AACC method (1mm flat perspex knife blade (A/LKB-F) and the following settings: pre test speed: 0.5mm/s, test speed: 0.17 mm/s, post-test speed: 10.0 mm/s; distance: 4 mm). Each test was performed on five strands of pasta and 15 replicate tests were performed for each type of pasta. A typical curve is presented in the Appendix 2.1c) and it gives information about maximum cutting force (peak force) and total work to cut (area under the curve). The AACC method (16-50) defines pasta firmness as the work in grams-centimetre required to shear one piece of pasta (e.g. one strand of spaghetti) (AACC, 1983); however, both parameters were found to be related to pasta firmness (Walsh, 1971).

2.2.1.5 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was used to measure thermal parameters (T_o - onset temperature, T_p - peak temperature, T_c - endset temperature, ΔH - enthalpy of starch gelatinisation) of raw and cooked pasta to evaluate the changes in starch at the molecular level during cooking, and in the same time to asses the influence which DF might have on the properties of the starch fraction. A typical DSC curve showing starch gelatinisation is presented in Appendix 2.2. Indium was used to calibrate the instrument (Metler Toledo - DSC 12E). Pastas were freeze-dried and then milled (0.5mm screen size). Samples and distilled water in proportion of 1:4, with a total weight of 15±0.3 mg were sealed hermetically in aluminum pans and left to equilibrate for 1 hour prior to the tests (Zhang and Hamaker, 1998). An empty aluminium pan was used as a reference. The temperature range of the scan was 4^0C - 110^0C with a 10^0C/min heating rate.

2.2.1.6 *In vitro* digestibility of starch.

Samples of cooked pasta were cut in pieces of approximate $1mm^3$ and 30g of each sample mixed with 100 ml enzyme solution [51 g of alpha amylase from porcine pancreas - EC. 3.2.1.1, type VI-B (Sigma Chemical, UK) were dissolved in 1 litre of phosphate buffered saline, pH 6.9, containing 0.12 M NaCl, 2.7mM KCl and 0.01M phosphate buffered salts], according to Brennan et al. (1996). The mixtures prepared were incubated at 37°C in a shaking water bath and samples of pasta suspensions were taken after 30, 60 and 90 minutes. Sample aliquots (25 ml) taken every 30 min were cooled immediately in ice, and centrifuged at 15000rpm for 10 min. The supernatants were withdrawn and stored in the freezer (at -60^0C) prior to glucose content analysis that was used as an indicator to monitor the extent of starch hydrolysis by α-amylase.

Glucose content was determined in triplicates using the Megazyme Glucose Assay Kit (Megazyme International Ireland Ltd., Ireland) using the glucose oxidase-peroxidase (GOPOD) reagent. The results were determined as mg glucose/100 ml supernatant, and for comparison purposes they are presented as mg glucose/g dry matter and mg glucose/g starch available in the sample.

2.2.1.7 Scanning electron microscopy (SEM[1])

Microscopy techniques have been previously used (Pagani et al., 1986; Fardet et al., 1998) in order to gain information about size, shape, arrangement of the particles, which can be further correlated with other pasta characteristics like texture, cooking behaviour and digestibility. In the present study, the microstructures of raw and cooked pasta were investigated by Scanning Electron Microscopy (SEM JEOL - JSM6100, Oxford, UK, resolution 4nm) of gold coated (using an Emitech K550 sputter coater) freeze dried pasta samples.

2.2.1.8 Statistical analysis

The results from all the tests were calculated as means ± S.E.M. Analysis of variance (One way ANOVA) followed by Tukey's test of Minitab 12 software were used for statistical analysis. Data expressed as percentages, were arcsin transformed prior to statistical analysis.

2.2.2 Stage 2

2.2.2.1 Pasta making

Durum wheat pasta was made using as raw materials: the same commercial durum semolina (Allied Mills Ltd., UK), water and an extended range of DFs: inulin (Frutafit HD, Calleva Ltd., UK), guar gum (E412) (Calleblend GUA, Calleva Ltd., UK), pea fibre (Exafine, Cosucra, Belgium), locust bean gum (E410) (Calleblend LBG, Calleva Ltd., UK), xanthan gum (Kelco®F, CP Kelco, UK), bamboo fibre (Qualicel 41B, CFF, Germany), β-glucan enriched flour (HiSol, HiSol Ltd., UK). DF characteristics as provided by the suppliers are presented in Table 2.3.

The fibres were incorporated into recipes at replacement levels for durum wheat flour at the following proportions (w/w): 2.5%, 5%, 7.5% and 10%. For the formulations containing HiSol (with only 12% β-glucan) semolina flour was replaced with the appropriate amount of HiSol, so the β-glucan in the final formulation would be 2.5%, 5%, 7.5% and 10% (w/w). Control pasta with no DF included was also prepared. The mixture was extruded as spaghetti (1.5 mm diameter) using a pilot size pasta maker (La Monferrina, Model P6, Italy), and following a standard formula (1.5 kg semolina flour and 510 g water) and the same storage procedure as mentioned at 2.2.1.1..

2.2.2.2 Cooking procedure

Optimum cooking time was determined using the same procedure as mentioned at 2.2.1.2, and it was found to be 7 min. 100 grams of each pasta sample were then cooked for 7 min in 1000 ml of boiling distilled water. After draining, pasta samples were analysed for the swelling index, dry matter, textural properties (firmness, adhesiveness, stickiness and elasticity), and microstructure following the same protocols as described for stage 2.1. Aliquots of cooking water were used further for determination of cooking losses as described previously.

2.2.2.3 Differential Scanning Calorimetry (DSC)

Sample preparation and the scanning rates were the same as described at 2.2.1.5.

2.2.2.4 Chemical analysis of pasta

Cooked pasta samples were prepared as for DSC tests (section 2.2.1): freeze dried, milled to particle size of less than 0.5mm and used for determination of starch, protein, moisture and total DF content.

Starch content of cooked pasta was determined as previously described in section 2.2.1.3.

Total DF content of cooked pasta (except for the samples containing inulin) was evaluated using the 'total DF assay procedure' (Megazyme International Ireland Ltd., Ireland), which is based on the AOAC and AACC official methods (AOAC method 991.43, AACC method 32-07, AACC method 32-21, AOAC method 985.29). The determinations were performed in duplicates.

Table 2.3. Dietary fibres' characteristics as provided by the suppliers

	Pea fibre (Exafine 250)	Inulin (Frutafit HD)	Guar gum (Calleblend GUA)	Locust bean gum (Calleblend LBG)	Xanthan gum (Kelco®F)	Bamboo fibre (Qualicel 41B)	Beta glucan (HiSol)
Chemical composition							
Dry matter content, %	min. 90.0	95.0	min. 90.0	min. 90.0	min. 90.0	min. 90.0	min. 88.0
Carbohydrates, % db	min. 89.0	99.0	99.0	99.0	99.0	95.0	93.75
of which:							
▪ Total DF, %db	min. 88.0 (of which: cellulose 50%, hemicellulose 20%, pectin 10%, lignin 5%)	95.0 (inulin)	99.0 (guar gum)	99.0 (locust bean gum)	99.0 (xanthan gum)	min. 95.0 (cellulose)	17.04 (of which: beta glucan 13.64%)
Protein, %	<4.0	0.0	0.0	0.0	0.0		6
Ash, %	2.0	<0.1	<0.1	<0.1	<0.1	<0.3	-
Physico-chemical aspects							
Particle size	<250µm	10<80%<85µm	-	-	-	80%<32µm 98%<75µm	100%<180µm
Dispersability	-	Good	-	-	-	-	-
pH	-	Neutral	5.0-7.0	5.0-7.0	6.0-8.0	4.0-6.0	-
Colour	Light beige	White	Light cream	Light cream	Light cream	White	White, slightly speckled
Taste	Neutral	Neutral, slightly sweet	Neutral	Neutral	Neutral	Neutral	Neutral

Protein content in cooked pasta was determined by Dumas combustion method (AOAC 968.06) on a LECO® FP-2000 nitrogen/protein combustion analyzer (LECO Corporation, Michigan, USA) (AOAC, 1995) according to the manufacture's instructions. The LECO® FP-2000 analyzer determines the nitrogen content in a variety of biological materials. The instrument was calibrated using ethylenediaminetetraacetic acid (EDTA 9.56% nitrogen). A factor 6.25 was used to convert nitrogen values to proteins. Triplicate determinations were conducted for each pasta sample.

Moisture content of cooked and dried pasta samples was determined using the same methods described at 2.2.1.3. The determinations were performed in triplicates for each pasta type and the results were used to calculate the percentage of starch, protein and TDF on dry matter for each pasta sample, and to calculate the amount of these components in freshly cooked pasta.

2.2.2.5 Texture characteristics of pasta

Textural characteristics of cooked pasta (elasticity, firmness, adhesivness and stickiness) were evaluated using methods described in paragraph 2.2.1.4.

2.2.2.6 *In vitro* digestibility of pasta as affected by DF

In vitro digestibility of starch was evaluated using a modified multi-enzymic method of Brighenti et al. (1995) involving simulated mastication, a proteolytic stage followed by incubation with pancreatic α-amylase restricted by dialysis tubings. This method allowed the calculation of a hydrolysis index (HI) and a predictive glycaemic index (GI) for each pasta sample. Duplicate samples of product (4g of cooked pasta) were mixed with 20ml sodium potassium phosphate buffer (pH 6.9). Thereafter, the pH was adjusted to 1.5 (using 8M HCl), and 5ml pepsin solution (pepsin 115U/ml, EC 3.4.23.1, 452 U/mg solid, Merck,

UK) were added to the samples, followed by incubation at 37^{0}C for 30 minutes. Before addition of porcine pancreatic α-amylase solution (amylase = 110 units/ml buffer, EC 3.2.1.1, 15.8 U/mg solid, Sigma Chemicals, UK) the pH was readjusted to 6.9 with 10% NaOH and the sample brought to 49 ml with sodium potassium phosphate buffer (pH 6.9). Then 1ml of α-amylase solution was added and the mixture transferred into dialysis tubes. The dialysis tubes were made from 250mm strips of dialysis tubing (width 20mm, molecular weight cut-off 12,000-14,000 Daltons; Medicell International Ltd., London, UK) knotted at both ends. Each tube was placed into a beaker containing 450 ml potassium phosphate buffer and, to stimulate peristalsis, glass balls were inserted into the tubes and used to mix the contents during the incubation (5h at 37^{0}C) by inverting the tubes every 15 min. A sample blank (with deactivated enzyme) and a maltose blank (with deactivated enzyme plus a known amount of maltose - 1ml of 20% maltose solution) were run with each sample digestion, which allowed for the measurement of sugar diffusivity through the dialysis tube in the presence of food (diffusion). Every 30 min for 5 hours, aliquots of 1 ml from dialysate were withdrawn in triplicates for analysis of reducing sugar content using the 3,5 dinitrosalicylic acid (DNS) method. The withdrawn dialysate was replaced each time with sodium potassium phosphate buffer. A standard curve using maltose was prepared.

White reference bread

A white wheat bread (WWB) was included as a reference when evaluating the rate of starch hydrolysis *in vitro* and for calculation of HI and GI values. The recipe was that of Liljeberg and Bjorck (1994): 300 g white wheat breadmaking flour (Allied Mills Ltd., UK), 200g water, 3g dry yeast, 3g salt, 3g of a mixture of soybean and palm oil. The breadmaking method as well as flour characteristics is more fully described in Chapter 3,

section 3.2.1. After cooling the bread was cut into slices, placed in sealed polyethylene bags and stored at -40°C until used. Before the tests, the breads were thawed at ambient temperature for 3 h.

The whole exercise was repeated twice, and results obtained were used to calculate the following parameters:

- **the amount of reducing sugars released (RSR),** consisting of the dialysed fragments of digested starch plus native reducing sugars was calculated as maltose equivalents, as percentage of the total available carbohydrates present in the sample:

$$RSR = \frac{A_{Sample} \times 500 \times 0.95}{A_{Maltose} \times Carbohydr} \times 100, \qquad \text{(Equation 2.1)}$$

where: A_{sample} represents the value of the absorbance at 546nm of sample, $A_{Maltose}$ represents the absorbance value of a solution containing 1 mg of pure maltose/ml phosphate buffer, *Carbohydr* represents the amount (in milligrams) of starch plus sugars contained in the sample, 500 ml is the total volume of solution and 0.95 is the conversion factor from maltose to starch.

- **the percentage of maltose able to diffuse out of the bag in the presence of sample (DIFF):**

$$DIFF = \frac{(A_{Blank+Maltose} - A_{Blank}) \times 500}{A_{Maltose} \times 200} \times 100, \qquad \text{(Equation 2.2)}$$

where $A_{Blank+Maltose}$ represents the absorbance value of Blank + Maltose, A_{Blank} represents the value of absorbance of Blank, and 200 represents the weight of maltose added to the Blank + Maltose sample (in milligrams); DIFF was also calculated for a control solution consisting of 1 g of pure maltose dissolved in 50ml of phosphate buffer in absence of sample.

- The values of diffusion of pure maltose and of Blank + Maltose were used to calculate for each sample a **sugar diffusion index (SDI)** - diffusion results reported to those of a pure maltose solution in absence of food: $SDI = \frac{DIFF_{Maltose}}{DIFF_{Sample+Maltose}}$; (Equation 2.3)

- **Percentage of digested starch (DIG):**

$$DIG = \frac{(A_{Sample} - A_{Blank}) \times 500 \times 0.95}{A_{Maltose} \times St} \times SDI \times 100; \quad \text{(Equation 2.4)}$$

- **Digestion index (DIGI)** - digestion results minus blank expressed as a percentage of those obtained for the reference white bread;

- **Reducing sugars released index (RSRI)** - calculated values of RSR expressed as a percentage of those obtained for the reference white bread;

- **'Hydrolysis index' (HI)** calculated as the area under the hydrolysis curve (0-180min) with the product as a percentage of the corresponding area with a reference white bread (Granfeldt et al., 1994b; Akerberg et al., 1998).

- **Predicted glycemic index** using a predictive equations:

  - $GI_{predicted}$ = 105.52xfibre/carbohydrate - 76.46xprotein/ carbohydrate + 1.23x $RSRI_{at\ 150\ min}$ +69.41x $SDI_{at\ 270\ min}$ - 83.87, (to be referred to as GI*) (Equation 2.5)

    taking into account that the index of physical form for pasta is considered as 1 (Giacco et al., 2001; Brighenti et al., 1995); or

  - $GI_{predicted}$=0.862HI+8.189, (to be referred to as GI**) (Equation 2.6)

    where HI=hydrolysis index calculated as previously mentioned Granfeldt (1994) (Granfeldt, 1994a) cited by (Akerberg et al., 1998).

2.2.2.7 Scanning electron microscopy of pasta

SEM[1] was used as described earlier at 2.2.1.7, but using a Scanning Electron Microscope with a resolution of 3.5nm (JEOL 5600LV, Oxford, UK) to obtain information about pasta microstructure (both before and after *in vitro* digestion) as affected by the type of DF added to the formulation.

2.2.2.8 Statistical analysis

Results from all the tests were calculated as means ± SD. Analysis of variance (GLM) followed by Tukey's test of Minitab 13.1 software (Minitab Inc., USA) were used for statistical analysis to investigate the effect of two factors: type of DF and the level of DF used. The results for the control were not included in the GLM model for the statistical analysis.

2.3 Results and discussions

2.3.1 Stage 1

2.3.1.1 Pasta cooking and compositional characteristics

Pasta quality is influenced by a range of characteristics: physical, chemical, textural and nutritional. For consumers, cooking quality is the most important quality attribute (Feillet and Dexter, 1996) including optimal cooking time, swelling or water uptake during cooking, texture of the cooked product, extent of disintegration of the cooked product, stickiness, aroma and taste.

Results related to pasta composition and cooking attributes (swelling index and cooking loss) of pasta are presented in Table 2.4. Generally, the swelling indices of DF enriched

pasta were not significant different from the control ($p>0.05$, Table 2.4), except for the sample containing guar 10%. Values ranged from 1.67% (for pasta containing inulin 15%) to 2.28% (for pasta containing guar 10%), with the control sample showing a swelling index of 1.85 (Table 2.4). However, the mean values indicate that for formulations containing pea fibre, and especially guar gum, there was a trend in increasing swelling index values with increasing levels of DFs. The higher swelling indices obtained for the pasta containing guar gum, may be explained by the higher capacity of guar gum to absorb and retain water within a very well developed starch-protein-polysaccharide network, in comparison to the pea and inulin fibres.

Cooking loss is a commonly used predictor of overall spaghetti cooking performance by both consumers and industry. Previous research has demonstrated a clear link between the protein content of durum wheat and the cooking quality of pasta (Fardet et al., 1998; D'Edigio et al., 1990). In a review, Feillet (1988) explained that in pasta, semolina proteins are linked together by disulphide, hydrogen and hydrophobic bonds to form a matrix, which gives contributes to cooked pasta viscoelastic properties. The continuity and strength of the protein matrix is dependent on the nature of inter- and intra-molecular bonds. During the cooking process this matrix may disintegrates gradually releasing exudates during starch granule gelatinisation which in turn contributes to an increase in adhesivness and stickiness of the surface of cooked pasta.

Table 2.4. Composition and water uptake of pasta containing different types of DF

Dietary fibre addition	(%)	DM of raw pasta, (%)	DM of cooked pasta (%)	Swelling index, (g water/g dry pasta)	Cooking loss, (g/100g raw pasta)	Starch in pasta (% dmb)	
						raw	cooked
Control	**0.0**	66.1±1.22^{b,c,d}	35.2±0.53^{a,b}	1.8±0.04^{b,c}	7.4±0.34^{c,d,e}	73.6±1.31^{a}	76.0±0.56^{a}
Pea fibre	**7.5**	67.3±1.17^{b}	34.0±0.71^{a,b,c}	1.9±0.06^{a,b,c}	8.3±0.79^{b,c,d}	68.0±1.01^{b,c}	69.2±0.59^{b,c}
	10.0	66.6±0.64^{b,c}	36.5±0.58^{a,b}	1.7±0.04^{b,c}	8.3±0.45^{b,c,d}	66.8±1.69^{b,c}	71.6±1.25^{b,c}
	12.5	67.3±1.40^{b}	33.5±0.75^{b,c}	2.0±0.07^{a,b}	10.1±1.15^{a,b}	66.6±0.80^{b,c}	72.0±1.85^{b}
	15.0	68.7±1.92^{a,b}	34.6±0.77^{a,b}	1.9±0.06^{b,c}	9.9±1.01^{a,b,c}	66.1±0.85^{b,c}	65.0±1.13^{c,d}
Inulin	**7.5**	67.0±0.63^{b,c}	34.2±0.64^{a,b,c}	1.9±0.05^{a,c}	10.3±1.54^{a,b}	61.4±0.91^{d}	62.5±1.12^{d,e}
	10.0	67.0±1.39^{b,c}	33.2±0.72^{b,c}	2.0±0.07^{a,b}	9.8±1.18^{a,b,c}	60.0±1.02^{d}	61.3±0.94^{e}
	12.5	70.2±0.93^{a,b}	36.0±0.40^{a,b}	1.8±0.03^{b,c}	11.0±0.84^{a}	61.0±1.45^{d}	62.5±0.94^{d,e}
	15.0	73.9±0.88^{a}	37.8±0.54^{a}	1.6±0.03^{c}	9.3±1.55^{a,b,c}	57.3±1.03^{e}	60.3±0.92^{e}
Guar gum	**3.0**	58.9±1.44^{e}	35.9±0.99^{a,b}	1.8±0.14^{b,c}	5.3±0.88^{f}	70.7±0.38^{a,b}	73.4±1.11^{b}
	5.0	60.9±1.24^{c,d,e}	32.9±0.98^{b,c}	2.0±0.09^{a,b}	6.2±1.08^{f}	70.1±0.58^{b}	69.4±0.61^{b,c}
	7.0	59.8±1.55^{e}	32.9±0.79^{b,c}	2.0±0.07^{a,b}	7.9±0.58^{d,e,f}	66.2±0.47^{b,c}	67.5±0.62^{c}
	10.0	60.3±1.35^{d,e}	30.6±0.83^{c}	2.3±0.09^{a}	7.8±0.99^{d,e}	64.0±0.59^{c}	64.2±0.45^{d}

*means ± S.E.M.

**within columns means with the same superscript are not significantly different (p>0.05).

The results presented in Table 2.4 indicate that increased cooking losses were obtained for the samples containing pea fibre and inulin (for certain levels of addition, $p<0.05$), while pastas containing guar gum showed reduced (for guar gum at 3%, 5%) or similar cooking losses (for guar gum at 7% and 10%) in comparison to the control. The behaviour of pastas containing inulin or pea fibre during cooking suggests that the DF used physically disrupted and weakened the protein-starch matrix similar to what was previously reported in tortilla dough (Seetharaman et al., 1997). In comparison, the presence of guar gum seemed to favourably influence the cooking properties of pasta and this may be related to its viscosity characteristics. Thus hydrated guar gum may form a network encapsulating the starch granules, restricting their excessive swelling during cooking, with effects on the structural integrity of the pasta. This explanation is in agreement with changes in the microstructure of cereal products previously reported by Brennan et al. (1996) studying the interaction of guar gum in bread products.

2.3.1.2 Pasta textural attributes as influenced by DF

The textural characteristics of pasta play an essential role in determining the final acceptance by consumers; pasta products that retain acceptable texture characteristics not only within normal cooking time, but also with over cooking are preferred. Pasta firmness, elasticity, adhesivness and stickiness as evaluated using texture tests are presented in Table 2.5, and statistical analysis showed that the values of each textural attribute measured were significantly affected by the type and percentage of DF inclusion into pasta ($p<0.05$, Table 2.5). Tukey's test performed after ANOVA, revealed the specific origins of differences.

Generally, the firmness of pastas containing DFs and control were similar ($p>0.05$); the exceptions were formulations containing the highest level of pea fibre (15%) or guar gum (10%) for which significantly reduced firmness values were obtained. In these cases, the

reduction in pasta firmness may be associated with the disruption of the protein-starch matrix, and thus weakening of pasta structure, which may occur at high levels of DF. A softer texture in comparison to the control was also shown by pasta with 7.5% on db pea fibre, but this may be an outlier.

Table 2.5. Textural characteristics of cooked pasta with added DF

DF addition (%)		Firmness		Stickiness	Adhesiviness	Elasticity
		peak force (N)	area (N*s)	peak force (N)	area (N*s)	peak force (N)
Control	**0.0**	1.01±0.04^{a}	9.46±0.39^{a}	3.61±0.11^{c,d,e}	0.21±0.01^{g,h}	0.24±0.01^{a}
Pea fibre	**7.5**	0.78±0.03^{b,c,d}	6.99±0.31^{c,d}	3.56±0.12^{c,d,e}	0.23±0.01^{f,g,h}	0.22±0.01^{a,b,c}
	10.0	0.86±0.03^{a,b,c}	7.66±0.32^{b,c}	3.11±0.31^{e,f}	0.18±0.02^{h,i}	0.24±0.01^{a,b}
	12.5	0.95±0.04^{a}	8.13±0.35^{a,b,c}	3.41±0.09^{d,e,f}	0.18±0.01^{h,i}	0.21±0.01^{a,b,c}
	15.0	0.77±0.02^{c,d}	6.93±0.21^{c,d}	2.76±0.06^{f}	0.14±0.01^{i}	0.19±0.01^{c,d,e,f}
Inulin	**7.5**	1.00±0.03^{a}	9.37±0.37^{a}	3.37±0.09^{e,f}	0.22±0.01^{g,h}	0.14±0.01^{g}
	10.0	1.01±0.03^{a}	9.48±0.38^{a}	3.54±0.10^{c,d,e}	0.25±0.01^{e,f,g}	0.16±0.01^{g}
	12.5	0.96±0.03^{a}	8.92±0.30^{a,b}	4.93±0.20^{a}	0.37±0.01^{a,b}	0.16±0.01^{e,f,g}
	15.0	0.94±0.05^{a}	8.29±0.37^{a,b,c}	4.70±0.19^{a,b}	0.33±0.01^{b,c,d}	0.18±0.01^{d,e,f}
Guar gum	**3.0**	1.00±0.04^{a}	8.93±0.29^{a,b}	4.70±0.15^{a,b}	0.40±0.01^{a}	0.20±0.01^{b,c,d}
	5.0	1.01±0.04^{a}	8.60±0.31^{a,b}	4.07±0.12^{b,c,d}	0.34±0.01^{a,b,c}	0.20±0.01^{b,c,d}
	7.0	0.94±0.03^{a,b}	8.70±0.27^{a,b}	4.19±0.11^{b,c}	0.30±0.01^{c,d,e}	0.16±0.01^{f,g}
	10.0	0.67±0.03^{d}	5.92±0.31^{d}	3.76±0.11^{c,d,e}	0.28±0.01^{d,e,f}	0.14±0.01^{g}

*means ± S.E.M.

**within columns means with the same superscript are not significantly different ($p>0.05$).

The elasticity of DF enriched pasta showed significant differences. The values obtained for pastas containing pea fibre (7.5%, 10% and 12.5%) were comparable to the control (Table 2.5), while the addition of either inulin or guar gum in the formulation led to significant reductions in elasticity in comparison to the control. Similarly, the adhesiveness and stickiness of pastas containing pea fibre were similar to those of control (except for pasta with 15% pea fibre addition). This was not the case for inulin and guar gum, which when

present in the formulations resulted in increased values of the same parameters in comparison to the control.

Addition of DFs appears to interfere with the structure of pasta, possibly disrupting the continuity of the protein starch matrix; this was indicated by reduced firmness values in comparison to the control, especially for high levels of DF. These results are in agreement with those obtained by Edwards et al. (1995) who reported that fortification of pasta with pea fibre altered its structure, resulting in moderate reduction in pasta firmness and in an increase in cooking losses. The explanation offered was related to the disruptive effect pea fibre inclusion had on the protein matrix which allowed starch granules to rupture during cooking, hence releasing high levels of amylose into the cooking water. Results from the present study support this theory, in particular the effect of pea fibre (mainly insoluble fibre) on cooking losses and the swelling index of pasta.

Inulin however, seems to influence the structure of pasta differently. Firmness values were similar to the control, suggesting that the structure remains compact, but elasticity values were significantly lower in comparison to the control ($p<0.05$) indicating that inulin may interfere with the formation of protein strands leading to weaker structures. Adhesivness and stickiness of pastas containing inulin were found to be higher than the control, and this may be explained by the physico-chemical nature of inulin. Being highly hydrophilic inulin may compete with the starch and protein for water upon hydration leading to starch and protein fractions of the pasta being more discrete and less incorporated in a matrix. Hence during cooking the starch which is not fully encapsulated within the protein matrix may form a 'starchy' layer at the surface of the product resulting in higher levels of stickiness and adhesiveness, and part of it may be lost in the water, hence the cooking losses observed (Table 2.4).

The interaction between guar gum, protein and starch within pasta matrix appear to follow a complex pattern. Low levels of guar were found to have no influence on the firmness of pasta. However, higher levels of guar (10% on db) lead to a significant decrease in pasta firmness (Table 2.5). This observation may be related to the significantly higher moisture content ($p<0.05$), and swelling index ($p<0.05$) observed with increasing guar concentrations (Table 2.4). The higher moisture content may have an impact on mechanical properties of the system - water acting as a plasticizer of composite materials hence influencing their flow properties. Adhesivness and stickiness were significantly higher than the control ($p<0.05$), while the elasticity values significantly decreased with increasing levels of guar gum used in the formulations. The explanation for the increased adhesivness and stickiness values is probably different from the one given for inulin, since they were not supported by an increase in cooking losses. Thus the effect is more likely to be related to gel forming/viscosity development characteristics of guar gum, which may lead to the formation of a highly viscous, sticky layer surrounding/interfering with starch - protein matrix as observed by Brennan et al. (1996). The internal structure of pasta is potentially affected and this is indicated by its decreased elasticity, which is equivalent with a reduced strength.

2.3.1.3 Influence of DF on the pasta microstructure

Scanning Electron Microscopy techniques were used to investigate the internal structure of both raw and cooked pasta as affected by DF added in the formulations. Figures 2.1 and 2.2 show representative SEM micrographs obtained for both raw and cooked pasta, and the structures observed appear to support the aforementioned theories.

Micrographs of the control raw pasta samples show a well formed protein-starch matrix, with strong and continuous protein strands entrapping large starch granules (Figure 2.1a).

The starch granules within this pasta appear slightly swollen and irregular in size and shape, possibly indicating a certain level of gelatinisation during the extrusion process.

The addition of pea fibre into the product appears to disrupt the continuity of the protein matrix. Protein-fibre matrix within pasta containing pea fibre at 7.5% (Figure 2.1b) and 15% (Figure 2.1c) appears to be less developed than in the control, resulting in an open appearance with discrete starch granules 'uncovered' and potentially exposed to enzymatic attack. The degree of disruption appears to increase with the amount of pea fibre added to the product. A similar effect was observed in samples containing inulin (Figures 2.1d and e). However in the case of inulin addition the protein-fibre-starch matrix had a more continuous appearance than for the samples containing pea fibre.

Micrographs of pasta containing guar gum at 3 % (Figure 2.1e) show a similar matrix to the control, with protein-starch-fibre integrating into a compact network. However, the starch granules within this matrix show no signs of deformation or swelling, indicating that they have not been prone to swelling or gelatinisation during extrusion. SEM images of pasta with 10% guar gum on db (Figure 2.1g), show starch granules embedded in a highly developed network (probably of protein and fibre), illustrating that at higher inclusion rates, soluble DF affects the overall structural organisation of pasta.

Images of cooked pasta (Figure 2.2) also show differences related to the type and quantity of DF used. Gelatinised starch granules within control pasta (Figure 2.2a) appear integrated in a developed protein matrix to form a relatively compact structure. In contrast, pea fibre inclusion at both 7.5% and 15% (on db) (Figure 2.2b, c) appear to reduce starch swelling (visible especially at 10% level of addition) and to disrupt the protein network giving rise to a discontinuous, weak structure. This structure may explain the decreased firmness and elasticity observed (Table 2.5) and hence the overall textural quality of the pasta.

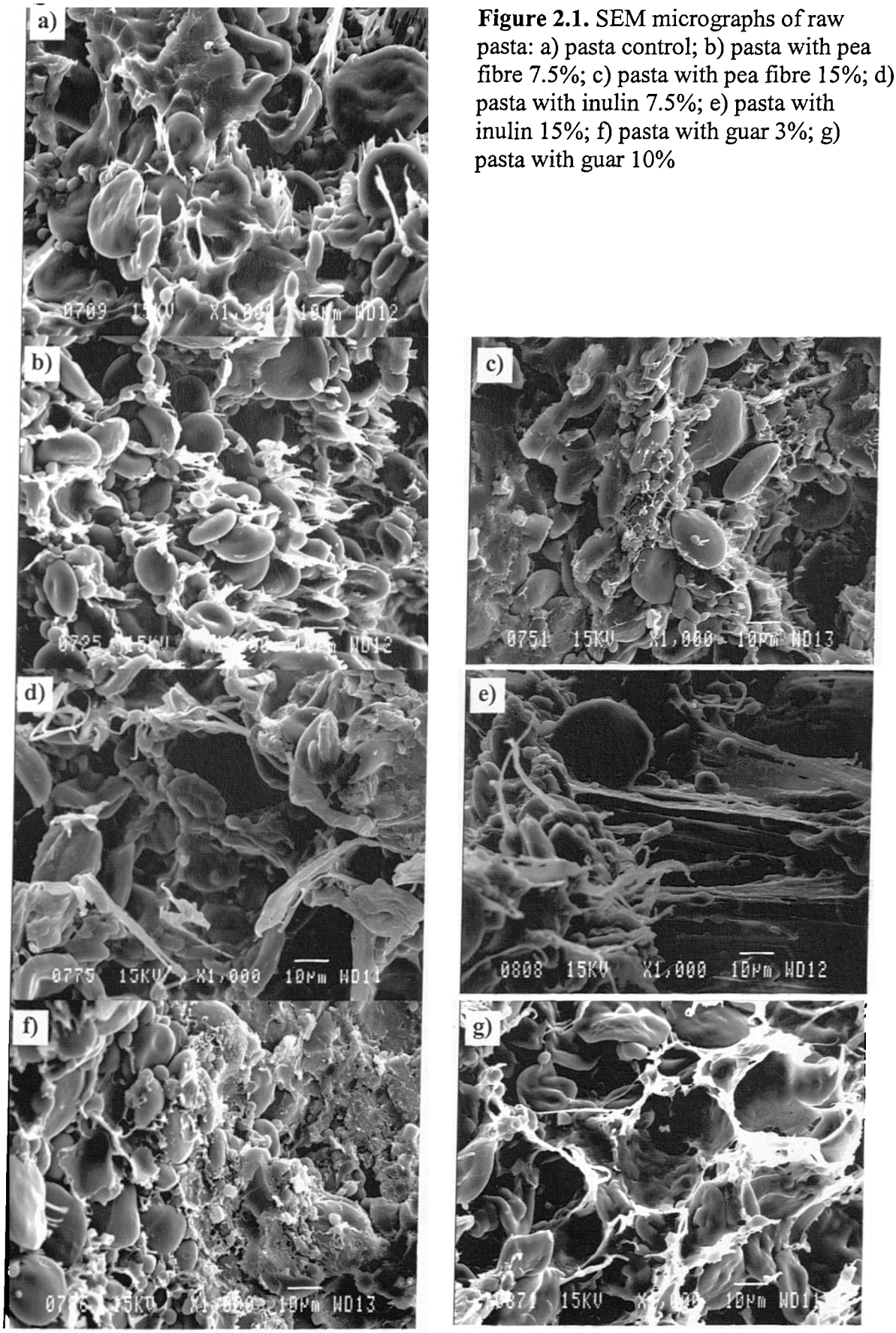


Figure 2.1. SEM micrographs of raw pasta: a) pasta control; b) pasta with pea fibre 7.5%; c) pasta with pea fibre 15%; d) pasta with inulin 7.5%; e) pasta with inulin 15%; f) pasta with guar 3%; g) pasta with guar 10%

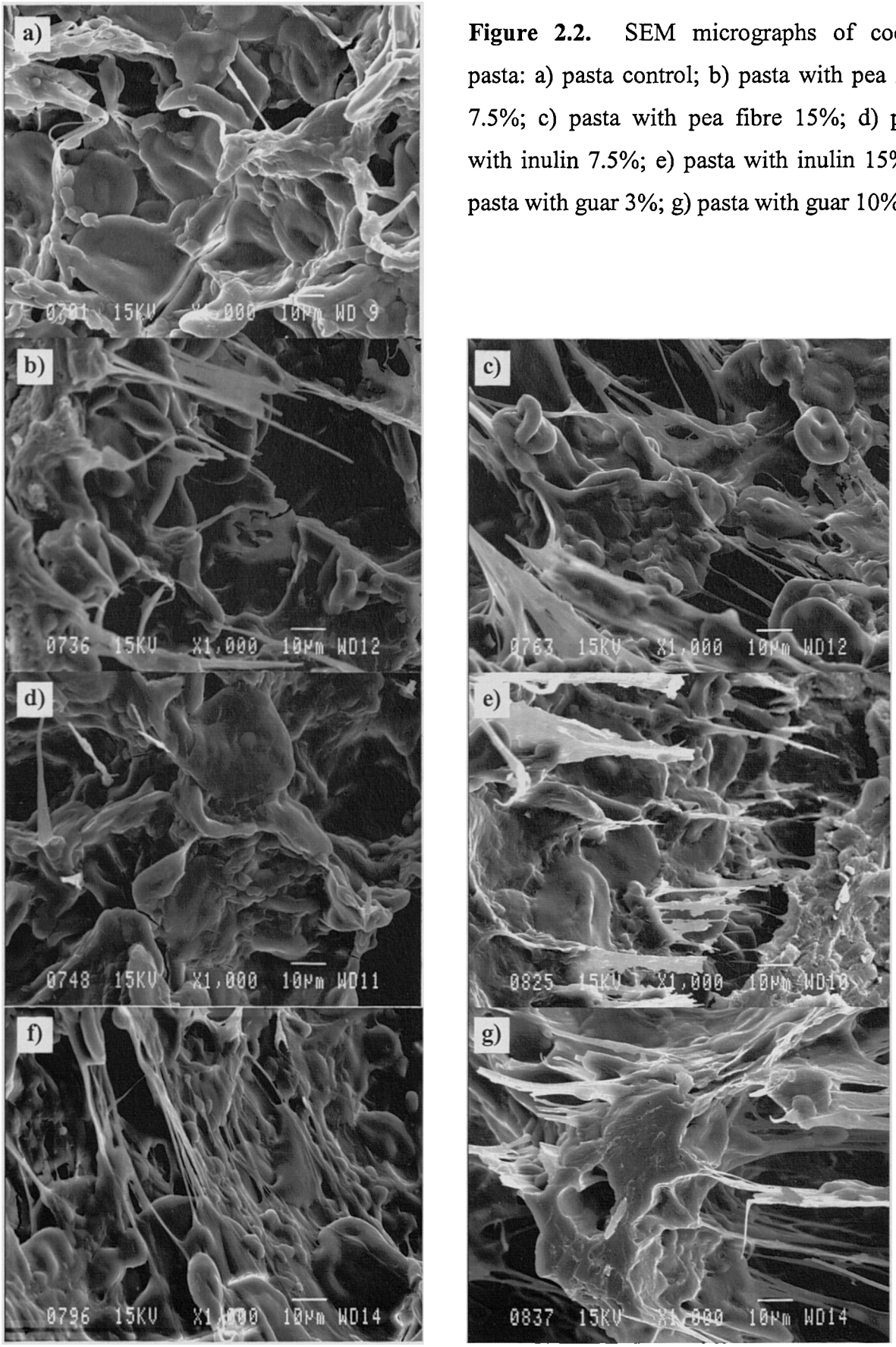


Figure 2.2. SEM micrographs of cooked pasta: a) pasta control; b) pasta with pea fibre 7.5%; c) pasta with pea fibre 15%; d) pasta with inulin 7.5%; e) pasta with inulin 15%; f) pasta with guar 3%; g) pasta with guar 10%

Additions of inulin at 7.5% and 15% (Figures 2 d and e respectively) appear not to significantly affect pasta structure. The protein-fibre-starch network is highly developed with discrete starch granules highly swollen. The similarities in the internal structure of inulin and control pasta samples may explain the similarity in their firmness as presented in Table 2.5.

It is important to note the internal structure of pasta containing guar gum (Figure 2f and g) which appears to be very different in comparison to the control. In particular, the appearance of the starch granules is different to that observed in the control with the granules being less swollen and more regular in shape than the control samples. Moreover, micrographs of pasta with a 10% guar on db (Figure 2.2g) show a highly compacted pasta structure where starch granules appear to be entrapped within a continuous protein-fibre network. This may explain not only the reduced cooking losses (Table 2.4) but also the significantly altered textural characteristics (Table 2.5). These results support the data obtained by Fardet et al. (1999a) in a study on pasta containing soluble DF.

2.3.1.4 The effects of DF on thermal characteristics and *in vitro* digestibility of pasta

Results from DSC analysis on raw pasta products (Table 2.6) show that DFs had no significant effect on the starch gelatinisation temperatures as indicated by the onset, peak, and endset temperatures (Table 2.6). An exception appears to be the addition of inulin at 12.5%, which resulted in higher peak temperature in comparison to all the other samples. However, taking into account that no significant difference was found for T_{onset} and T_{endset} between the samples, it is fair to assume that the value obtained may due to experimental error and hence an outlier. Nevertheless it is interesting to observe that the mean T_{endset} values are higher for the samples containing DF than the control, although statistical significance was not obtained. This trend is consistent with previous research (Eerlingen et

al., 1996; Ferrero et al., 1996), which showed that the inclusion of soluble non-starch polysaccharides leads to an increase in starch gelatinisation temperature. This is partly due to the soluble fibres competing with starch for water and hence limiting starch swelling and gelatinisation events, resulting in higher than expected T_{endset} values.

Table 2.6. Thermal properties (DSC measurements) for raw pasta

DF addition (%)		T_{onset} (°C)	T_{endset} (°C)	Enthalpy (ΔH) (J/g starch)	Gelatinisation temperature (°C)
Control	**0.0**	55.10±0.2	66.43±0.3	7.49±0.46^{a}	60.73±0.22^{b}
Pea fibre	**7.5**	54.30±0.4	67.05±0.2	6.69±0.05^{a,b}	61.10±0.1^{b}
	10.0	53.65±0.4	67.35±0.3	6.27±0.25^{a,b,c}	60.95±0.05^{b}
	12.5	53.05±0.6	67.25±0.2	5.66±0.04^{b,c,d}	60.85±0.05^{b}
	15.0	53.30±0.1	66.85±0.2	5.20±0.05^{c,d}	60.60±0.2^{b}
Inulin	**7.5**	55.70±1.3	67.10±0.3	6.99±0.05^{a,b}	60.85±0.55^{b}
	10.0	55.90±1.8	69.70±1.5	4.84±0.22^{d,e}	61.85±0.65^{b}
	12.5	55.80±0.4	68.65±1.4	3.14±0.09^{e}	64.05±0.85^{a}
	15.0	54.70±0.8	68.00±0.5	5.39±1.02^{c,d}	62.55±0.05^{a,b}
Guar gum	**3.0**	53.90±0.3	67.07±0.5	5.97±0.61^{a,b}	61.27±0.03^{b}
	5.0	53.70±0.6	67.90±0.5	5.88±0.39^{a,b}	61.00±0.2^{b}
	7.0	54.57±1.8	68.70±1.5	5.33±0.18^{b,c}	61.60±0.4^{b}
	10.0	52.55±0.1	67.00±1.1	5.74±0.12^{b,c}	61.65±0.15^{b}

*means ± S.E.M.

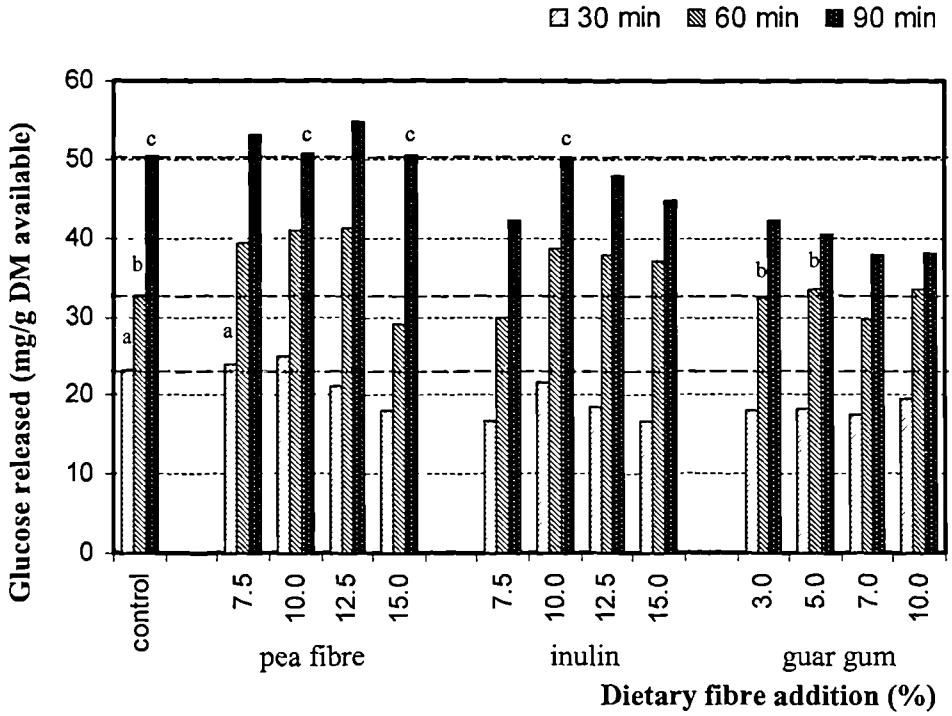
**within columns means with the same superscript are not significantly different ($p>0.05$).

The results obtained for the enthalpy necessary to melt starch crystallites in the presence of DFs (Table 2.6) appear inconsistent with previous research data reported by Ferrero et al. (1996) who found that enthalpy of starch / guar and xanthan gum mixtures increased with increasing concentrations of gum used. The results obtained in this experiment suggest that the enthalpy of a flour / DF complex decreased with increasing concentration of DF (Table 2.6). Liu and Lelievre (1992) have obtained similar trends for mixtures of rice starch,

gellan and locust bean gum. One explanation might be incomplete gelatinisation of starch in the presence of DF, but this is highly unlikely at the ratio pasta:water used in the DSC runs. Alternatively, restrictions to the extent of conformational disordering might have occurred (Liu and Lelievre, 1992). Nevertheless more work needs to be conducted on this topic to characterise the actual gelatinisation events during the cooking of pasta products with added DF.

DSC analysis of cooked pasta products were also conducted. No gelatinisation peak was observed between 4 and 100^{0}C (results not shown) indicating that during pasta cooking the starch was fully gelatinised.

Figure 2.3. Glucose released *per* unit DM (* Values labelled with the same letter are not significantly different (p>0.05); for the sake of clarity, the graph shows only the comparisons to the control)

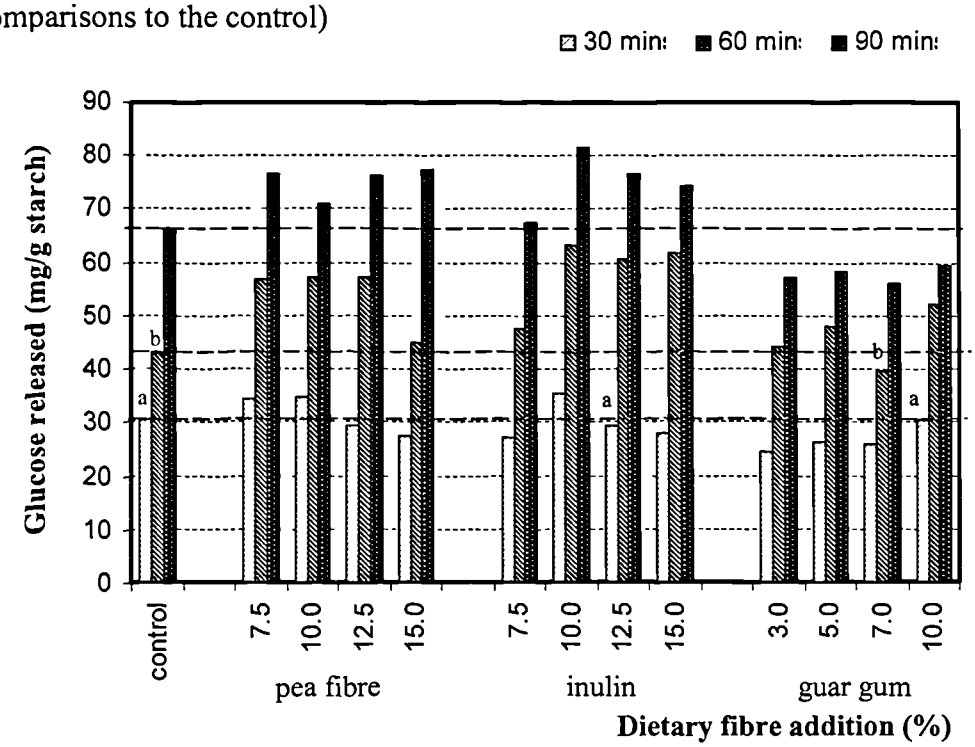


Pasta digestibility as influenced by the presence of DFs was investigated using an *in vitro* method (Brennan et al., 1996), and the results expressed as amount of glucose released are presented in Figures 2.3 and 2.4. Statistical analysis indicate that inulin and guar gum

significantly decreased the digestibility of pasta ($p<0.001$), especially after 90min of digestion (Figure 2.3). Pea fibre however, appears to increase the overall glucose released from pasta, and this was seen at each sampling point.

The decrease in glucose released observed for samples containing inulin or guar gum may be partially due to lower starch content within the DF enriched pasta, accounting for DF inclusion. In order to evaluate starch digestibility in the presence of DF, the actual starch content within the pasta complex was taken into account and the glucose released was then expressed as mg *per* gram of starch (Figure 2.4). Statistical analysis indicates in this case that the amount of glucose released, and thus starch digestibility is significantly higher for pasta containing pea fibre or inulin, than for control ($p<0.001$); this was observed after both 60 and 90min of *in vitro* digestion. However, after 90min of amylolysis a significant decrease in starch digestibility was observed for pasta containing guar gum, regardless of the level of addition ($p<0.001$, Table 2.4).

Figure 2.4. Glucose released *per* 1g starch available (* Values labelled with the same letter are not significantly different ($p>0.05$); for the sake of clarity, the graph shows only the comparisons to the control)



Since the DSC measurements on cooked pasta showed no peak in between 4^0 and 100^0C, the decrease of starch digestibility in samples containing guar gum, can not be related to an incomplete gelatinisation of starch. A possible explanation of the results may relate to the observations made on pasta microstructure. The SEM images of cooked pasta enriched with either pea fibre or inulin showed structures with disrupted protein strands or similar to the control pasta respectively; in either situation the starch granules were distinct from the protein-fibre matrix, and appeared to be highly exposed and thus easily accessible and more susceptible to α-amylase attack. This may explain the results for the amount of glucose released which was higher (for pasta containing pea fibre) or comparable (for pasta with inulin) to the control pasta.

In comparison, guar gum appeared to alter the internal structure of pasta through the formation of a continuous, mucilaginous looking layer that encapsulates starch granules (Figures 2.2.f and g), and may act as a physical barrier offering protection against the enzymatic action. Thus, the potential degradation of starch may be reduced and as a consequence the overall amount of glucose released may be decreased (Figures 2.3 and 2.4). Similar observations were made by Brennan et al. (1996) during an experiment on bread enriched with guar gum; the authors also reported a decrease of the starch hydrolysis rates, which were attributed to the layer formed by guar gum, surrounding starch granules. This hypothesis on starch encapsulation is also supported by the decreased cooking losses in pasta containing guar gum, presented and discussed earlier in this chapter.

These preliminary results suggest that it is possible to obtain pasta products enriched with DF and maintain relatively acceptable cooking and textural characteristics, in most cases comparable with the control product (with no DF added). It appears that a relationship

between the level of DF addition and textural qualities exists: the results showing that increasing levels of DF in the formulation result in more pronounced differences in terms of structure and texture in comparison to the control. The type of DF (soluble *vs* insoluble) was also found to significantly alter the microstructure of the products and thus their cooking properties, texture and, equally important, the starch hydrolysis rates. Pasta products enriched with certain DF (guar gum in this case) appear to bring in additional potential health benefits, most notably the reduced rate of starch digestion/(sugars released) in comparison to control pasta. The mechanisms involved are complex and could not be uniquely attributed to the structural characteristics of the protein network, but more likely to interaction between protein, starch and DF at the microscopic level.

Bearing in mind that the final product needs to be acceptable to the consumers, these results indicate that on average formulations with 10% DF on db are expected to give the most benefits: relatively high amount of DF and slow release of sugars during digestion without impairing though the textural and cooking properties.

2.3.2 Stage 2

Based on the results from the previous stage, this second set of experiments focused on producing pasta with a maximum level of added DF of 10% (on db); their quality attributes together with potential nutritional benefits were assessed. The range of DFs used was widened to include also locust bean gum (lbg), xanthan gum, bamboo fibre, and β-glucan. This decision was made for two reasons. One was to validate the results of Stage 1, and the second to investigate if the observations made are due to the existence of generic

behaviour, which may relate the solubility of DFs to their technological and nutritional effects, or they are the result of specific effects of individual DFs.

Additionally, for this set of experiments pasta was produced using a pilot scale extruder as a necessary step toward simulation of the industrial process. These DF enriched pasta products were again compared against a control (no added DF) in terms of cooking qualities, texture properties, internal structure, and starch digestibility. At this stage, it became necessary to mimic more closely the digestion that takes place in the gastrointestinal tract. Therefore the digestibility of starch was assessed using an *in vitro* digestion procedure comprising several steps: simulated mastication, proteolysis, followed by restricted amylolysis. A predicted GI was also calculated for each product.

2.3.2.1 Cooking qualities of DF enriched pasta

The results obtained for cooking properties of pasta, and for chemical composition of the cooked product are summarised in the ANOVA Table 2.7. Dry matter of the pasta cooked for the optimum time ranged between 25.4% (for the pasta made with 10% xanthan gum on db) and 33.2% (for pasta made with 7.5% inulin on db). As the values presented in Table 2.7 indicate, dry matter values of cooked pasta were significantly affected only by the type of DF used in the formulation ($p<0.01$). The use of xanthan gum resulted in pasta products with dry matter values significantly lower than those of pastas containing guar gum, pea fibre, bamboo fibre, locust bean gum, β-glucan, or inulin. Although not introduced in the model for statistical analysis, the dry matter of control pasta appear to be similar to those of pastas containing pea fibre, bamboo fibre, locust bean gum, β-glucan, or inulin (Table 2.7). The level of DF used in the formulation appeared to have no significant effect on the dry matter of the final product ($p>0.05$). Nevertheless, the results presented in Table 2.7 suggest there is a trend towards a decrease in the dry matter of the final product

with increasing levels of DF addition. This is not surprising since many DFs through their nature are known to be able to retain high amounts of water within their structure.

Swelling index of pasta was significantly affected by both type of DF used in the formulation ($p<0.001$) and the level of usage ($p<0.01$). The highest swelling index was obtained for pastas containing xanthan gum, while all the other values were similar to that of control pasta. These results agree with those obtained for the dry matter: high swelling index associated with pastas containing xanthan gum indicate higher amounts of water *per* gram of solids and thus lower percentage of dry matter in the cooked product. The higher the level of DF used, the higher the swelling index for pasta - a trend to be expected if the water holding capacity of DF is considered.

The type of DF used in pasta formulation had a significant effect on the cooking characteristics (dry matter in the cooking water and the cooking loss - $p<0.05$ in both cases - Table 2.7). The highest percentage of solids in the cooking water was found for pastas containing β-glucan, while the lowest value was found for pastas containing xanthan gum. Taking into account that values higher than 2.1g/100g cooking water were previously related to low cooking quality while values lower than 1.4g/100g cooking water to very good cooking quality (D'Edigio and Nardi, 1996), it can be concluded that overall, none of the DFs used in the formulations produced unacceptable cooking qualities for pastas.

Table 2.7. ANOVA table summarising the cooking characteristics of pasta and chemical composition of cooked pasta (the values represent means of a values at a given treatment level)

Sample	Dry matter (%)	Dry matter of the cooking water (%)	Swelling index (g water/g DM)	Cooking losses (%)	Protein content (%)	Starch content (%)	Available carbohydrate content (%)	TDF (%)
Control	*32.5±1.03*	*0.93±0.06*	*2.18±0.24*	*8.8±1.2*	*4.9±0.04*	*25.7±1.37*	*25.7±1.39*	*1.5±0.08*
Effect of the type of DF								
Pea fibre	31.8^{a}	$0.96^{a,b}$	2.15^{b}	$10.2^{a,}$	$4.7^{a,b}$	$23.4^{a,b}$	$23.6^{a,b}$	2.6^{c}
Inulin	32.4^{a}	$0.90^{a,b}$	2.13^{b}	9.7^{a}	$4.7^{a,b,c}$	24.1^{a}	24.3^{a}	$3.1^{a,b,c}$
Guar gum	$29.6^{a,b}$	$0.88^{a,b}$	$2.39^{a,b}$	9.3^{a}	$4.5^{b,c,d}$	$21.8^{b,d}$	$21.9^{b,c}$	$3.0^{a,b,c}$
Bamboo fibre	31.5^{a}	$0.93^{a,b}$	2.21^{b}	9.9^{a}	$4.5^{a,b,c}$	$22.4^{a,b,c}$	$22.5^{a,b,c}$	$3.1^{a,b}$
β-glucan (as Hi Sol)	$31.4^{a,b}$	1.08^{a}	2.19^{b}	11.5^{a}	$4.3^{c,d}$	$21.2^{b,d}$	21.5^{c}	3.4^{a}
Xanthan gum	28.4^{b}	0.75^{b}	2.72^{a}	7.6^{b}	4.1^{d}	$20.8^{c,d}$	20.9^{c}	$2.7^{b,c}$
Locust bean gum	32.2^{a}	$0.84^{a,b}$	2.11^{b}	9.3^{a}	4.9^{a}	$23.5^{a,b}$	$23.6^{a,b}$	3.4^{a}
Significance	**	*	***	*	***	***	***	***
SEM	0.70	0.06	0.08	0.63	0.09	0.04	0.43	0.11
Effect of the level of DF addition								
2.5%	31.8	0.88	2.18^{B}	9.6	4.8^{A}	23.6^{A}	23.8^{A}	1.8^{A}
5.0%	31.6	0.87	2.18^{B}	9.7	$4.7^{A,B}$	$23.1^{A,B}$	$23.3^{A,B}$	2.8^{B}
7.5%	30.8	0.92	$2.30^{A,B}$	10.6	$4.4^{B,C}$	22.2^{B}	22.4^{B}	3.4^{C}
10.0%	30.1	0.96	2.44^{A}	10.7	4.2^{C}	20.8^{C}	21.0^{C}	4.2^{D}
Significance	NS	NS	**	NS	***	***	***	***
SEM	0.53	0.04	0.06	0.48	0.07	0.32	0.32	0.08
Effect of the interaction								
Type of DF*level of addition	NS	NS	NS	NS	NS	NS	NS	*

- within the same column, the values with the same letter are not significantly different
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

Cooking losses were significantly affected by the type of DF used ($p<0.05$), with xanthan gum leading to the lowest values. High cooking losses are usually associated with disruption of the gluten matrix (which promotes water absorption and expose starch granules to swelling and rupture, leading finally to the release of solids from the structure). Thus, the results presented in Table 2.7 would suggest that in comparison with all the other DF, xanthan gum consolidates the structure strength. Similar results for pasta containing xanthan gum (at 1 or 2%) have been previously obtained by Edwards et al. (1995) based on iodine binding capacity of the cooking water. Their explanation was that xanthan gum forms a network around the starch granules trapping them in place during cooking, and restricting excessive swelling and diffusion of amylose.

Nevertheless, it is worthwhile to note that overall DFs appear to have no significant effect on cooking losses in comparison to control pasta (Table 2.7); the exception was β-glucan (as HiSol). Similarly, statistical analysis showed that no significant effect was related to the level of DF used in the formulations ($p>0.05$), although the increasing levels of DF appear to result in increasing mean values suggesting a trend towards deterioration in cooking qualities.

Chemical composition of cooked pasta (protein content, starch content, carbohydrate content and total DF) was significantly affected by both type of DF used in the formulation ($p<0.001$) and the level of usage ($p<0.001$). This was to be expected since various DF led to differences in the moisture content of the final product, and the initial formulation was based in the replacement of flour with various levels of DF. Thus, the higher the level of DF in the initial formulation, the lower the protein, starch and carbohydrate content and the higher the level of DF determined in the final product ($p<0.001$; Table 2.7).

2.3.2.2 Textural characteristics of DF enriched pasta

The main criteria generally accepted to assess the overall quality of cooked pasta is based on the textural evaluation. Taste panels are time consuming and impractical when a large number of samples need assessing. Instrumental methods have been proved to successfully estimate the textural characteristics of pasta which are recognised as being important for the consumers thus dictating product acceptability: firmness, stickiness/adhesivness and elasticity/breaking strength. The mean values of these parameters for cooked pasta as evaluated using the Texture Analyser are summarised in Table 2.8 and illustrated in Figures 2.5, 2.6, and 2.7. Statistical analysis indicate that all textural parameters characterising the cooked product were significantly affected by the type of DF used and also by the level of their usage ($p<0.001$ in all cases).

The firmness of cooked pasta generally decreased with increasing levels of DF in the formulation ($p<0.001$) (Table 2.8 and Figure 2.5); this trend is in accordance with the results obtained for the dry matter, swelling indexes and protein content of cooked pasta. Higher moisture contents and swelling indexes associated with higher levels of DF in the formulation will result in lower firmness of the end product - water acts as a plasticiser of composite materials reducing stiffness. In addition, lower levels of protein (in high DF pasta) are also associated with reduced firmness, being known that the quantity and quality of proteins in pasta formulation have direct implication for textural attributes of the cooked product (D'Edigio and Nardi, 1996).

Generally, the firmness of DF enriched pastas was lower than of the control (except for pastas containing locust bean gum or xanthan gum); these results could again be related to the relatively increased moisture contents and increased swelling indexes of these products in comparison to the control (Table 2.7). However, this assumption does not necessarily

apply in all the cases; for example pastas containing locust bean gum or xanthan gum, had moisture values similar or respectively higher than the control pasta, and at the same time their firmness showed increased values (Table 2.8). This would suggest that locust bean gum and xanthan gum contribute to the structure strength. In the case of xanthan gum, this hypothesis could be also related to the low values obtained for cooking losses, indicating a well formed structure from which few solids are released during cooking.

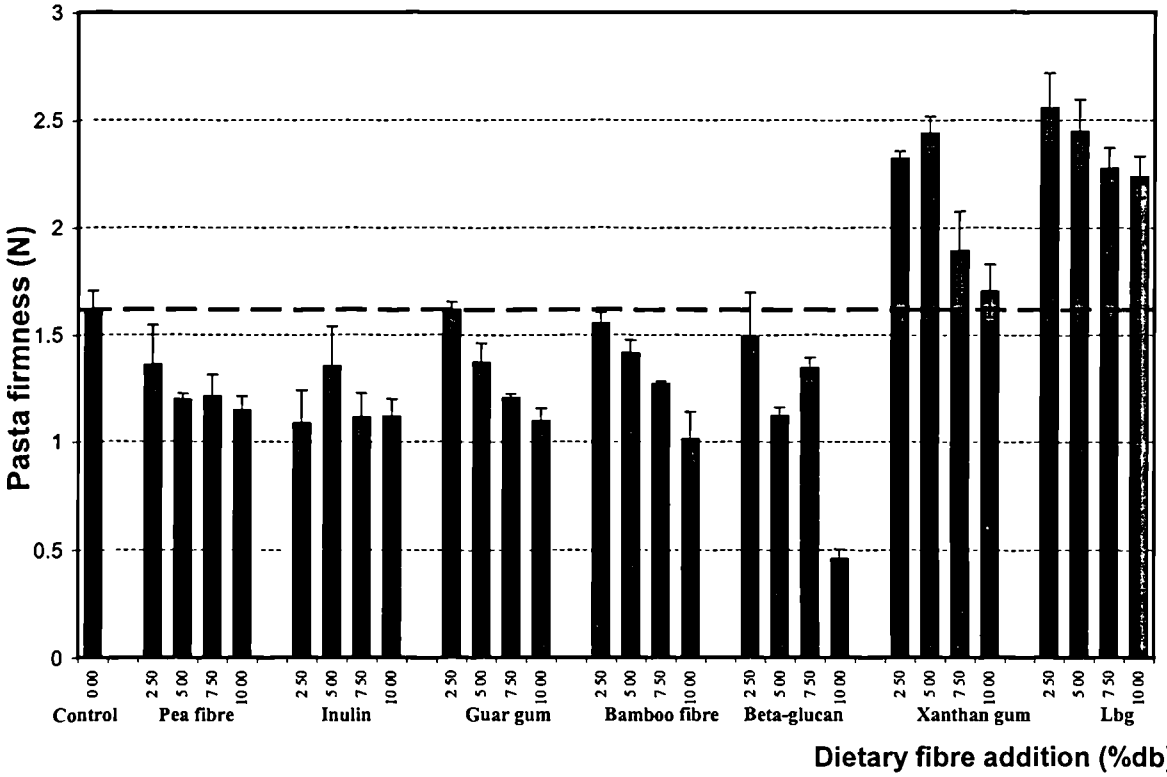
Table 2.8. ANOVA table summarising the textural attributes of cooked pasta (the values represent means of all values at a given treatment level)

	Firmness (N)	**Stickiness (N)**	**Adhesiveness (N*s)**	**Elasticity (N)**
Control	*1.60±0.10*	*3.0±0.28*	*0.22±0.03*	*0.17±0.00*
Effect of the type of DF				
Pea fibre	$1.23^{c,d}$	$2.9^{c,d}$	0.17^{d}	$0.16^{b,c}$
Inulin	$1.17^{c,d}$	3.3^{c}	$0.31^{c,d}$	0.14^{c}
Guar gum	1.32^{c}	4.2^{b}	$0.38^{b,c}$	$0.15^{b,c}$
Bamboo fibre	1.31^{c}	2.6^{d}	0.19^{d}	$0.16^{b,c}$
β-glucan (as β-glucan)	1.10^{d}	10.9^{a}	1.35^{a}	0.10^{d}
Xanthan gum	2.09^{b}	2.4^{d}	0.22^{d}	0.22^{a}
Locust bean gum	2.38^{a}	4.0^{b}	0.53^{b}	0.18^{b}
Significance	***	***	***	***
SEM	0.046	0.14	0.015	0.008
Effect of the level of DF addition				
2.5%	1.71^{A}	3.6^{A}	0.4^{B}	0.18^{A}
5.0%	1.62^{A}	4.1^{B}	0.45^{B}	$0.16^{A,B}$
7.5%	1.47^{B}	4.1^{B}	0.37^{B}	0.15^{B}
10.0%	1.25^{C}	5.5^{C}	0.59^{A}	0.14^{B}
Significance	***	***	***	**
SEM	0.035	0.11	0.012	0.006
Effect of the interaction				
Type of DF*level of addition	**	***	***	NS

- within the same column, the values with the same letter are not significantly different;
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

These findings support the work of Edwards et al. (1995) who reported an improvement in pasta firmness without the alteration of cooking qualities when xanthan gum was added at levels of 1% or 2%. The results have been explained by the authors through the cellulosic chain backbone structure of xanthan gum, which has a stiff conformation and readily gives a network structure in solution contributing to the structure strength. Similar results were reported by Fardet et al. (1999) for pasta made from freeze-dried flour fractions in which soluble fibres accounted for 7%. They found that the firmness of the resulting pasta increased and cooking loss was reduced, the proposed explanation being the formation of a network by the soluble DF around the starch granules, leading to a stronger cohesiveness between starch and protein within the pasta structure.

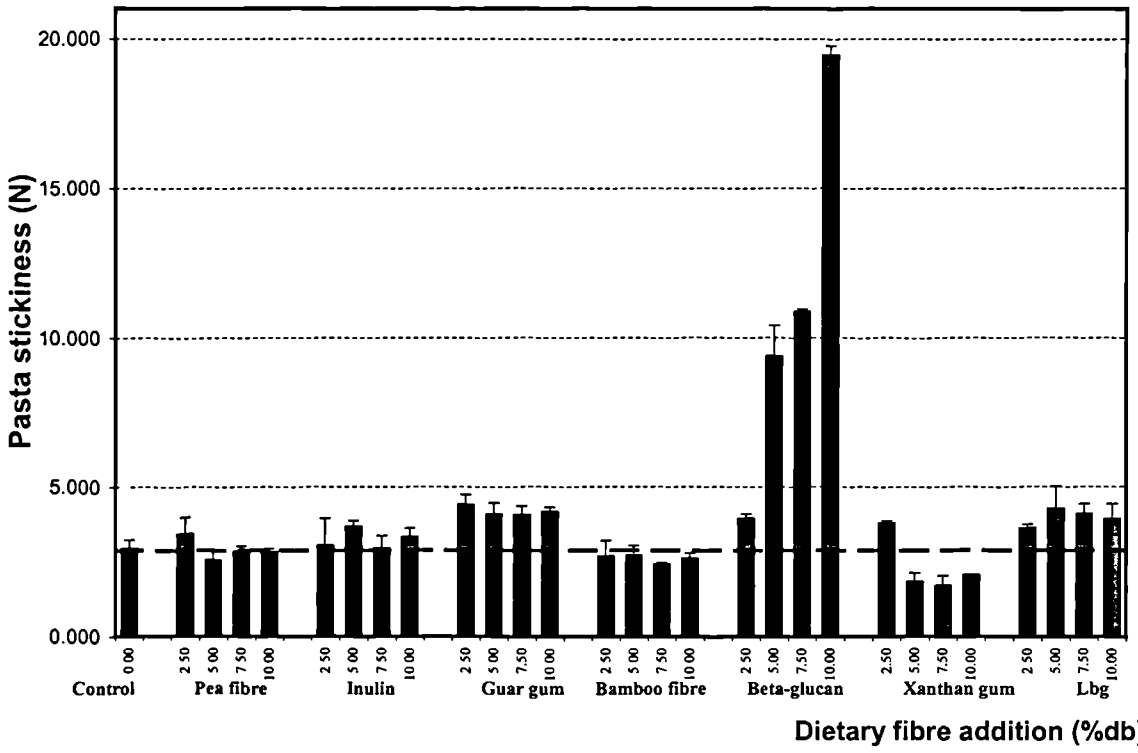
Figure 2.5. Firmness of pasta samples as determined using the Texture Analyser



The average firmness values for pastas containing pea fibre, inulin, guar gum, bamboo fibre or β-glucan (as HiSol) were lower than for control pasta (trends generally similar to what was observed at 2.3.1.2), indicating weaker structures possibly due to the interference

of these DFs with the gluten matrix. However, as Figure 2.5 indicates, at low levels of addition these DFs do not produce significant changes in the firmness when compared to the control. Such effects have been previously reported for pea fibre and oat bran (Edwards et al., 1995), the authors suggesting that the moderate reduction in pasta firmness was due to a disruption of the continuity of the protein-starch network in pasta promoted by the presence of DFs.

Figure 2.6. Stickiness of pasta samples as determined using the Texture Analyser

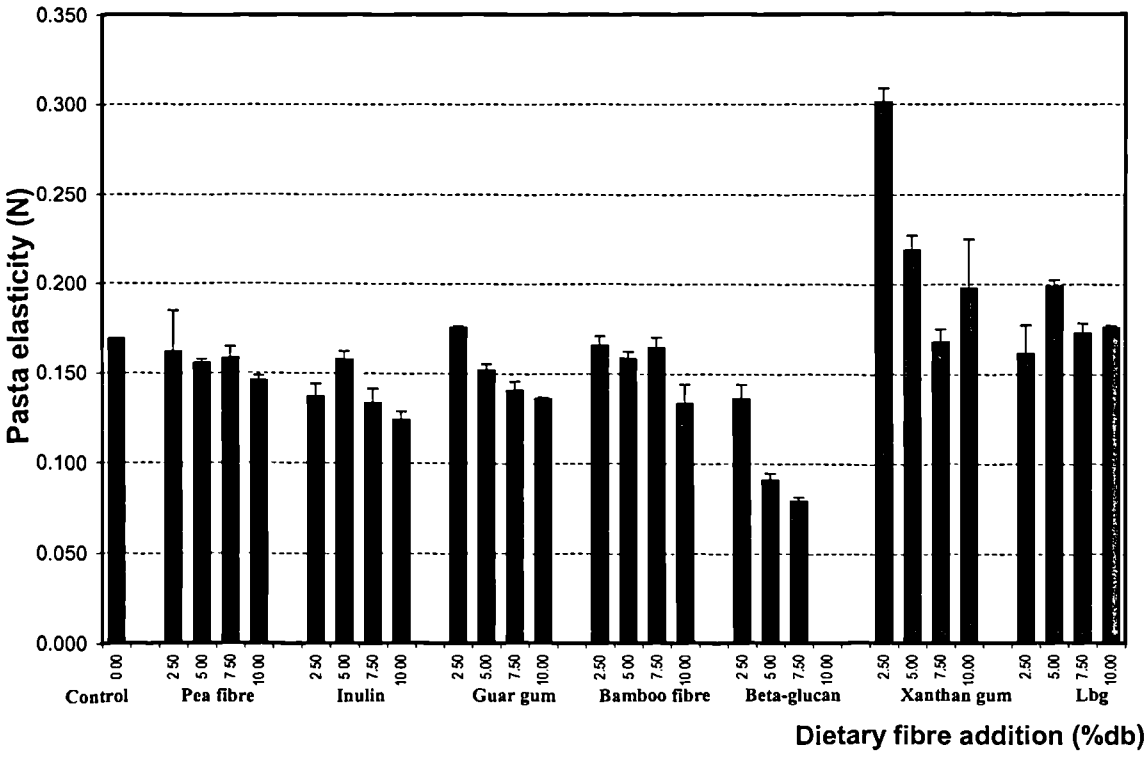


Statistical analysis showed that the stickiness and adhesiveness of cooked pasta increased with increasing levels of DF included in the formulation ($p<0.001$; Table 2.8). These outcomes are in concordance with the firmness results if the relationship structure-texture is taken into account. Generally, the higher the level of DF in the formulation, the more pronounced the disruption of the gluten matrix would be expected, which causes not only changes in firmness (reduced firmness), but also on surface characteristics (becoming stickier, more adhesive). However, the examination of Figure 2.6 leads to the conclusion

that the trend shown by the statistical analysis appears to be valid only for pastas containing β-glucan and to a lesser extent for lbg containing pasta. For all the other types of pastas, as Figure 2.6 shows, increasing amounts of DF did not have an effect on the surface properties. Moreover, the stickiness and adhesivness of pastas containing pea fibre or inulin were similar to those of control pasta. Higher values for these textural parameters were found for pastas containing guar gum (similar to what was found at 2.3.1.2), locust bean gum, or β-glucan ($p<0.001$).

The most obvious differences in comparison to the control pasta were shown by pastas containing β-glucan (as Hi Sol) at levels higher than 2.5% (on db), where the extremely high values recorded for these textural parameters would suggest a significant deterioration of product quality, making it unacceptable. The use of bamboo fibre or xanthan gum seem to have a positive impact on surface characteristics, leading to pastas less sticky than the control ($p<0.01$).

Figure 2.7. Elasticity of pasta samples as determined using the Texture Analyser



The results obtained for pasta elasticity/strength from the tensile test offer a similar picture of the effect of DFs on product structure. Increasing levels of DF lead to decreased elasticity (Table 2.8), indicating a weakening effect on the structure. Moreover, different types of DF behaved differently within pasta structure. For example, while the majority of them caused a slight decrease in pasta elasticity in comparison to the control, xanthan gum and lbg seemed to give extra strength to the structure, shown by increased elasticity values in comparison to the control (Table 2.8 and Figure 2.7). These results complement those obtained for pasta firmness/strength following a different type of test (compression test rather than extension test).

Generally speaking DF enriched pasta showed reasonably good textural characteristics, in many cases very similar to the control. Xanthan gum and lbg seemed to consolidate the strength of the structure (shown by increased firmness and elasticity values) which may be an important advantage as a selling point; for consumers this would mean that a DF enriched product would still have an *al dente* texture. On the other hand, from a technological point of view, slight overcooking would be expected to not dramatically change the final quality of the product; the texture will probably still be similar to the control.

The inclusion of β-glucan in pasta formulation seemed to alter the textural attributes of the final product considerably; at levels of β-glucan exceeding 2.5% (on db), cooked pasta became significantly less firm and stickier, the values indicating very poor quality for the final product from texture point of view. These were due to the incorporation of β-glucan into pasta in the form of HiSol, which is a barley flour containing 13.64% β-glucan. As mentioned in the paragraph 2.2.2 of 'Materials and methods', for β-glucan containing pasta semolina flour was replaced with the appropriate amount of HiSol, so that the content of β-

glucan added was 2.5, 5.0, 7.5 and 10% on db. Thus, a significant amount of HiSol was used in the formulations to replace semolina flour (ranging from 18.32% to 73.3%). Moreover the protein content of HiSol was only 6%, in comparison to 13.7% of semolina flour, and also the proteins of barley, although they have the ability to aggregate into large molecules, don't have the ability to form a gluten gel (Eliasson and Larsson, 1993). It is known that cooked pasta viscoelastic properties are given by the ability of semolina proteins to link together by disulphide, hydrogen and hydrophobic bonds to form a matrix. In addition, the differences in pasta cooking quality and texture can be almost completely explained by differences in the protein content and protein composition of semolina. It is therefore understandable why the inclusion of HiSol into the formulation led to a weaker structure of the cooked product and also to high cooking losses and increased surface stickiness. Similar findings have been mentioned by Marconi et al. (2000), and Knuckles et al. (1997). Knuckles et al. (1997) reported that a 40% replacement of semolina flour with a barley enriched flour (18.9% β-glucan) made the quality characteristics for pasta to fall below the acceptability range. Nevertheless, the texture and cooking properties could be improved by adding extra vital gluten or egg white.

2.3.2.3 Pasta digestibility as affected by DFs

The aspects related to pasta digestibility as monitored *in vitro* are summarised in ANOVA Table 2.9 and Figures 2.8 - 2.12.

Complete gelatinisation during cooking of pasta is extremely important in obtaining highly digestible starch. DSC can be used as a means to evaluate the existence of starch crystallites in the samples. DSC runs on cooked pasta samples found no peaks between 20°C and 100°C where the endothermic peaks are usually detected for starch (Table 2.9). This indicates that native starch was fully gelatinised (starch crystallites were all melted)

during cooking of pasta irrespective of the type of DF incorporated in the recipe or of the level of DF used (Table 2.9).

The *in vitro* procedure used at this stage to monitor starch digestibility offers the advantage that it more closely resembles the behaviour of foods as eaten; the preincubation with pepsin was performed because interactions with proteins have been suggested to reduce enzyme availability in pasta (Colonna et al., 1990). Nevertheless, the ranking of the products digested with or without pepsin was previously found to be similar (Granfeldt and Bjorck, 1991). The use of a dialysis system also has certain advantages as it helps taking into account the effect of the viscosity of digesta on the rate of appearance of reducing sugars (as it was suggested to happen *in vivo*). It is also important to highlight that previous research reported a close correlation between the rate of starch uptake *in vivo* as judged from the postprandial glucose response, and the rate of *in vitro* amylolysis when employing the dialysis system (Granfeldt et al., 1994) or not (Goni et al., 1997).

Table 2.9 presents several parameters calculated following *in vitro* digestion of cooked pasta samples (proportion of starch digested at different digestion times, hydrolysis indexes and predicted GIs), which are thought to be representative for understanding the behaviour of the products during digestion. Statistical analysis revealed that all parameters describing the rates of starch digestion were significantly affected by both the type and the levels of DF used in the formulation ($p<0.001$). Moreover, the interaction between the type of DF and the level used also had a significant effect on starch degradability (generally $p<0.01$, except for predicted GI* where the interaction was proved to not have a significant effect). The proportion of starch digested at different sampling times (150 min, 180 min, 300 min) was significantly lower for products containing DFs than for the control pasta, and it significantly decreased with increasing levels of DF present in the product ($p<0.001$, Table

2.9). It is worthwhile to note that after 150 min and 180 min of digestion, only the products with 10% (on db) added DF had significantly lower proportion of starch digested than the others. After 300 min of *in vitro* digestion the proportion of starch digested in both 7.5% and 10% DF containing samples was significantly lower than for samples with a lower level of DF addition (2.5% and 5% respectively).

Examining the results presented in Table 2.9 and Figures 2.8 and 2.9), it becomes evident that the starch from pastas containing DF (except for inulin) was digested at slower rates than the starch from the control (except pasta containing inulin). The lowest values for the proportion of digested starch were obtained when so called 'soluble DF' were used in the formulation (e.g. lbg, xanthan, β-glucan as HiSol, and guar gum). Interestingly, the use of bamboo fibre seemed to have a similar effect on the rate of starch digestion as the soluble DFs (Figures 2.8 and 2.9). The highest proportion of starch digested was obtained from the samples containing inulin (the values appear to be not significantly different from the control), followed by the pasta containing pea fibre (Table 2.9). These results are also illustrated by Figures 2.8 and 2.9.

A more complete picture about the differences in the rate and degree of starch hydrolysis is given by the area under the hydrolysis curve for each type of DF and level of addition. A useful indicator in this respect is the 'hydrolysis index' (HI) calculated (as previously mentioned in paragraph 2.2.2) as the ratio between the area under the hydrolysis curve (0-180 min) for a product and the reference white bread and expressed as a percentage. The results obtained are summarised in ANOVA Table 2.9 and Figure 2.10. As Table 2.9 indicates, both type of DF used and the level of usage significantly affected the HIs ($p < 0.001$ in all cases). In addition, an interaction between the type of DF and level of usage also appeared to have a significant effect on the HI values ($p<0.01$).

Table 2.9. ANOVA table summarising the digestion characteristics of cooked pasta and its thermal characteristics (the values represent means of al values at a given treatment level)

Sample	Enthalpy (J/g)	Starch digested after 150 min (%)	Starch digested after 180 min (%)	Starch digested after 300 min (%)	Hydrolysis Index (%)	Predicted GI* (Eq. 2.5)	Predicted GI** (Eq. 2.6)
Control	*0*	*12.9±0.3*	*19.2±0.2*	*35.8±2.7*	*42.9±3.5*	*45.0±3.3*	*44.8±2.7*
Effect of the type of DF							
Pea fibre	0	10.1^{b}	$15.6^{a,b}$	30.7^{b}	36.6^{b}	$39.2^{b,c}$	39.7^{b}
Inulin	0	12.8^{a}	17.8^{a}	33.9^{a}	46.6^{a}	$41.1^{a,b,c}$	48.4^{a}
Guar gum	0	$7.7^{c,e}$	$11.8^{c,d}$	$25.5^{c,d}$	22.3^{d}	$37.9^{b,c}$	27.4^{d}
Bamboo fibre	0	$8.3^{b,c,d}$	$12.4^{c,d}$	26.7^{c}	$24.6^{c,d}$	37.1^{c}	$29.4^{c,d}$
β-glucan (as Hi Sol)	0	$9.3^{b,c}$	$13.1^{c,d}$	$22.9^{d,e}$	$24.6^{c,d}$	42.9^{a}	$29.4^{c,d}$
Xanthan gum	0	$6.9^{d,e}$	10.2^{d}	21.3^{e}	$24.8^{c,d}$	$41.4^{a,b}$	$29.6^{c,d}$
Locust bean gum	0	$8.8^{b,c,d}$	$12.8^{c,d}$	$22.7^{d,e}$	27.9^{c}	37.0^{c}	32.3^{c}
SEM		0.45	0.59	0.69	1.06	0.93	0.91
Significance	NS	***	***	***	***	***	***
Effect of the level of DF addition							
2.5%	0	10.7^{A}	15.13^{A}	29.83^{A}	34.4^{A}	42.1^{A}	37.8^{A}
5.0%	0	9.4^{A}	14.33^{A}	28.08^{A}	30.4^{B}	$39.2^{B,C}$	34.4^{B}
7.5%	0	9.7^{A}	14.12^{A}	25.87^{B}	30.0^{B}	$40.1^{A,B,C}$	34.1^{B}
10.0%	0	6.6^{B}	10.01^{B}	21.20^{C}	23.7^{C}	37.2^{C}	28.6^{C}
SEM		0.34	0.446	0.525	0.80	0.70	0.69
Significance	NS	***	***	***	***	***	***
Effect of the interaction							
Type of DF*level addition	NS	**	**	**	***	NS	***

- within the same column, the values with the same letter are not significantly different; *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

Figure 2.8. Proportion of starch digested during *in vitro* digestion of cooked pasta: a) control; b) pea fibre; c) inulin; d) bamboo fibre; e) guar gum; f) β-glucan; g) LBG; h) xanthan gum

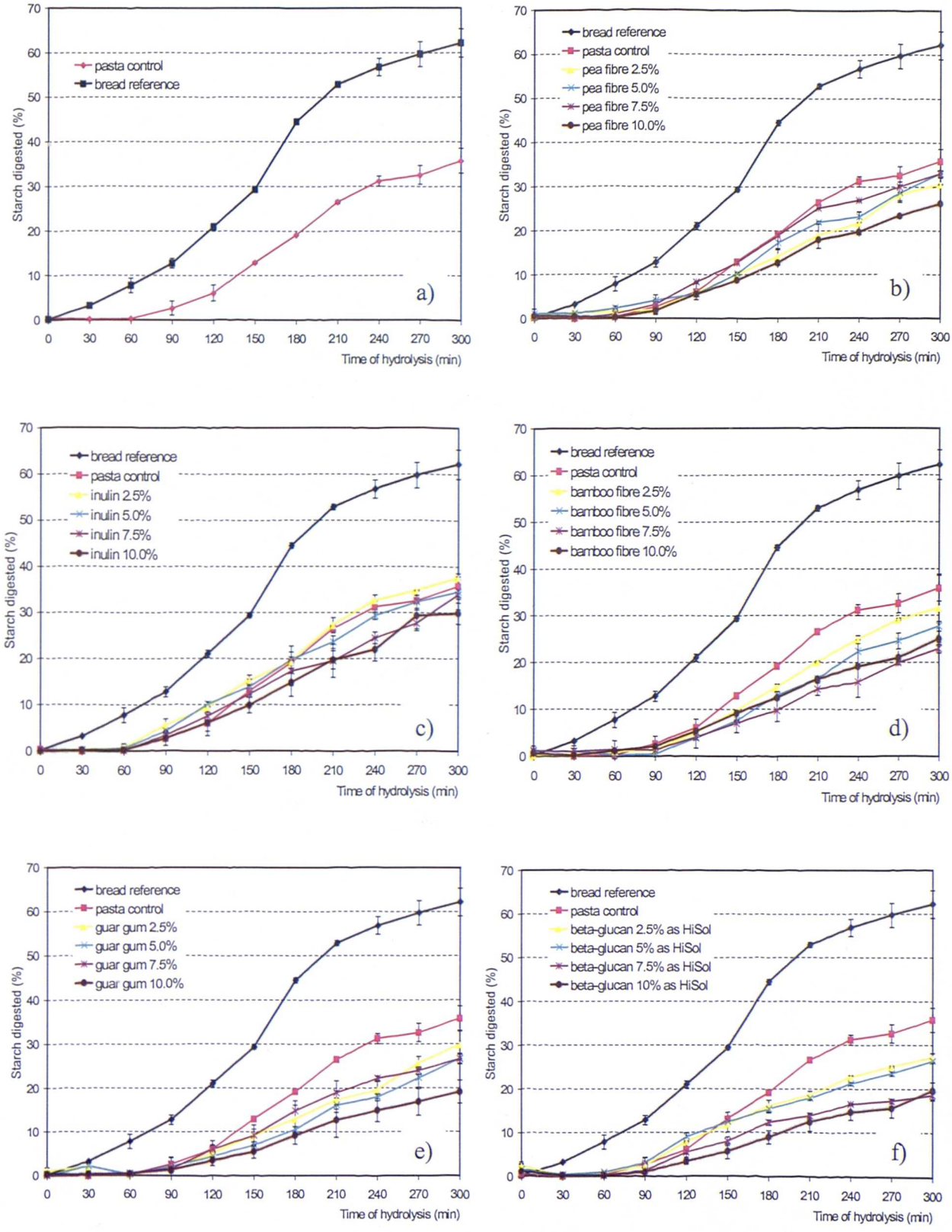
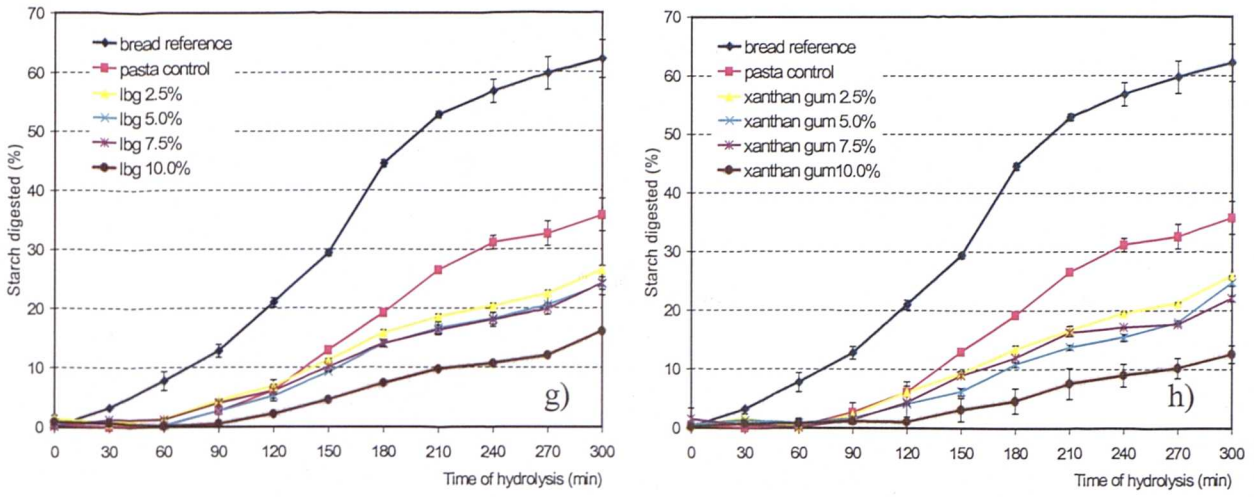


Figure 2.8. (continued)



Increasing levels of DF resulted in decreasing values of HI, indicating a retardation of starch digestion from pasta in comparison to the white reference bread (Table 2.9). For the control pasta the HI value was 42.9, while in the products containing DF, the HI values ranged from 34.4 to 23.7. Generally the values appears to be significantly smaller than the control. Noteworthy is the magnitude of the decrease in starch hydrolysis. HI indicates that on average the inclusion of DF in pasta formulation results in a reduction of the total amount of reducing sugars released with 19.8% (for 2.5% DF addition on db) up to 44.7% (for 10% on db DF).

The values presented in Table 2.9 and Figure 2.10 also indicate a strong effect of the type of DF on the HI values ($p<0.001$). The results suggests that except for inulin, the use of all the other DF chosen for this experiment generated reduced rates of starch hydrolysis, as shown by the smaller amounts of RSR in comparison to the control (HI=42.9).

Figure 2.9. Proportion of starch digested from cooked pasta at various *in vitro* digestion times: a - b) insoluble DF + inulin and soluble DF at 150 min; c- d) insoluble DF + inulin and soluble DF at 180 min e - f) insoluble DF + inulin and soluble DF at 300 min

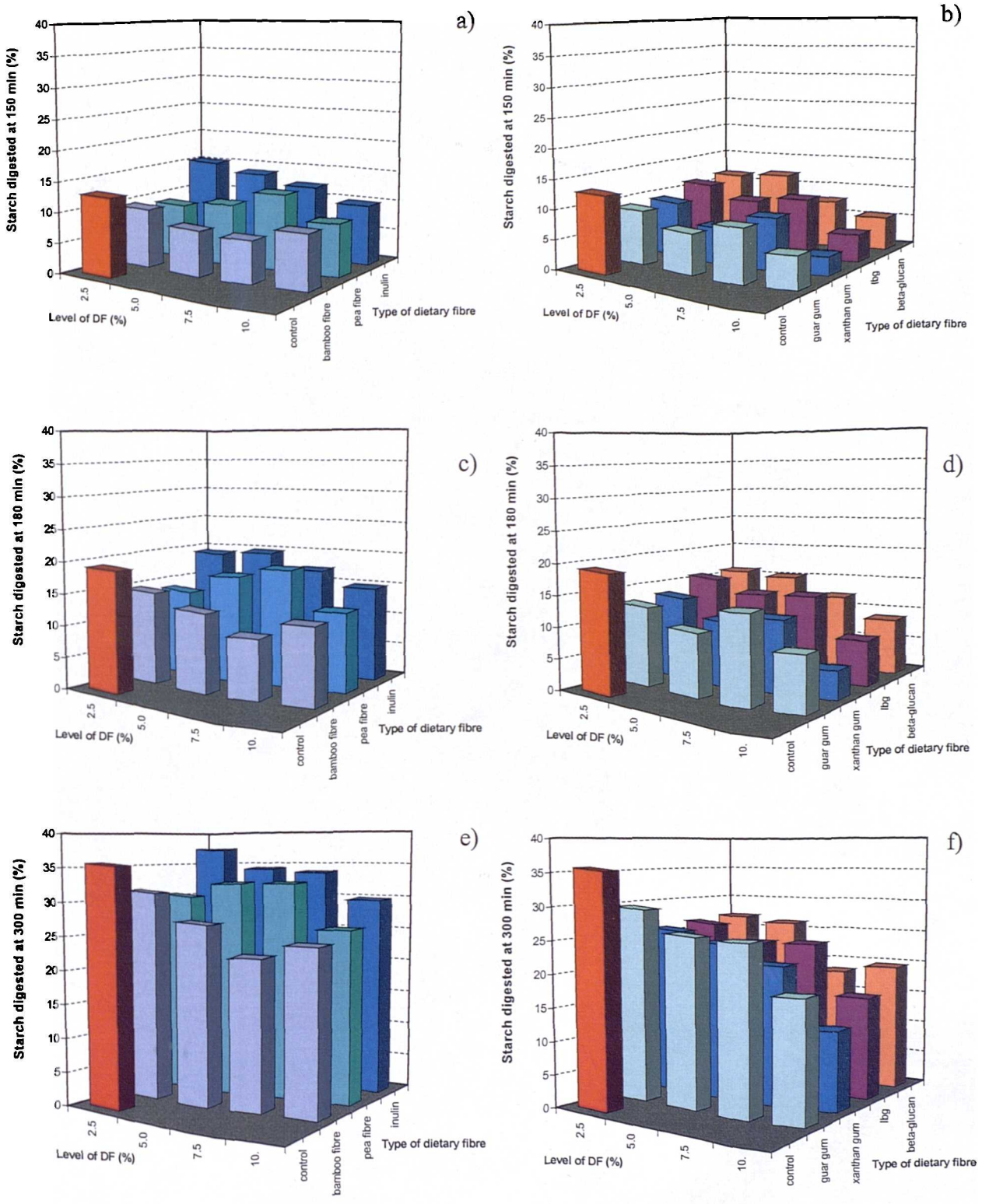
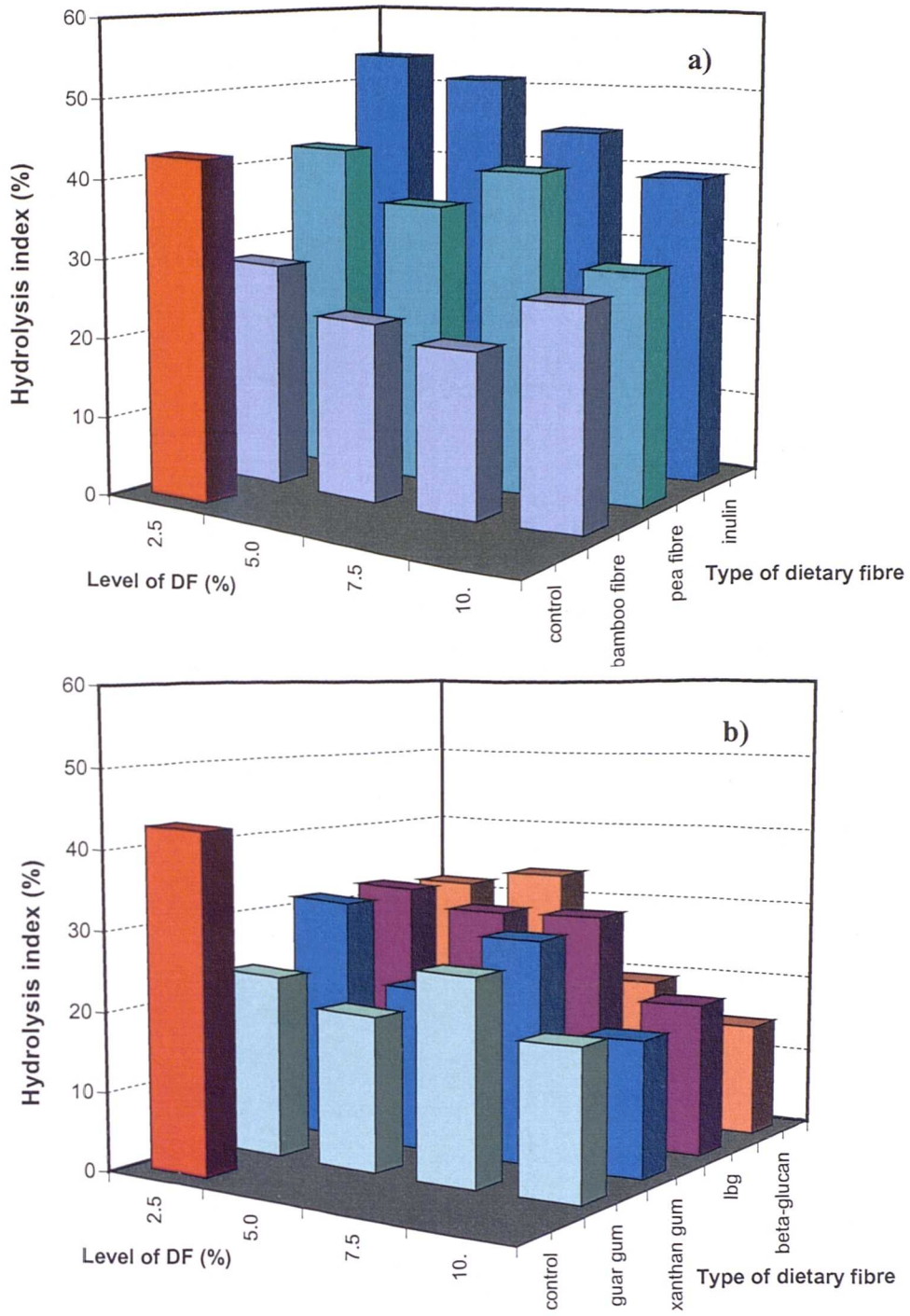


Figure 2.10. Hydrolysis indexes of cooked pasta (*based on RSR values): a) with insoluble DF + inulin; b) with soluble DF



The weakest effect was associated with the inclusion of pea fibre in the formulation, which decreased the HI by only 14% in comparison to the control. All the soluble fibres appeared to lead to relatively similar values for the HI, ranging from 22.3 (guar gum) to 27.9 (LBG), which are equivalent to decreases in the HI of 35 - 48% in comparison to the control pasta. The fact that different soluble DF used in the experiment produced relatively similar HI

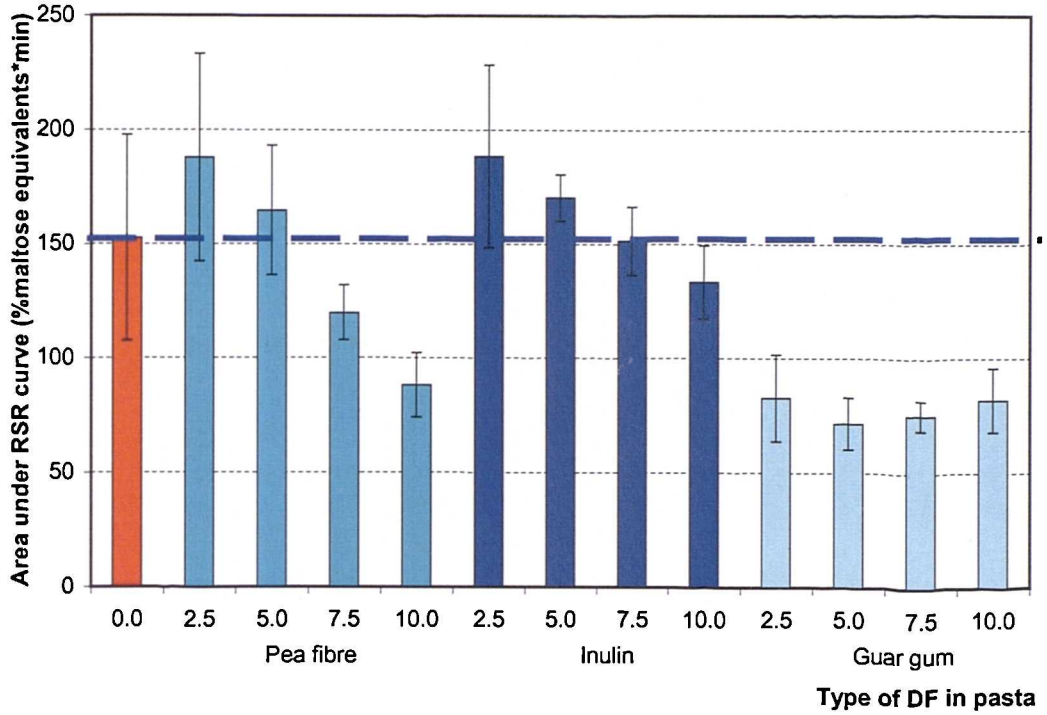
values (as shown in Table 2.9, $p>0.05$ according to Tukey's test) would imply that the mechanisms by which soluble DF affect starch digestion would be similar. However, this may not be completely true if the results for pasta containing bamboo fibre (an insoluble DF) are considered. Surprisingly, the overall HI of pastas containing bamboo fibre (24.6) was in the same range of values as those of pastas containing soluble DF (except for pasta containing inulin). This would suggest that bamboo fibre when incorporated in the structure of pasta could contribute as successfully as the soluble DF towards reducing starch digestibility.

The exception, as seen before in Figures 2.8 and 2.9 for the proportion of starch digested was made by inulin. The results indicate an overall increase in the proportion of starch digested associated with the presence of inulin in pasta structure (Table 2.9 and Figure 2.10a). However, at levels of 10% (on db), inulin appears to reduce the HI in comparison to the control (Figure 2.10). The HI values for pasta samples in relation to each type of DF and each level of addition are illustrated in Figure 2.10. They graphically show what was found out by statistical analysis: in all cases the strongest effect on decreasing the rate of starch degradation was obtained for 10% (on db) level of DF addition.

It is important to note that the results corresponding to pastas containing pea fibre, inulin and guar gum seem to confirm what was previously found in the first stage of the experiment. Since initially (the first stage of the experiment) the digestion was conducted for only 90 min, for comparison purposes it was natural to calculate the corresponding pasta the area under the hydrolysis curve between 0 and 90 min as well, as an indicator of the proportion of starch digested. The results are shown in Figure 2.11 and they indicate that on average, for the first 90 min of *in vitro* digestion, pea fibre and inulin appear to have no significant effect on the starch degradability (except for pea fibre at 10% db),

while guar gum significantly decreased the rate of starch digestion. These agree with the trend observed in the first stage of the experiment when a simpler *in vitro* method was used for pasta digestion.

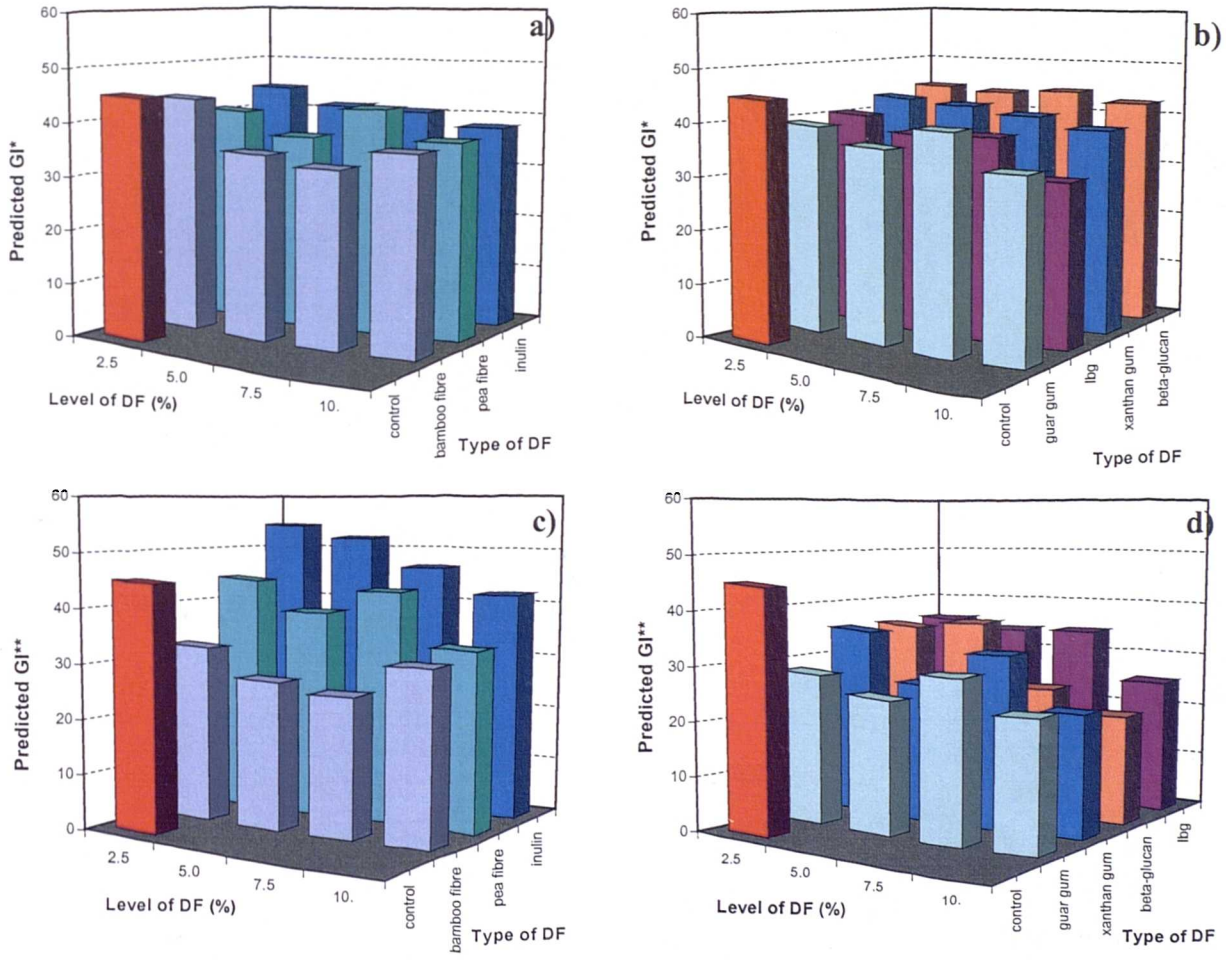
Figure 2.11. Area under starch hydrolysis curve (based on RSR, 0-90 min) calculated for pasta containing pea fibre, inulin and guar gum



The results obtained for the predicted GI** calculated according to equation 2.6 ($GI^{**}_{predicted}$=0.862HI+8.189) and summarised in Table 2.9 and Figure 2.12 c,d). According to this calculation the GI** of control pasta was 44.8, a value which is similar to the previously published data both *in vitro* and *in vivo* (as presented in Table 1.4, Chapter 1). Bearing in mind that these predicted GIs values are derived from the HI results, it is not surprising that the statistical analysis had similar outcomes: both type and level of DF addition significantly affected the predicted GI** of pasta containing DF ($p<0.001$ in both cases). Also the trends observed in the predicted GI** followed the same pattern as those of HI: GI** values decreased with increasing levels of DF in formulation. The predicted GIs** for pastas containing soluble DF were also comparable as values (ranging from 27

to 32), and all were significantly lower than the control (Table 2.9). As expected from the HI results, the smallest effect in decreasing the predicted GI** was related to the use of pea fibre (which resulted in an average GI** for pasta of 39), while inulin produced a small increase in the overall GI** (average value of 48) in comparison to the control. Nevertheless, this increase appeared to be not statistically significant. Pasta containing bamboo fibre had a predicted GI** of 29, which place is again within the range of products made with soluble DF. These results allow ranking of DF enriched pasta products in a descending order of predicted GI** as follows: (inulin, control)>pea fibre>(lbg, xanthan gum, β-glucan, bamboo fibre, guar gum).

Figure 2.12. Predicted glycaemic indexes of cooked pasta: a-b) GI* (Eq. 2.5) of pasta with insoluble DF and inulin and soluble DF (except inulin); c-d) GI** (Eq 2.6) of pasta with insoluble DF and inulin and soluble DF (except inulin)



The GI values of pasta were also calculated using the predictive equation 2.5 (paragraph 2.2) which takes into account not only the amount of sugars released (as proportion of those from white reference bread), but also the chemical composition of the products, and the diffusivity of the sugars produced through the dialysis tubings in the presence of sample. The calculations were used to generate the results presented in the ANOVA table (Table 2.9) and Figure 2.12 a-b) and they are labelled as GI*. The GI* for control pasta was 45, similar with the value resulted using equation 2.6, and which falls within the range of GI data previously published for this product (as presented in Table 1.4, Chapter 1). Statistical analysis indicated that GI* was significantly affected by the type of DF and the level of usage ($p<0.001$), as was found previously for GI** calculated using Equation 2.6. Also similar to what was found for GI** values, the GI* of DF containing pasta samples (including pasta containing inulin in this case) were on average lower than the GI* of control pasta, and they decreased with increasing levels of DF in the formulation.

The differences in the GI* values in relation to the type of DF used appeared to not follow the same pattern as observed earlier for GI**. The 'solubility' of DF did not result in a clear trend for GI* values as before; the lowest GI* pastas were those containing guar gum, lbg, bamboo fibre and pea fibre, while inulin, β-glucan (as HiSol) and xanthan gum produced significantly higher values. It is encouraging though that the two equations produced generally comparable GI.

2.3.2.4 The microstructure of pasta as affected by DF

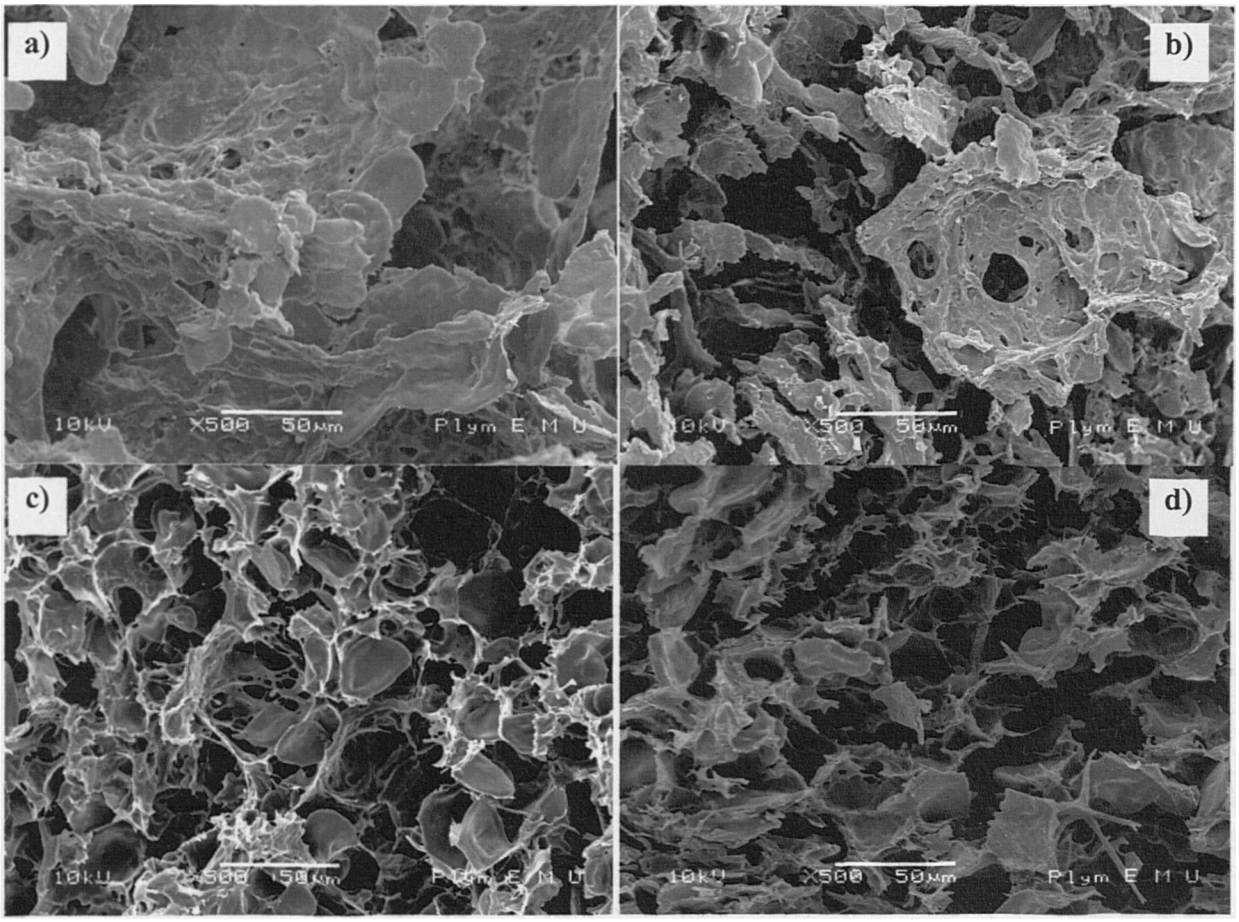
The internal structures of pastas before and after *in vitro* digestion for all types of DF used in the formulations at lowest (2.5%) and highest (10%) levels of addition are illustrated by the SEM micrographs presented in Figures 2.13 - 2.15.

Figure 2.13 presents the structure of white reference bread and control pasta before and after 300 min of *in vitro* digestion. The differences in structure of the two products is evident and it is in agreement with what has been previously reported by several studies (Cunin et al., 1995; Brennan et al., 1996; Hug-Iten et al., 1999). Control bread (Figure 2.13a) is characterised by an open structure with large holes, which correspond to the air bubbles entrapped in the structure during proofing/baking. The starch granules visible in this structure appear to retain their granular identity although they are swollen and elongated, and most of them seem to be aligned parallel to the pore surface. They also appear to be fused with neighbouring granules as part of a continuous network forming the crumb known to be made from coagulated (or denatured gluten) and leached amylose (Hug-Iten et al., 1999). After 300 min of digestion, the structure of the white bread (Figure 2.13b) changed: the integrity of the crumb was destroyed, and no intact starch granules appear to exist, indicating a high proportion of starch having been digested.

In contrast, the internal structure of control pasta was notably different, and it appears similar to the one described by Cunin et al. (1995). The first impression is of a denser structure in comparison to that of bread, formed by starch granules (both A and B types) surrounded by a continuous and dense protein network (Figure 2.13c). In the centre part of the pasta starch granules preserved their form due to a limited water absorption in the centre of the strand and were significantly less swollen than the granules present in bread structure. Amylose leaching appeared to not occur in the centre of the strand; thus starch granules seemed independent from one another and not connected through a gel formed by leached amylose. It was shown by Cunin et al. (1995) that in pasta, amylose diffuses primarily into the centre of starch granule due to outer layers of the granule acting as a barrier. After 300 min of *in vitro* digestion the internal structure of pasta seems altered as well, but not at the same extent as that of white bread. In digested pasta there were still

starch granules visible either intact or partially damaged (Figure 2.13d). This indicates a lower proportion of starch being degraded during the same period of digestion and it well visualises what was found during the *in vitro* digestion of pasta (Figure 2.8): lower digestibility of starch in pasta as compared to white bread.

Figure 2.13. SEM micrographs (x 500) for white reference bread and cooked pasta before and after 300 min of *in vitro* digestion: a) bread control; b) bread control digested; c) pasta control; d) pasta control digested



Pastas containing pea fibre, inulin or bamboo fibre (as shown in Figures 2.14 a,c,e,g,i,k) appeared to have similar internal structures to the control pasta, irrespective of the level of addition (2.5% or 10%). A matrix formed from gluten and DF surrounded starch granules, which were again less swollen than in bread, and presenting a well maintained shape possibly due to limited water available. Moreover, on average the starch granules in pasta containing

containing these DFs seemed to be even less swollen than in control pasta (especially at 10% addition). In control pasta the average diameter of the 'A starch' granules appeared to be higher than 25μm, while especially in pasta containing 10% pea fibre or bamboo fibre, the average size of starch granules seemed to be lower than this value. The explanation could be the competition for the available water between starch and the DF present in the system. It is also worthwhile to note that pasta containing bamboo fibre was characterised by a denser internal structure than the control or pastas containing inulin or pea fibre; the matrix which encapsulates starch granules appeared to have thicker walls and to tighter surround the starch granules.

The corresponding digested samples (Figures 2.14 b,d,f,h,j,l) appeared to be different from the digested control pasta. Generally, all micrographs except for those of pasta containing pea fibre and inulin at 2.5%, showed starch granules still undigested or partially digested after 300 min of *in vitro* digestion; moreover, the average size of these granules was similar to those in the corresponding pasta prior to digestion, indicating that no further starch swelling occurred during digestion. The highest proportion of undigested starch after 300 min of hydrolysis seemed to be related to the pasta containing bamboo fibre at 10% addition as shown by the micrograph presented in Figure 2.14l. In this case, as well as in pasta containing 10% pea fibre (Figure 2.14d), the proportion of the granules that remained intact after digestion appears to be significantly higher than in digested control pasta (Figure 2.13d).

Figure 2.14. SEM micrographs (x 500) for cooked pasta containing insoluble DF and inulin before and after 300 min of *in vitro* digestion: a) pea fibre 2.5%; b) pea fibre 2.5% - digested; c) pea fibre 10%; d) pea fibre 10% - digested; e) inulin 2.5%; f) inulin 2.5% - digested; g) inulin 10%; h) inulin 10% - digested; i) bamboo fibre 2.5%; j) bamboo fibre 2.5% - digested; k) bamboo fibre 10%; l) bamboo fibre 10% - digested

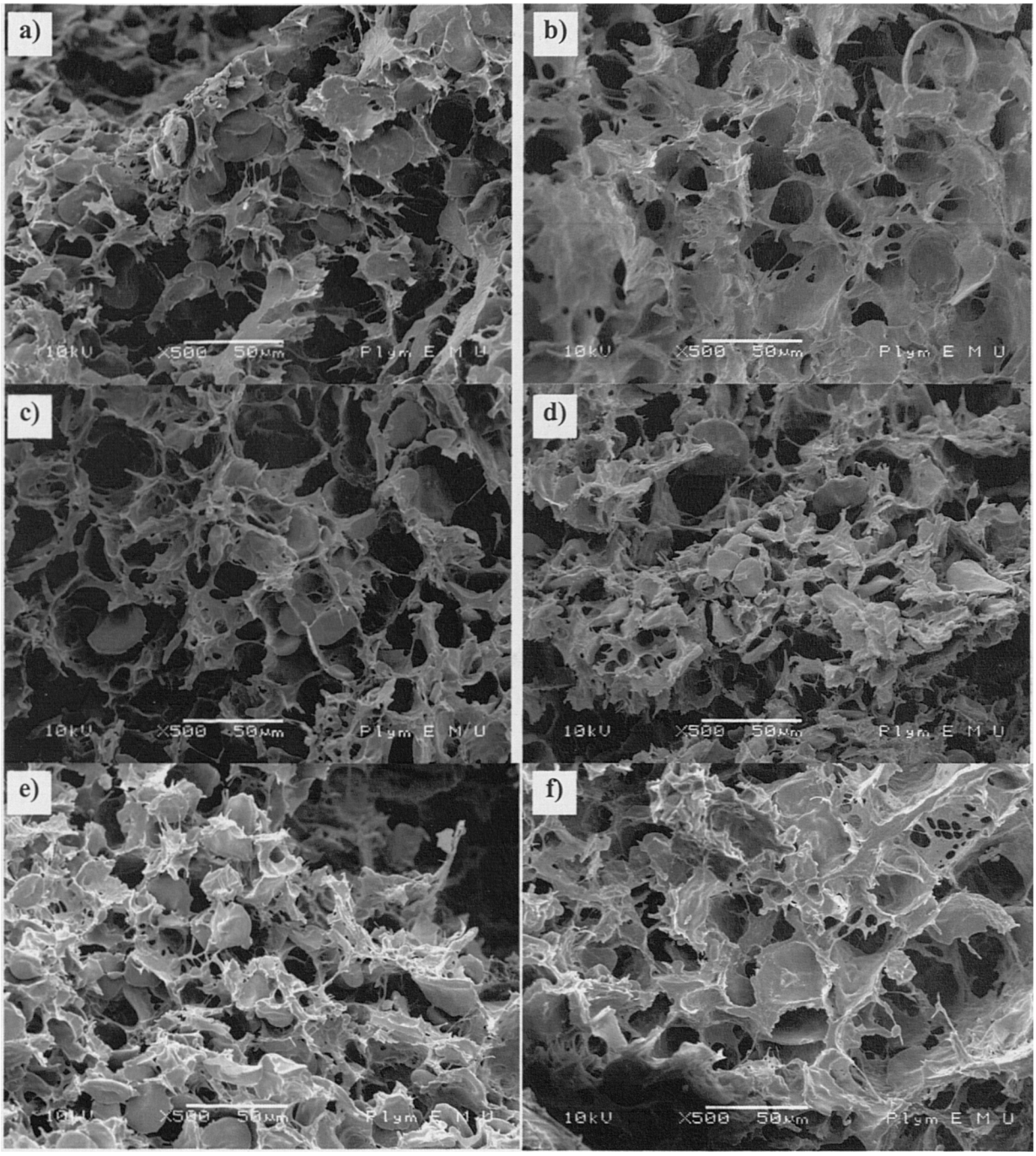


Figure 2.14. (continued)

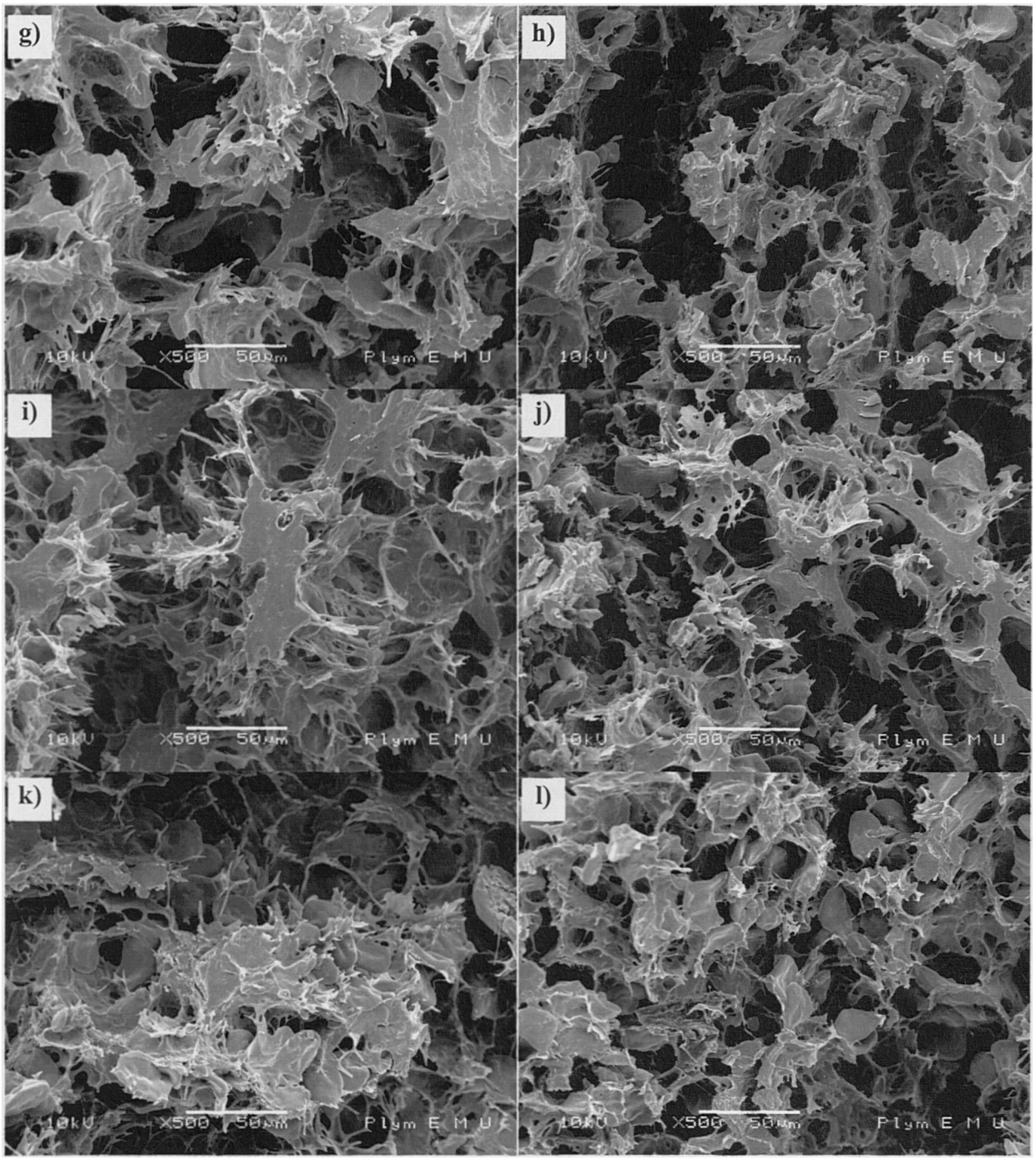


Figure 2.15. SEM micrographs (x 500) for cooked pasta containing soluble DF (except for inulin) before and after 300 min *in vitro* digestion: a) guar gum 2.5%; b) guar gum 2.5% - digested; c) guar gum 10%; d) guar gum 10% - digested; e) β-glucan (as HiSol) 2.5%; f) β-glucan (as HiSol) 2.5% - digested; g) β-glucan (as HiSol) 10%; h) β-glucan (as HiSol) 10% - digested; i) LBG 2.5%; j) LBG 2.5% - digested; k) LBG 10%; l) LBG 10% - digested; m) xanthan gum 2.5%; n) xanthan gum 2.5% - digested; o) xanthan gum 10%; p) xanthan gum 10% - digested

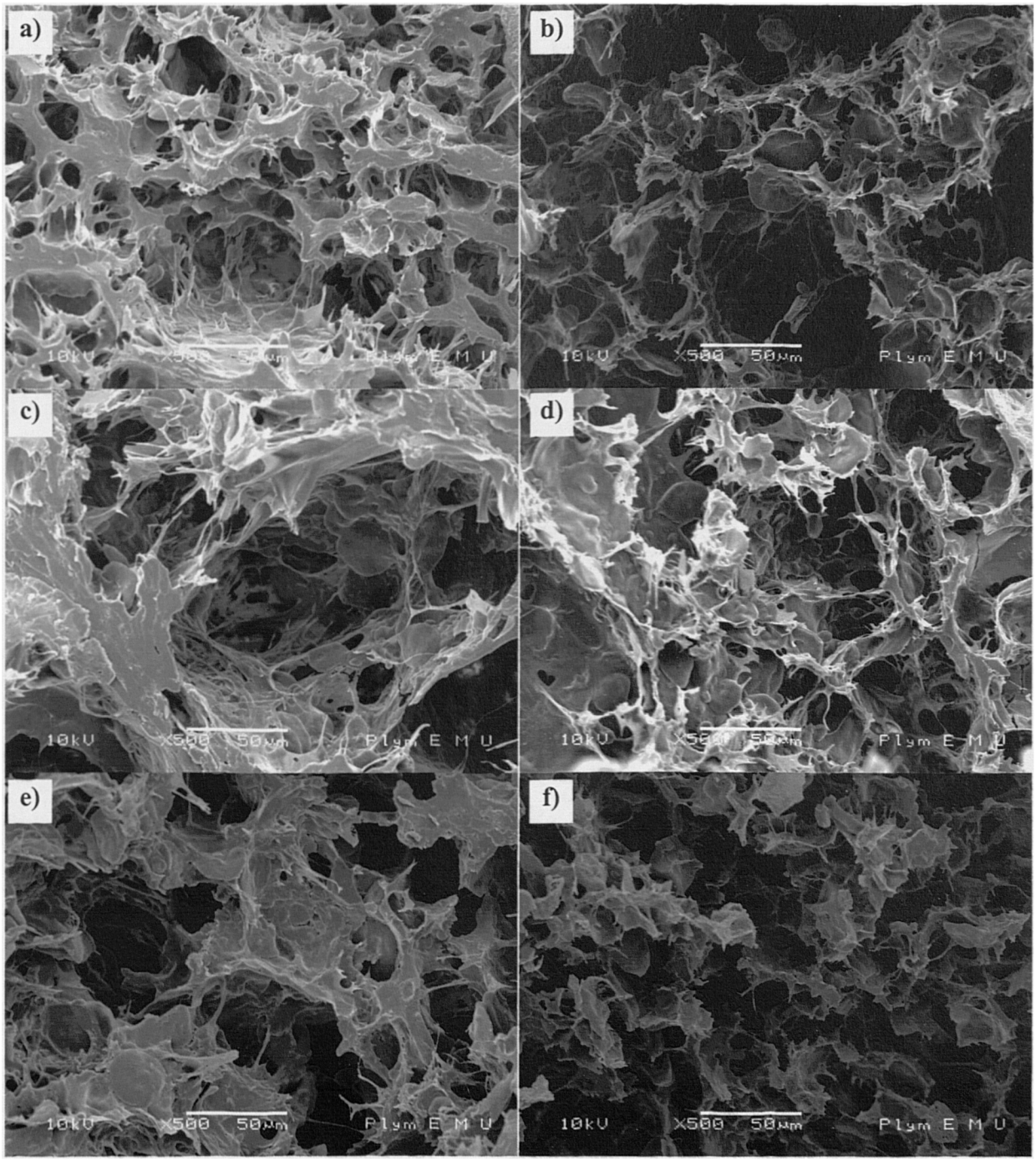


Figure 2.15. (continued)

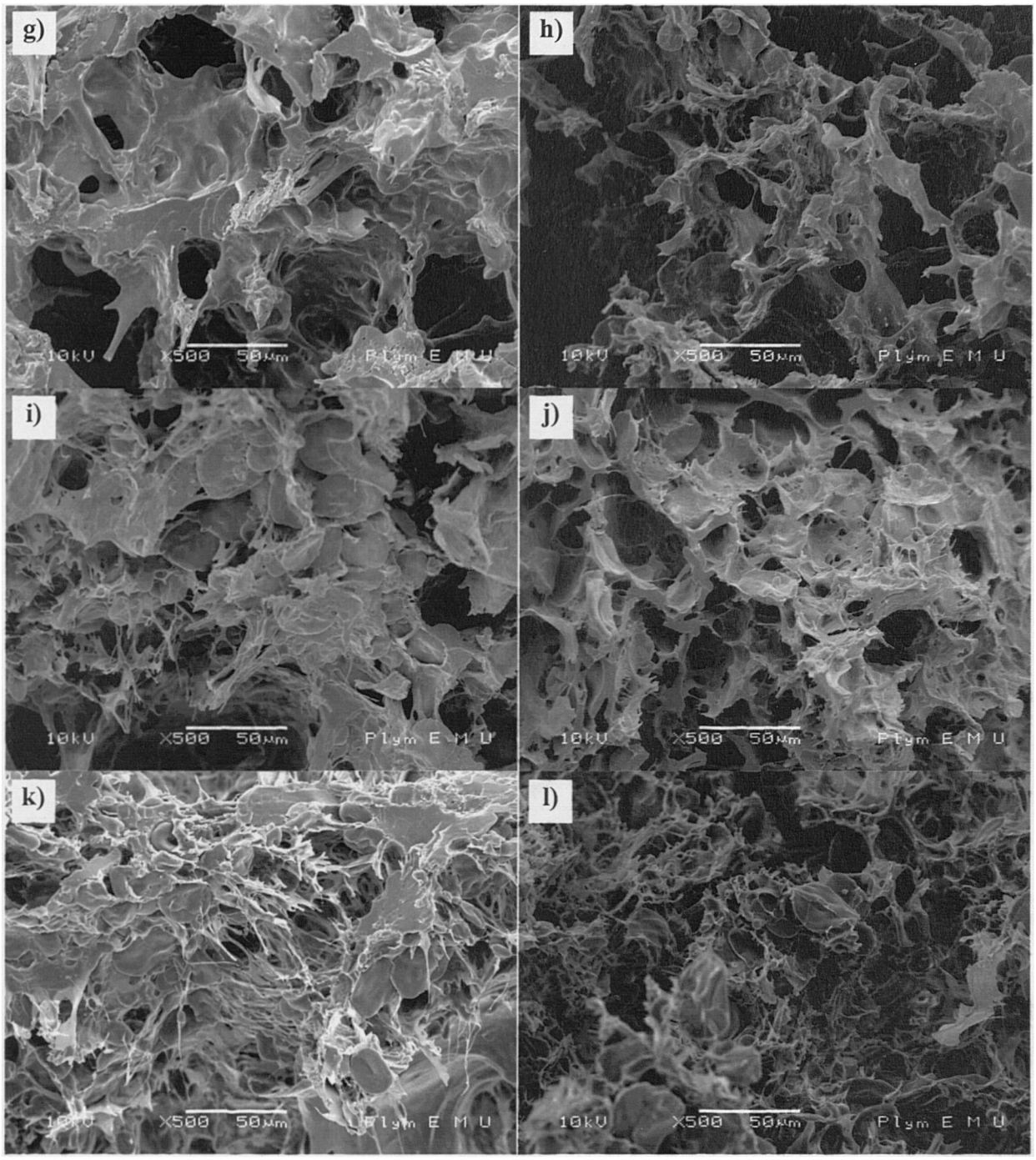
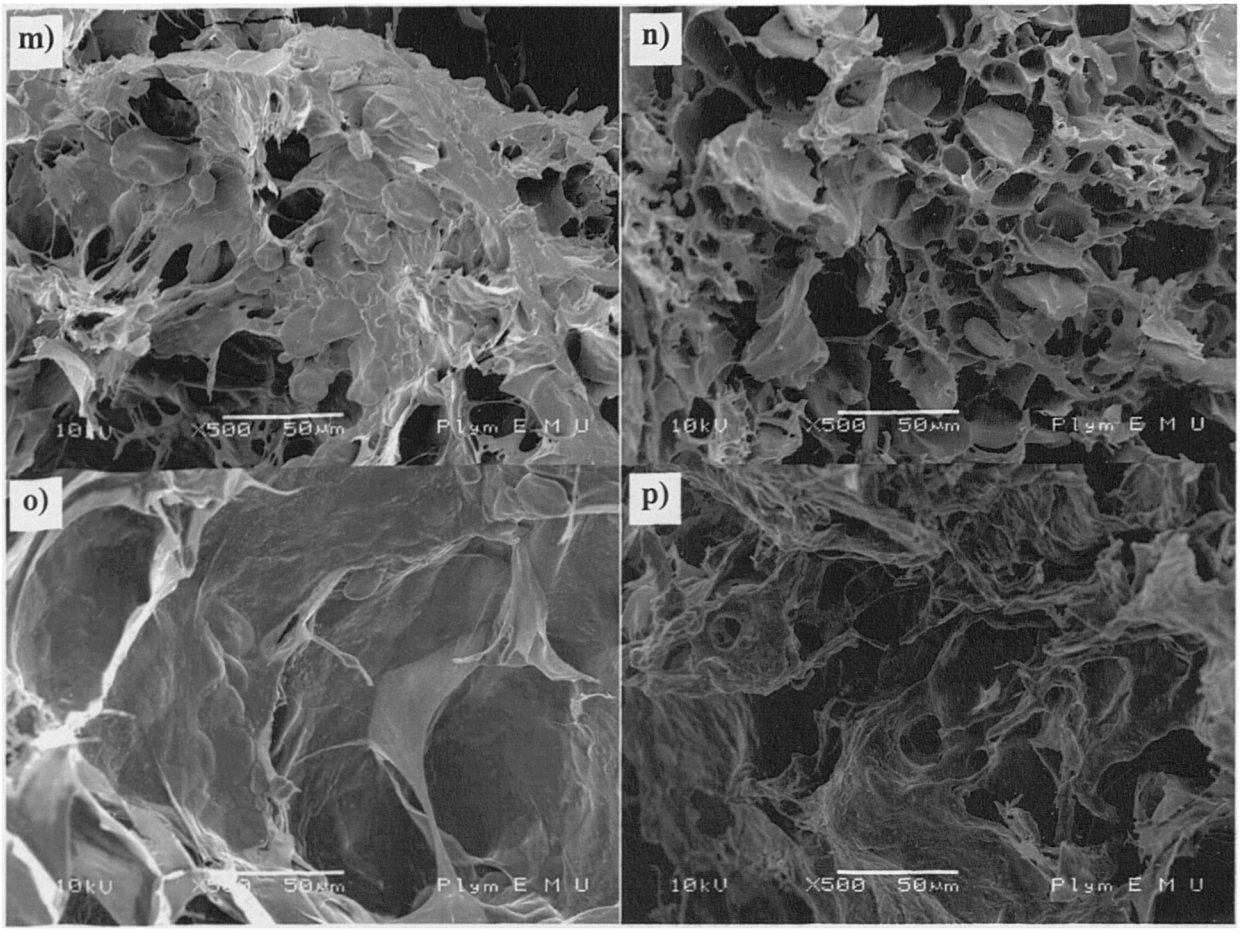


Figure 2.15. (continued)



These SEM micrographs of digested pasta are in agreement with the parameters resulted following *in vitro* digestion (%starch digested, HI and GI), and provide a visualisation of what was concluded following these calculations. They indicate that the proportion of starch digested from pasta containing inulin is similar to the control, while the incorporation of pea fibre or bamboo fibre at 10% considerably reduces the percentage of starch digested; this confirms the results presented in Figures 2.9 e and 2.10 a. At a level of 2.5%, pea fibre does not influence starch digestibility (Figure 2.14 b), but bamboo fibre seems to be effective in reducing the proportion of starch digested, and these are in agreement with the HI for these products illustrated in Figure 2.10 a, and predicted GI (Figure 2.12 c).

Figure 2.15 presents the internal structure of cooked pasta containing soluble DF, both before (Figures 2.15 a, c, e, g, i, k, m, o), and after *in vitro* digestion (Figures 2.15 b, d, f, h, j, l, n, p). These micrographs indicate a different arrangement in comparison to the control or pastas containing insoluble DF. While in those cases the wheat starch granules were distinct within the protein matrix (DF - gluten matrix respectively), in pastas containing soluble DF the starch granules appear to be coated in a mucilaginous looking layer. A similar observation was made following a research study investigating the effect of guar gum on the digestibility of bread and where the coating layer was identified as being formed by guar gum (Brennan et al., 1996). In addition, although starch granules within samples containing soluble DFs appear to maintain their shape and be distinct from one another, they became part of the matrix, which forms pasta structure, and they do not seem to act as fillers as in control or insoluble DF containing pasta. Consequently the thickness of the matrix walls appears to be higher on average than within control pasta. These observations were especially evident for samples containing 10% soluble DF addition. In these cases it could also be noticed that the average size of the starch granules was smaller than those in control pasta, indicating a lower degree of swelling (Figures 2.15 a, c, e, g, i, k, m, o). This again can be related to reduced water availability due to the competition for water between starch and soluble DFs.

What is interesting to note and it is shown so obviously by the micrographs (Figures 2.15 b, d, f, h, j, l, n, p) is the relatively high proportion of undigested starch remaining at the end of 300min of *in vitro* digestion in comparison to control pasta. Moreover, the overall initial structure of pasta appears to be relatively preserved, still presenting starch granules as part of the global structure-forming matrix, and still embedded in the mucilaginous coating. This high proportion of undigested or partially digested starch granules is even

more remarkable when regarded in conjunction with the initial formulation: pastas containing DF have lower starch content in comparison to the control since proportions of semolina were replaced by corresponding proportions of DF. Consequently, these micrographs seem consistent with data showing a significant decrease in starch digestibility in formulations containing soluble DFs (except for inulin) in comparison to the control and also with the majority of pasta containing insoluble DFs (except for bamboo fibre). Of likely importance, is that soluble DFs appear to be effective even at low levels of addition (2.5% on db) as shown by the micrographs (Figures 2.15 b, f, k, o), while the majority of insoluble DFs have no effect at similar level (Figures 2.14 b, f, j). The information gathered from the micrographs is in agreement with the parameters calculated following the *in vitro* digestion: the HI (Figure 2.10b) and predicted GI (Figure 2.12d), all indicating a reduction in starch digestibility associated with the presence of soluble DF in pasta formulation.

As an overall conclusion, the results of all these experiments showed a reduced digestibility of pasta products in comparison to a standard white bread (which is in agreement with previous research). Moreover, as demonstrated by the *in vitro* digestion parameters and also by the SEM micrographs, starch digestibility and predicted GIs were even lower for the products incorporating DFs; the exception was made by pasta containing inulin and insoluble DFs at low levels of addition.

The slower rates of sugars released/starch digested in pasta products in comparison to white bread were reported previously by several researchers and the explanation was usually related to the product structure. It is known that starch accessibility to digestive

enzymes has a strong influence on its digestibility, thus modulating the postprandial glucose response (Brighenti et al., 1995). In highly porous products such as bread the crumb structure created due to entrapment of gas bubbles that expand during baking greatly enhances the surface exposed to enzyme activity. Moreover, as illustrated in the SEM micrographs, starch granules within the bread are highly swollen and aligned parallel to the pores surface, making them easy targets for amylolysis.

In contrast, due to the extrusion process pasta shows a compact structure, with a dense firm texture formed by starch granules trapped within the protein matrix, and this is in agreement with previous research (Cunin et al., 1995; Fardet et al., 1998). This internal structure may restrict the susceptibility of starch to α-amylase, with a direct effect on its resistance to digestion. The importance of food structure, and of the ratio between surface area to mass have been highlighted by studies comparing the glycaemic responses of ground spaghetti, spaghetti, bread or native starch. It was previously found that the GI of ground spaghetti was significantly higher than of spaghetti, but still lower than that of white bread (Granfeldt and Bjorck, 1991) or purified starch (Colonna et al., 1990). Thus, although food structure plays a major role in the glycaemic response, there must be other factors involved.

In this context it is worthwhile to look into more detail at the formation of the internal structure of pasta during processing and cooking. It has been suggested that the surface of the freshly extruded pasta is formed by a continuous protein film, while the inner part is a compact structure of starch granules embedded in a continuous protein matrix aligned in layers parallel to the protein film (Resmini and Pagani, 1983). Thus pasta structure has been described as composed of a starch reduced surface layer, and a 'starch filled' central layer. The explanation for this structure given by Tolstoguzov (2003) is based on the 'ball-

bearing' effect starch has in flowing pasta dough that can decrease the friction between adjacent gluten layers flowing at different rates, and contribute to the formation of the three dimensional structure of pasta. Moreover, Tolstoguzov (2003) concluded that during extrusion, the revolving starch granules will move towards the central layers of the flowing dough which are moving faster and therefore are characterised by the least pressure (according to Bernoulli's principal).

During cooking, major structural changes occur in pasta due to starch gelatinisation and protein denaturation, with direct impact on the final texture. Both transformations occur at the same temperature and moisture level and they are competitive and antagonistic (Cunin et al., 1995). Microscopic observations showed that in cooked pasta starch changes vary from the outer layer to the inner layer. In the outer zone greatly swollen starch and a starchy filamentous network were observed while in the central zone starch granules showed a limited degree of swelling although they were fully gelatinised (Cunin et al., 1995).

Taking into account that restricted swelling of starch during cooking of the pasta also limits the α-amylase susceptibility of starch (Tester and Sommerville, 2001), these structural characteristics of pasta could further clarify the reduced starch digestibility in pasta in comparison to starch in white bread.

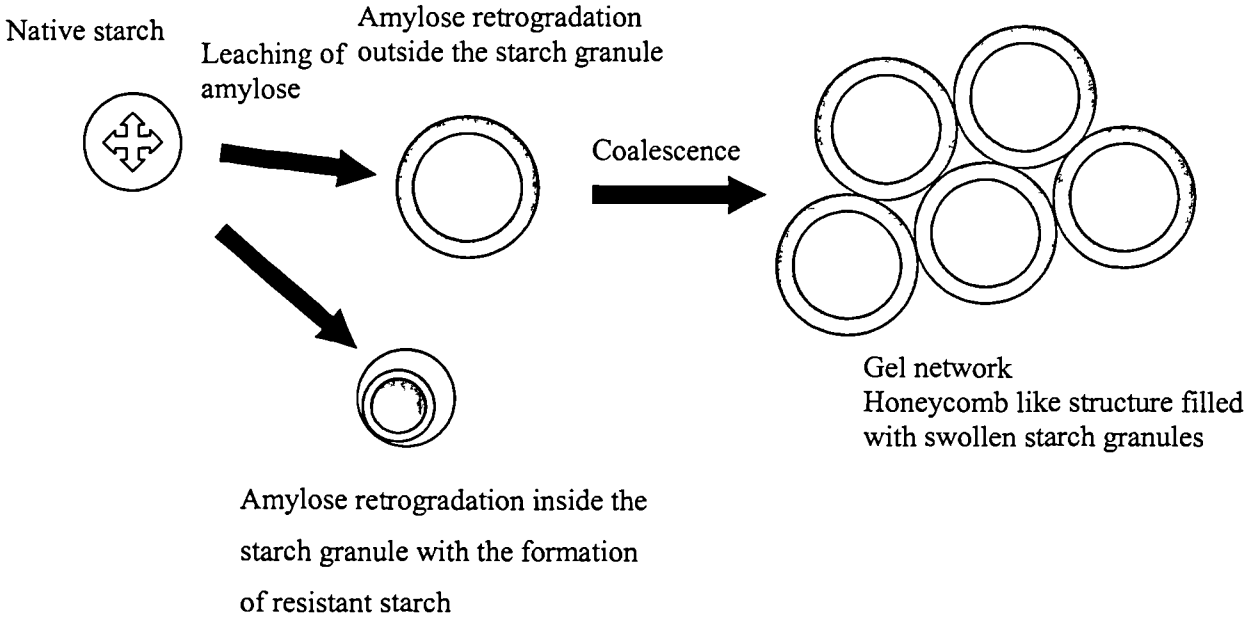
The relationship between starch swelling and its digestibility could be associated to the thermodynamic incompatibility that exists between the two components of the starch: amylose and amylopectin (Tolstoguzov, 2003) and their behaviour during heat treatment. Bowler et al. (1980) indicated that during heating in excess of water, the swelling of wheat starch granules is a combination of two stages: radial expansion which form a flattened

disc, followed by tangential expansion with the formation of puckered (wrinkles, creased) granules with amylose leaching out from the granules. As suggested by Tolstoguzov (2003) this is a consequence of the high local concentration and high excluded volume of amylopectin molecules inside the swollen granules. The rate of leaching is known to decrease with increasing concentration of starch dispersion, and since the rate of leaching could be either greater or less than the rate of amylose retrogradation, amylose can retrograde either between or within the starch granules as illustrated in Figure 2.16. Therefore a possibility exists that thin layers of leached and retrograded amylose could encapsulate swollen starch granules, and through coalescence could form a three-dimensional gel, with the swollen granules acting as an active filler; this is the case for the starch granules situated in the outer zone of the pasta strand. At the centre of pasta strand the increased concentration of the macromolecules leads to a reduction of their excluded volume and reduction or stopping of amylose leaching outside the granules; hence, an increase in starch concentration would promote self-association, gelation and crystallisation (Tolstoguzov, 1997) with the formation of resistant starch (Figure 2.16). This theory is supported by previous microscopy studies on cooked pasta which have reported that the starch granules in the central zones were stained dark blue indicating that amylose enrichment occurred in the centre of the granules without any external leaching (Cunin et al., 1995).

The study conducted by Tester and Sommerville (2001) has shown that there is a strong correlation between the swelling of the starch and the extent of amylolysis (associated with increased porosity of the granule and generation of amorphous material, more readily digestible than starch crystallites, or because amylose facilitates α-amylase access to the swollen starch). This finding can be extrapolated to the case of pasta for the starch found in

the interior of the structure; thus, a high concentration of starch which is gelatinised as shown by DSC (Table 2.9), but with low swelling, and no apparent leaching of amylose (Figure 2.13) could be related to decreased digestibility of pasta in comparison with bread (Table 2.9 and Figures 2.9 and 2.11).

Figure 2.16. Schematic representation of changes occurring in starch during heating in presence of water ((from Tolstoguzov, (2003))



However, the results presented earlier in this chapter on the digestion characteristics of pastas containing DFs (Table 2.9, Figures 2.9, 2.11, 2.13) indicate an even further decrease of starch digestibility in comparison with white bread and control pasta. This in turn suggests an influence due to the presence of DFs in the formulation. The strongest effect was associated with soluble DFs as well as bamboo fibre.

The results obtained for soluble fibres confirm what was previously reported by Fardet et al. (1999) (7% soluble fibre led to reduced starch digestibility in pasta), and Brennan et al.

(1996) (guar gum decreased the rate of starch digestibility of wheat bread in comparison with a control bread). The authors suggested that wheat starch granules were coated with a layer of guar galactomannan which would act as a physical barrier during starch digestion protecting the starch granule from enzyme attack (Brennan et al., 1996). This mucilaginous layer formed by the soluble DFs around the starch granules was also seen in the present study (micrographs presented in Figure 2.15 for both cooked and cooked and digested pasta) and it was related to decreased rate of starch digestion.

The formation of a layer of polysaccharide coating the starch granules is also supported by the theory of thermodynamic incompatibility typical for bio-polymers (Tolstoguzov, 2003). According to this theory, the macromolecules show a preference to be surrounded by their own type in mixed solution. Consequently the self-association is intensified in the presence of other macromolecules. Taking the case of pasta dough, gluten and starch are thermodynamically incompatible, forming separate continuous aqueous phases: the first is a concentrated phase of gluten proteins and the second a viscous mixed solution of polysaccharides (soluble pentosans and starch) containing most of the soluble proteins. When DF are included in the formulation they also may lead to phase separation due to thermodynamic incompatibility (Closs et al., 1999) as explained by Tolstoguzov (2003) for addition of guar gum. In the case of soluble DF this phase separation is likely to be accompanied by encapsulation of starchy phase by soluble DF enriched phase, interfering with granule swelling and stopping/reducing amylose leaching from the starch granule. This would result in an increase of macromolecules concentration inside the starch granules, and consequently an increase in the rate and degree of starch retrogradation and thus formation of resistant starch. Thus, besides the starch granules situated in the central part of pasta strand showing reduced swelling and increased rates of starch retrogradation

(as previously discussed), more starch granules located towards the outer part of pasta strand may be restricted to swell and to leach amylose (due to the coating layer formed by soluble DF). Moreover, the presence of soluble DF may restrict even further the swelling of starch granules situated in the central zone, as suggested by the micrographs presented in Figure 2.15 and discussed previously, making them even less accessible to the α-amylase attack. These could explain lower starch digestibility in pastas containing soluble DF in comparison to control pasta.

Incomplete gelatinisation of starch has also been suggested in the past to be associated with a reduction in the glycaemic response of starchy foods. However, in this study this option could be ruled out as the reason for lower digestibility of pasta containing certain DFs, since the DSC tests conducted on cooked pasta showed no endothermic peak in the region 55-80°C (which would correspond to melting of the starch crystallites).

The magnitude of the effect of DF on starch digestibility can be better understood when the percentage of proteins present in pasta formulation is taken into consideration. It is known that the wheat proteins form a network, which constitutes the backbone of the pasta structure and closely entraps the gelatinised and partly swollen starch granules (Cunin et al., 1995). Previous studies on pasta digestibility have found that the protein network induced a retarded hydrolysis of starch but did not constitute a total physical barrier to α-amylase (Fardet et al., 1998). Enrichment of the pasta formulation with gluten resulted in lower rates of sugars released, which was explained by an increased degree of starch encapsulation and increase in pasta diameter due to protein enrichment, therefore the path to reach starch in the pasta structure has been lengthened (Fardet et al., 1999). A reduction in the protein content of pasta would be expected to result in increased rates of starch degradation accompanied by increased rates of sugars released. Hence, within this

experiment from the values presented in Table 2.7 showing the protein content of DF enriched pasta, it would be expected a higher rate of sugars released in pasta containing DF (due to lower protein content in comparison to the control). However, the parameters characterising starch hydrolysis (Table 2.9 and Figures 2.10, 2.11) suggests that DFs not only compensate for reduced levels of proteins in the formulation, but they reduce even further the degree of starch degradability.

The internal structure of pastas containing insoluble DF or inulin do not show the coating layer covering starch granules which is so characteristic for pasta containing soluble DF. Instead, the internal structure is closer to that of control pasta; the differences appear to be in the degree of swelling of starch granules, which seems to be reduced in comparison to the control, and also in the increased thickness of cell walls surrounding starch granules (Figure 2.14). These observed differences may also contribute to the lower rates of starch digestion (as indicated by smaller proportions of starch digested, smaller HI and predicted GI in comparison to control pasta - Table 2.9) especially at the high level of addition (10% db). The explanation could also partially rely on thermodynamic incompatibility between the DF and starch. Similar to soluble DF, insoluble DF and inulin are thermodynamically incompatible with starch exhibiting a preference to be surrounded by their own type, and although they do not form a coating layer for the starch granules, they seem to aggregate in structures characterised by thicker cell walls in comparison to the control. This, in addition to their highly hydrophilic character, would affect the degree of starch swelling (as seen in Figure 2.14) and rate of amylose leaching (starch granules in Figure 2.14 preserved their shape and no apparent leached amylose can be seen within the structures) with direct effect on the rate on starch digested. Since insoluble DFs were seen to not form a coating layer, it

was to be expected that they would not be as effective as soluble DFs in lowering the degree of starch digestibility.

The effect of bamboo fibre was surprising and unpredicted: although it is an insoluble fibre (cellulose) its use resulted in rates of starch digested similar to those of soluble DFs. This may be due to the internal structure of pasta formed following the use of bamboo fibre. As discussed previously, it appears that pasta containing bamboo fibre have a denser structure in comparison to the control or pasta containing pea fibre, which possibly could retard the action of α-amylase. Another exception was shown by the use of inulin; although it is a soluble fibre, its effect on pasta structure and starch digestibility was minimal especially during the first 180 min of digestion and levels of addition less than 10% (Figure 2.8c). At high level of addition (10% db) it resembles more the effect of pea fibre (insoluble DF). Nevertheless, the use of inulin at levels lower than 10% resulted in digestion parameters (HI, GI) either comparable or slightly higher than the control. This may be related to the characteristics of inulin. It is known that generally a concentration of 40-45% inulin is needed to form a gel that is far above the levels used in pasta formulation. Moreover, the degree of polymerisation of the inulin used was on average of 9, which is giving it good dispersability in water. Thus, it is probable that part of the inulin may have been be dissolved and lost in the cooking water with implication for the protein network (weakening effect) and starch digestibility (leaving it possibly more exposed to the α-amylase attack).

The mechanisms discussed above in relation to reduced rates of starch digestibility in pasta containing DFs offer only part of the explanation for the results obtained. The effects of DFs on starch digestibility in pasta are probably even more complex than viscosity and thermodynamic incompatibility of polymers alone. This could be observed by comparing

the values of starch digested, HI and predicted GI for pastas containing different types of DFs, and especially different types of soluble DFs. If the retardation of starch digestibility would be related solely to the ability of soluble DF to form a coating layer around starch, to restrict their swelling capacity, and amylose leaching, then the percentage of decrease in starch digestibility and HI values would be very similar if not the same for all products containing the same level of soluble DF. However, different DFs behave differently in relation to sugar response (Table 2.9), and this suggests that other mechanisms may be involved. In a study on the effect of guar galactomannan on starch hydrolysis, Slaughter *et al.* (2002) concluded based on kinetic data that the effect of guar gum on lowering the glycaemic response to food may result from a non competitive inhibition of α-amylase (Slaughter et al., 2002). The authors indicated that a region of α-amylase may possess some affinity for DF, and they demonstrated that in the case of guar galactomannan a direct binding with α-amylase exists; lower starch digestibility was suggested in this case to result from the absorption of α-amylase to the galactomannan (Slaughter et al., 2002). Inhibition of α-amylase activity and thus the retardation of sugars released from the starch was also suggested by another study investigating the potential mechanisms for DF to lower postprandial serum glucose (Ou et al., 2001). Following several experiments conducted *in vitro* the authors stated that their results indicated that besides a possible inhibition of α-amylase activity, another two pathways that may be involved in lowering postprandial glucose response. One was related to the increase viscosity in the small intestine that retards the diffusion of the glucose (therefore glucose was presented to the small intestine with delay - which was suggested previously by other studies), and the other with the potential of DF to absorb glucose and thus to prevent its diffusion. This later suggestion was derived from their observations that diffusion rates did not slow down until

60 min and they were also reduced by the inclusion of insoluble fibres which are known to have little effect on the viscosity. Moreover, there was a difference between the diffused glucose between dialysate from DF and control even when the dialysis reached equilibrium (300 min). These mechanisms involved in starch digestibility may contribute to explain the results obtained in this present study. However, further investigations need to be conducted in those directions before a definitive conclusion can be drawn.

2.4 Conclusions

Pastas are appealing foods consumed worldwide that combine cheapness, ease of preparation, and long shelf life. In addition it is a source of slow released carbohydrates (Bornet et al., 1989). The only objection to an increased consumption of pasta from a nutritional point of view is its low DF content.

In an attempt to improve its nutritional qualities, the present work aimed to produce DF enriched pasta and to evaluate the effects various DF (soluble and insoluble) might have on the cooking properties, textural characteristics and starch digestibility.

The results obtained showed that DF could be used successfully in pasta formulation since it was generally possible to achieve products with acceptable cooking and textural attributes (in many cases similar to the control pasta). Inferior quality attributes were related to increased levels of DF addition (more than 10% on db), though even in these cases the quality of the final product could be improved by either increasing the protein content of the formulation (adding gluten) or by reducing the cooking time. These

suggestions were not the subject of the present study and therefore would need further investigation.

At least as important were the results related to the rate of starch digestion/sugars released during *in vitro* experiments conducted. In agreement with other *in vivo* and *in vitro* studies (Wolever et al., 1986; Casiraghi et al., 1992), this work indicates that pasta leads to a significantly lower glucose response than white wheat bread. More importantly, this work proved that various DF when used in pasta formulation could lead to even slower rates of sugars released than from control pasta, and that the effects are dependant on the type of DF used (thus no generic behaviour seemed to be applicable).

The effect of DFs on the rate of starch digestion could not be related to incomplete gelatinisation, protein content or amylose/amylopectin ratio. Most likely the reduced rate of starch digestibility is the outcome of a combination of factors:

- The formation of a layer coating starch granules and which may act as a barrier between starch and α-amylase;
- Reduced starch swelling and rate of amylose leaching out of the granule, resulting in an increased proportion of resistant starch, due to thermodynamic incompatibility with DF present in the formulation;
- Potential inhibition of α-amylase by DF and potential absorption of the sugars by the DF, thus impeding their diffusion through the membrane.

This work describes the behaviour of pasta products containing various DF during cooking and *in vitro* digestion, and provids a way to rank the products according to their predicted GI. The results indicate that it is possible to obtain pasta products with even lower HI and GI than the conventional ones. This not only represents good news for people suffering of

diabetes but also for healthy individuals who are concerned about possible risks of developing metabolic disorders. It is equally important though that the final products enriched in DF have reasonably good cooking and textural attributes that would contribute to their acceptability by the consumers and which represents the most important step in becoming part of a daily diet.

Chapter 3. Dietary fibre and cereal products. II - The effect of dietary fibre on the nutritional, textural and structural attributes of bread products

3.1 Introduction

Alongside pasta, bread is a staple cereal product consumed worldwide; moreover, it is part of the daily diet of the majority of European and USA households, white bread being at the top of consumer preferences. While extremely popular, white bread is known as a high glycaemic response food (Foster-Powel and Miller, 1995), which provides only small amounts of DFs (less than 3g/100g) (Endress and Fisher, 2001).

In recent years low GI diets have been shown to improve fasting blood glucose concentration in individuals with type 2 diabetes (Fontvieille et al., 1992), and thus may have a role in both prevention and management of this condition. Conversely, diets with high glycaemic load (defined as the product of the GI value of a food and its carbohydrate content) have been reported to increase the risk of developing type 2 diabetes in men (Salmeron, 1997a) and women (Salmeron, 1997b). Moreover, these studies show an inverse relationship between the intake of cereal fibre and the risk of diabetes, and recommend that grains should be consumed in a minimally refined form. The wide implementation of low GI diets needs to be supported by the presence on the market of such products. However, there is a shortage of low GI bread products on the market.

Means by which the GI of the bread could be modulated include: use of cereal kernels, sourdough fermentation, addition of organic acids, or DFs, use of cereal genotypes with high amylose content, or of selected time/temperature to promote starch retrogradation (Bjorck and Elmstahl, 2003). Amongst these, the use of DF in bread formulation would also bring the health benefits related to the consumption of a DF enriched product and

would represent a step forward towards meeting the daily requirements for DF consumption.

However, the data accumulated so far and presented in the literature suggests that the effect of DF on the glycaemic response of bread is controversial; several research studies reported no effect or variable effects of type or quantity of DF on glycaemic response. Insoluble DF are seen generally to have no effect on the glycaemic response (Hamberg et al., 1989). Moreover, it has been reported that although rich in DF, the GI of wholemeal bread is in the region of 99±10, which is similar to that of white bread (101±3) (FAO/WHO, 1998; Lu et al., 2000). Only mixed grain breads have a lower GI (64±17) (Lu et al., 2000), but they are more difficult to chew and therefore less acceptable to the consumers.

Soluble DF are known to beneficially affect the carbohydrate metabolism and their effects when in bread are well documented for certain fibres such as guar gum, β-glucan, pectin or psyllium. For example guar gum has been shown to improve blood glucose and/or insulin response both in healthy subjects (Ellis et al., 1991; Landin et al., 1992), and in patients with type 2 diabetes (Gatenby et al., 1996; Groop et al., 1993). Other soluble DF such as β-glucan (Cavallero et al., 2002; Pick et al., 1998), pectin (Leeds, 1983), or psyllium (Wolever et al., 1991) have been also shown to have positive effects. Nevertheless, similar data are not available for the physiological effects of a range of other dietary fibre such as LBG, xanthan gum, purified cellulose from various sources etc.

Despite the above mentioned physiological benefits, the use of these DF (especially high molecular weight guar gum) in breadmaking at levels which have been shown to be clinically effective remains limited due to the poor palatability of the final product (Apling

and Ellis, 1983), especially in relation to the use of viscous DF. Hence, a contradiction arises between the clinical and general health recommendations, and the acceptability of the final product. Strategies to improve the palatability of the breads enriched with DF have been suggested and amongst these the use of low molecular weight grades of guar gum resulted in encouraging results (Ellis et al., 1991; Blake et al., 1997).

However, poor palatability is not the only issue arising from the incorporation of DFs in bread formulations. Increased levels of DF in breads are generally associated with changes in dough characteristics and consequently bread quality; certain DFs are known to produce shorter doughs with reduced fermentation tolerance which would result in decreased loaf volume, increased crumb firmness and sometimes modified taste (Wang et al., 2002a). As mentioned in Chapter 1, these effects are thought to be due to the 'damage' of the gluten network, with a direct consequence on gas retention within the structure and thus final crumb structure/texture and loaf volume (Pomeranz et al., 1977). Interestingly, at very low level of addition (i.e. 0.5%, which, unfortunately, is far below the level needed for any physiological effect to occur) certain DF have been found to improve bread specific volume and reduce crumb firmness (Rosell et al., 2001). The challenge remains to produce DF enriched bread that exhibit both physiological benefits as well as good quality, thus making it appealing to the consumers.

This part of the study investigated the potential use of a range of DF in bread formulations with regard to its quality attributes as well as nutritional benefits. The results presented in Chapter 2 showed that it is possible to obtain good quality pasta products enriched with DF. An additional benefit brought by the use of certain DFs in pasta was a decrease in the rate of starch digestibility, and thus a reduction of the product GI. Therefore, it appeared interesting to study the effect of the same range of DF in bread products. Dough and bread

characteristics were investigated in relation to the type and level of DF added; a predicted GI was also evaluated for each of the products made following the same *in vitro* digestion method used in Chapter 2 (paragraph 2.2.2.6). At that stage, a further step was taken and the products which exhibited the most positive characteristics as resulted from the assessment of both quality attributes and *in vitro* starch digestibility were used for sensory analysis and for the determination of their GI *in vivo.* These assessments were felt to be essential in order acquire information about consumers perception of the quality of DF enriched bread and also to validate the potential benefits of certain DFs incorporation in bread when consumed by healthy volunteers.

3.2 Materials and methods

3.2.1 Materials

Commercially milled wheat flour was used as the base for all formulations as well as being the control flour for all comparisons. The flour used was of breadmaking grade (Allied Mills, UK) and its properties as provided by the supplier are given in Table 3.1.

Table 3.1. Properties of the standard control flour used for breadmaking

Protein content (%)	11.7
Moisture content (%)	13.8
Farinograph water absorption (600 Line) (%)	62.0

The DFs used were the same range of soluble and insoluble fibres as used for the experiment described in Stage 2 of the Chapter 2. They were: inulin (Frutafit HD - Calleva Ltd., UK), guar gum (E412) (Calleblend GUA - Calleva Ltd., UK), pea fibre (Exafine -

Cosucra, Belgium), locust bean gum (E410) (Calleblend LBG - Calleva Ltd., UK), xanthan gum (Kelco®F, CP Kelco, UK), bamboo fibre (Qualicel 41B - CFF, Germany), β-glucan enriched flour (HiSol, - HiSol Ltd., UK). Their characteristics as provided by the suppliers are presented in Table 2.3 (Chapter 2). These DFs were incorporated into bread recipes at the same replacement levels as used previously for pasta making (Chapter 2, paragraph 2.2.2.1): 2.5%, 5%, 7.5%, and 10% (w/w). For the formulations containing HiSol (with only 12% β-glucan) bread making flour was replaced with the appropriate amount of HiSol, so the β-glucan in the final formulation would be 2.5%, 5%, 7.5% and 10% (w/w). Control bread with no DF included was also prepared. The yeast used was dried yeast (Hovis, UK), and the oil used was a mixture of soybean and palm oil (1:1).

3.2.2 Methods

3.2.2.1 Bread making method

The base bread recipe was that of Tovar et al. (1992) for reference white bread and consisted of: flour 300g, water 200g, salt 3g, dry yeast 3g, soybean + palm oil 1.5g (Tovar et al., 1992; Liljeberg and Bjorck, 1994). The water content was adjusted in the recipes containing DF to give manageable dough (Table 2.3) as estimated by using the probe P50 of the Texture Analyser (to give similar peak forces on doughs). The dry ingredients were mixed in a small scale high speed mixer - Robot Coupe R4 (Robot Coupe Ltd, UK) for 15s at high speed. The water was then added and the mixing was carried out for another 45s. The dough was divided into 450g pieces and then a 6 min intermediate proof was given. After hand moulding, dough pieces were put into tins and proofed at 37°C for 50 min. The bread was baked in a catering size convectional oven (Zanussi Combiwave FCVM/E62) at 220°C for 18 min (with steam for the first 2 min).

Table 3.2. Formulations used for bread making

Dietary fibre addition (%)		Bread making flour (g)	DF (g)	Water (g)
Control	0.0	300.0	0.0	200.0
Pea fibre	2.5	292.5	7.5	205.0
	5.0	285.0	15.0	210.0
	7.5	277.5	22.5	215.0
	10.0	270.0	30.0	225.0
Inulin	2.5	292.5	7.5	190.0
	5.0	285.0	15.0	185.0
	7.5	277.5	22.5	180.0
	10.0	270.0	30.0	170.0
Guar gum	2.5	292.5	7.5	210.0
	5.0	285.0	15.0	240.0
	7.5	277.5	22.5	280.0
	10.0	270.0	30.0	310.0
Bamboo fibre	2.5	292.5	7.5	205.0
	5.0	285.0	15.0	215.0
	7.5	277.5	22.5	225.0
	10.0	270.0	30.0	235.0
Xanthan gum	2.5	292.5	7.5	210.0
	5.0	285.0	15.0	230.0
	7.5	277.5	22.5	250.0
	10.0	270.0	30.0	270.0
Locust bean gum	2.5	292.5	7.5	210.0
	5.0	285.0	15.0	230.0
	7.5	277.5	22.5	250.0
	10.0	270.0	30.0	270.0
Beta-glucan (Hi Sol)	2.5	246.4	53.6	220.0
	5.0	192.9	107.1	250.0
	7.5	139.3	160.7	280.0
	10.0	85.7	214.3	310.0

The bread was left to cool at room temperature for 2 hours before any assessment was made. The loaves which were assessed during storage were packed in finger sealable polypropylene bags and stored at 4°C for 4 days. Samples from each loaf taken on the baking day were also stored at -40°C for the assessment of *in vitro* starch digestibility. Baking tests were run in triplicates.

3.2.2.2 Dough assessment

All the doughs produced were assessed for extensibility and stickiness using a Texture Analyser TA.XT2 (Stable Micro Systems, UK) calibrated for a load cell of 5 kg.

- *Dough extensibility* was evaluated using the tension test and the Kieffer Dough & Gluten Extensibility Rig (A/KIE) (settings: pre-test speed: 2.0 mm/s; test speed: 3.3 mm/s; post-test speed: 10.0 mm/s; distance: 75mm). Maximum force recorded when the elastic limit is exceeded and the dough separates is related to its resistance to extension (N), and the distance reached at this point (i.e. close to zero force) was used as an indication of dough extensibility. The test was performed on 6 replicates *per* sample (the doughs being made in duplicate). A typical curve is presented in Appendix 3.1a).

- *Dough stickiness* was determined using the SMS/Chen-Hoseney Dough Stickiness Cell (A/DSC) and 25mm perspex cylinder probe (P/25P) and an adhesive test (settings: pre-test speed: 2.0 mm/s; test speed: 2.0 mm/s; post-test speed: 10.0 mm/s; distance: 4mm; force: 40g; time: 0.1s). The typical curve gave information regarding: dough stickiness (related to the maximum force reading, i.e. the highest +ve peak); 'work of adhesion' (related to the +ve area), and dough cohesion/dough strength (related to the distance the sample is extended on probe return). The test was performed on 6 replicates *per* sample (the doughs being made in duplicate) and a typical curve is presented in Appendix 3.1b).

3.2.2.3 Bread quality assessment

Bread quality was assessed using the following techniques: loaf volume and specific volume; crumb brightness and crust colour; crust thickness and photography.

- *Loaf volume and specific volume* give an overall indication of the way in which the dough has retained the gas produced during proving and baking. Typically high quality flour and good processing results in larger values. Loaf volume was evaluated using a seed displacement method (Cavallero et al., 2002) and the specific volume calculated as the ratio between loaf volume and its weight. The measurements were performed in triplicate. *Crust thickness* was measured with a calliper (four measurements *per* loaf).

- *Crumb brightness* is thought to be a function of both the pigmentation of the crumb (due to the ingredients used) as well as its physical characteristics with smaller, shallower gas cells typically giving a brighter appearance (higher percentage reflectance). A Minolta colorimeter (Minolta Ltd., UK) was used to evaluate crumb and crust colour; the measurements (two *per* loaf) were made in the Yxy colour system, where the Y value (expressed as a percentage) is an indication of product lightness.

- *Images of bread* were taken with a digital camera.

3.2.2.4 Bread staling

The changes occurring in bread during storage were assessed by texture analysis, during 4 days storage period at 4°C, with measurements being made on days 0, 2 and 4. Textural properties of bread crumb (crumb firmness and crumb springiness) were determined using a TA-TX2 Texture Analyser (Stable Micro System, UK) calibrated for 5kg weight.

- *Crumb firmness* was determined using the AACC (74-09) standard method (AACC 36mm cylinder probe with radius* (P/36R) and the following settings: test speed: 1.7 mm/s; post-test speed: 10.0 mm/s; strain: 40%). A typical curve is presented in Appendix 3.2 a); according to this method, crumb firmness is defined as the force

required to compress the product by a pre-set distance (i.e. force taken at 25% compression of 25mm) (AACC, 1983). The test was run in 4 replicates *per* loaf; two loafs were used for each bread sample.

- *Crumb springiness* was determined using a force in compression test with the AACC 36mm cylinder probe with radius (P/36R) and following test settings: option: hold until time; pre-test speed: 1.0 mm/s; test speed: 1.0 mm/s; post-test speed: 10.0 mm/s; strain: 25%; time: 60s. A typical curve is presented in Appendix 3.2 b). Crumb springiness was calculated from the recorded force after 60s of holding, which was divided by the maximum force, and then multiplied by 100% (i.e. F60/ F_{max} x 100 = % recovery. The closer the resulting value is to 100% the more "spring-like" the product is). The test was run in 4 replicates *per* loaf; two loafs were used for each sample.

3.2.2.5 Differential Scanning Calorimetry (DSC)

DSC tests were performed on bread samples taken on the baking day. Sample preparation and the scanning rates were the same as described in Chapter 2, section 2.2.1.

3.2.2.6 Chemical analysis of bread

Moisture content of fresh bread samples was determined in triplicate measurements according to standard methods (AACC, 1996-926.07B; AACC, 1995) as presented in sections 2.2.1 and 2.2.2 of Chapter 2.

Bread samples were prepared as for DSC (Chapter 2, section 2.2.1): freeze dried, milled to particle size of less than 0.5mm and used for determination of starch, protein and TDF content. The methods and the number of replicates used were presented in sections 2.2.1 and 2.2.2 of Chapter 2.

3.2.2.7 *In vitro* digestibility of bread

The method used was as for evaluating pasta digestibility *in vitro* and described in section 2.2.2 (Chapter 2). Similarly, the whole exercise was repeated twice, and results obtained were used to calculate the following parameters, which were fully defined in Chapter 2: the amount of reducing sugars released (RSR); the percentage of maltose able to diffuse out of the bag in the presence of sample (DIFF); sugar diffusion index (SDI); percentage of starch digested (DIG); digestion index (DIGI); reducing sugars released index (RSRI); hydrolysis index (HI), and predicted GI using both predictive equations.

3.2.2.8 *In vivo* determination of bread's GI

Twelve healthy volunteers (four men and eight women), aged 24-42 years, with normal body mass indexes (22.1±1.8kg/m^2), and without any drug therapy took part in the study. The protocol was approved by the Ethics Committee of the Faculty of Science, University of Plymouth, and also by the Ethic Committee of South Devon Health Care - NHS Trust.

The breads to be tested were offered in amounts corresponding to 50g of available carbohydrates and served with cheese and butter to balance the fat (12g *per* portion) and protein (15g *per* portion); 250 ml of water and 150g of coffee/tea accompanied the meals (Skrabanja et al., 2001). The meals were given at breakfast after an overnight fast on separate mornings, one week apart; the meals were consumed over approximately 12 min. Finger prick capillary blood samples (3.5µl) were collected prior to the meal (time 0) and at 15, 30, 30, 90 and 120 min after the meal (using a MediSense pen - Medisense Ltd., UK), for determination of glucose concentration (with the Precision Q-I-D MediSense sensor - MediSense Ltd, UK, precision: ±0.1mmol/l).

The GI was calculated from the 0-90 and 0-120 min incremental postprandial blood glucose areas after test meals, as a percentage of the corresponding area after the meal using white reference bread (control) (GI=100). Glucose values below the baseline were considered equivalent to zero (Granfeldt et al., 1994).

3.2.2.9 Assessment of satiety

The extent and duration of satiety were also evaluated after the isoenergetic meals were served. After each blood sampling time point, the volunteers were asked to numerically assess the feeling of hunger/satiety on a scoring scale system ranging from -10 (extreme hunger) to +10 (extreme satiety) (Granfeldt et al., 1994). The form for the assessment of satiety is presented in the Appendix 3.3. The score at time 0 was used to set the baseline. A satiety score (satiety area - SA) for each product was calculated as the area under the satiety curve (0-120 min) above the x axis and divided by the corresponding area obtained with the white reference bread (SA=100).

3.2.2.10 Scanning electron microscopy

Scanning electron microscopy was used as described previously in section 2.2.2.7 of Chapter 2 to obtain information about bread microstructure as affected by the DF addition. Freeze-dried bread and digested bread were examined to get an insight of their structure. Randomised fields were evaluated in each sample.

3.2.2.11 Sensory evaluation of bread

Bread samples which had been selected to be used for *in vivo* starch digestibility were also judged for sensory characteristics by a panel formed by 12 members recruited from University of Plymouth staff and students. Prior to the testing day, the members of the panel were involved in 2 training sessions in which the list of descriptors (as well as their

definition) were decided for bread sensory evaluation. The evaluation was performed the day after the baking and the panel was asked to score: crumb colour, crust colour, crumb texture, flavour, taste, and overall acceptability. Test samples, identified by a three digit code, were presented to the panellists randomly on individual white paper plates. All samples were presented as slices (2cm thickness), at room temperature and shortly after being sliced; testing was conducted on duplicate samples. Each panellist was asked to provide a score from 1 (=dislike extremely) to 9 (=like extremely) with 5 (=neither like or dislike) for each attribute (Cavallero et al., 2002). The form, which was filled in by each panellist, is presented in the Appendix 3.4.

3.2.2.12 Statistical analysis

The results from the tests evaluating bread quality and nutritional attributes are presented as means ± SD. Analysis of variance (GLM) followed by Tukey's test of Minitab 13.1 software (Minitab Inc., USA) were used for statistical analysis to investigate the effect of two factors: type of dietary fibre and the level of dietary fibre used. A third factor - storage time - was introduced for analysis of crumb texture data. The results for the control bread were not included in the model for the statistical analysis. The sensory evaluation results were analysed using One Way ANOVA.

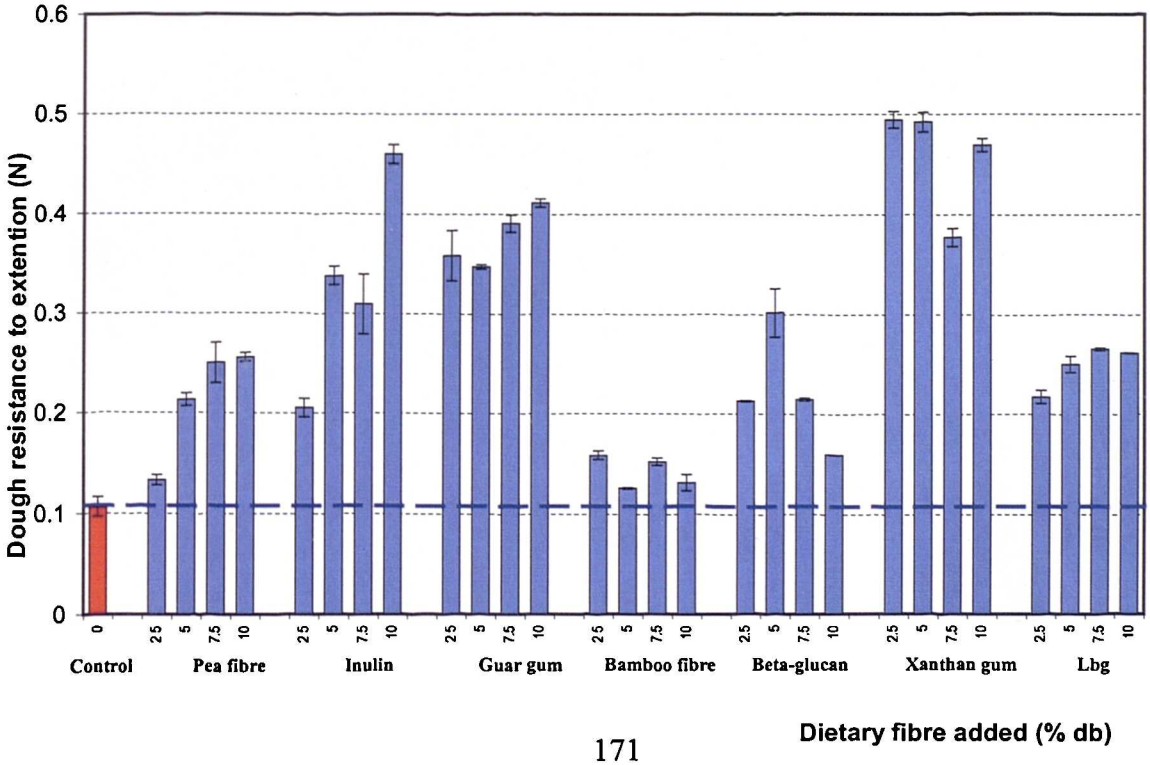
The results for the GI and SA were analysed by Student's t-test for paired samples, and also Wilcoxon test for paired observations. Differences in glucose response and satiety values were assessed using repeated measures design which had as experimental factors: diet, subject, and the sampling time; a value of $p<0.05$ was taken to indicate significant difference between tested meals (Gardinier, 1997a,b).

3.3 Results and discussions

3.3.1 Influence of DF on dough properties

Bread is a viscoelastic biopolymer foam system obtained from basic raw materials, most often wheat flour, water, yeast and salt. In order to convert these ingredients into a porous, palatable structure, several processing steps are involved: mixing and dough development, formation of a foam structure in the dough (through moulding, proofing, baking), and stabilisation of the porous structure by altering the molecular configuration of the polymeric components in the cell walls through heat during baking (Scalon and Zghal, 2001). An important step is the formation of a dough with such rheological characteristics that will allow it to expand, to retain gas and thus to produce a high volume bread loaf with even crumb structure. Thus, the properties of dough which are of concern in breadmaking are: resistance to extension, extensibility, and stickiness (Cauvain, 1998) .

Figure 3.1. Dough resistance to extension as determined using the Texture Analyser



The results on the effect of DF addition on dough characteristics are summarised in the ANOVA Table 3.3 and illustrated in Figures 3.1-3.3. They indicate that all the dough parameters as evaluated by Texture Analysis were significantly affected by both the type and the level of DF used in bread formulations ($p<0.001$). The exception was made by dough resistance to extension which appeared to be not significantly affected by the levels of DF used (Table 3.3).

The resistance to extension of bread dough containing DF was generally higher than the control (Figure 3.1). The lowest effect was shown by bamboo fibre inclusion, while the highest was observed for xanthan gum. As mentioned above, statistical analysis indicated no significant differences on dough resistance to extension introduced by the level of DF ($p>0.05$). Nevertheless, for the doughs containing either pea fibre, inulin, guar gum or LBG, there is a tendency to increasing resistance to extension with increasing levels of DF (Figure 3.1).

Dough extensibility was significantly decreased ($p<0.001$) by DF incorporation in the formulation (to less than half of the extensibility value of the control. Overall, the lowest effect was associated with the use of bamboo fibre, pea fibre, inulin, and LBG, while xanthan gum and β-glucan (as HiSol) showed the strongest effect. In addition as Figure 3.2 and Table 3.3 indicate, increasing levels of DF resulted in decreasing dough extensibility.

These results suggest that DFs greatly affect the rheological behaviour of the dough, and possibly the quality of the final product. Dough resistance to extension is thought to be an indicator of the dough's strengths and of its ability to retain gas, while its extensibility is a predictor of the processing/handling characteristics of the dough (Wang et al., 2002a) (Wang et al., 2002b). A good combination of these two parameters results in desirable

dough properties. The results obtained indicate that DFs addition appears to increase the strength of the dough and potentially its gas retaining capacity. At the same time the decrease in the extensibility of the dough suggests that DFs interfere with the gluten structure/development making it less extensible. Thus, the presence of DF may be expected to limit the free expansion of the dough and its ability to stretch in thin membranes during the fermentation/baking stages. These findings parallel those of Wang et al. (2002a) and Rosell et al. (2001) who studied the effect of various DF (carob fibre, pea fibre and inulin at 3% level of replacement and alginate, carrageenan, xanthan and carboxypropylmethylcellulose at 0.5% addition level respectively) on dough characteristics. The effects observed were assumed to be due to the dilution of the gluten network (Laurikainen et al., 1998) or to the interaction between DF and wheat flour proteins (Rosell et al., 2001).

Figure 3.2. Dough extensibility as determined using the Texture Analyser

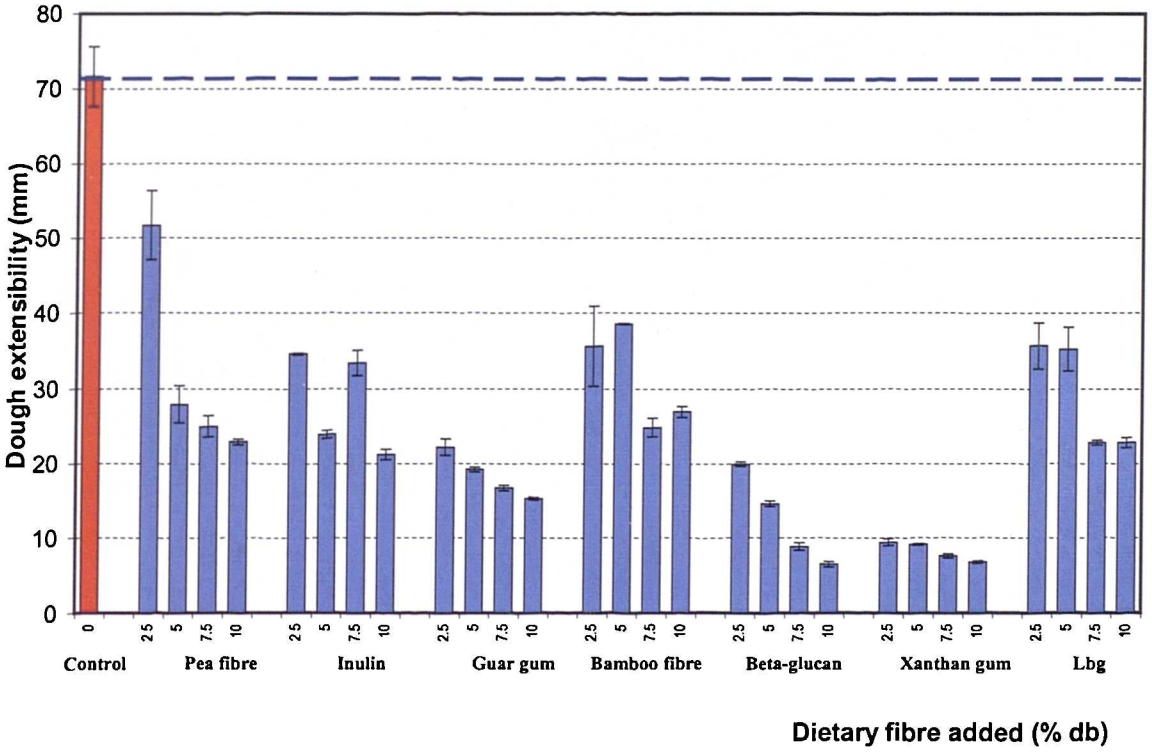


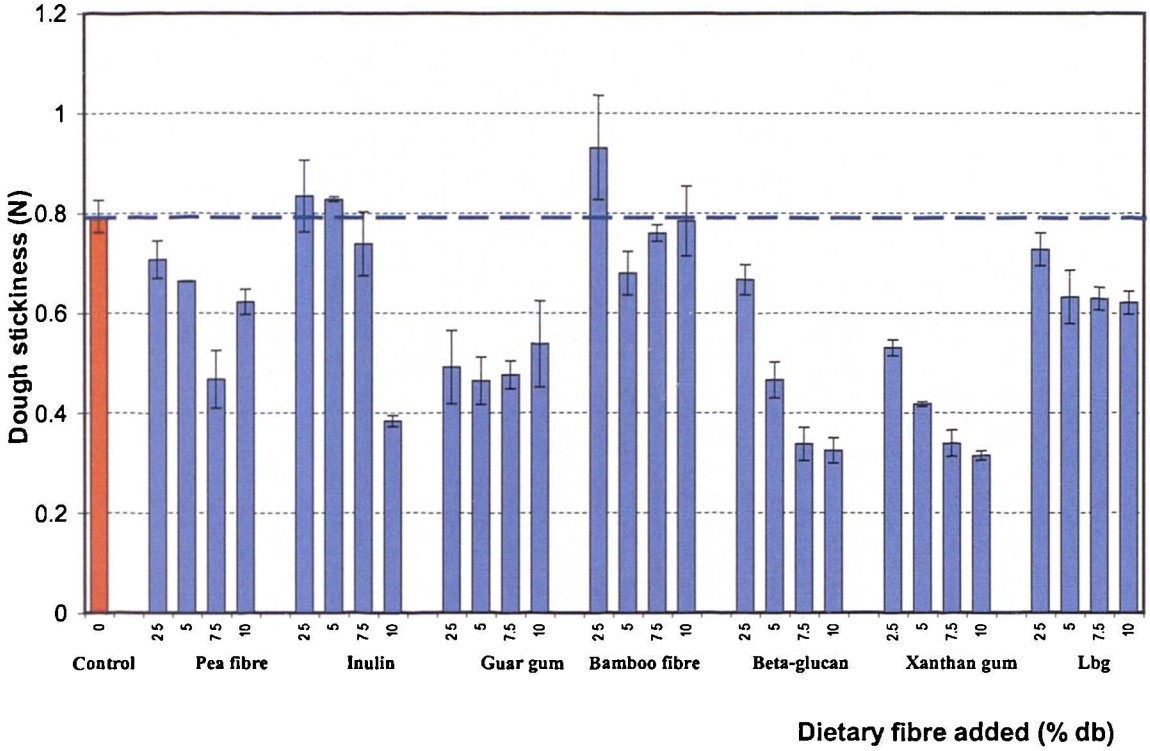
Table 3.3. ANOVA table summarising the textural attributes of bread dough (the values represent means of all values at a given treatment level)

Sample	Resistance to extension (N)	Extensibility (mm)	Stickiness (N)	Adhesion (N*s)	Cohesion (mm)
Control	*0.11±0.01*	*71.6±9.26*	*0.79±0.03*	*0.11±0.00*	*3.1±0.16*
Effect of the type of dietary fibre					
Pea fibre	$0.21^{d,e}$	31.8^{a}	$0.61^{b,c}$	$0.05^{b,c,d}$	$1.6^{c,d}$
Inulin	$0.33^{b,c}$	28.3^{a}	0.69^{a}	$0.06^{b,c}$	$2.0^{b,c}$
Guar gum	$0.38^{a,b}$	18.3^{b}	$0.49^{c,d}$	$0.04^{c,d}$	$1.5^{c,d}$
Bamboo fibre	0.14^{e}	31.5^{a}	0.79^{a}	0.09^{a}	2.7^{a}
β-glucan (as HiSol)	$0.22^{d,e}$	$12.4^{b,c}$	0.45^{d}	0.03^{d}	1.2^{d}
Xanthan gum	0.46^{a}	8.2^{c}	0.40^{d}	$0.05^{b,c,d}$	$2.4^{a,b}$
Locust bean gum	$0.25^{c,d}$	29.1^{a}	$0.65^{a,b}$	0.06^{b}	1.9^{c}
Significance	***	***	***	***	***
SEM	0.02	1.83	0.03	0.005	0.15
Effect of the level of DF addition					
2.5%	0.25	29.8^{A}	0.70^{A}	0.07^{A}	2.2^{A}
5.0%	0.29	24.0^{B}	0.59^{B}	0.05^{B}	$1.9^{A,B}$
7.5%	0.28	$19.8^{B,C}$	0.53^{B}	0.05^{B}	$1.9^{A,B}$
10.0%	0.31	17.4^{C}	0.51^{B}	0.04^{B}	1.6^{B}
Significance	NS	***	***	***	***
SEM	0.01	1.38	0.02	0.004	0.11
Effect of the interaction					
Type of fibre*level of addition	NS	***	***	***	***

- within the same column, the values with the same letter are not significantly different;
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

In comparison to the control dough, the stickiness, adhesiveness and cohesion were significantly decreased by DF addition ($p<0.001$); moreover, the higher the level of DF used, the more pronounced the decrease seemed to be (Table 3.3 and Figure 3.3).

Figure 3.3. Dough stickiness as determined using the Texture Analyser



In terms of dough stickiness, the lowest effect was observed when bamboo fibre, inulin or LBG were used; in these cases the overall dough stickiness values appeared to be not significantly different from the control. The other parameters were also least affected by the use of bamboo fibre. These results appear to contradict previously published results that have reported an increase of dough stickiness when bran (Laurikainen et al., 1998) or guar gum, xanthan gum or methylcellulose (Friend et al., 1993) were added to the formulation. However, it is fair to point out that in the case of the latter research study, the stickiness of the dough was sensory assessed during mixing rather than quantitatively. This overall reduction of the dough parameters related to its handling properties suggests that DFs may improve the machinability of the dough.

3.3.2 Influence of DF on bread chemical composition and quality characteristics

The composition of bread in terms of dry matter (DM), protein, starch, TDF content is summarised in the ANOVA Table 3.4. As expected, the DM of bread containing DF was lower than of the control bread due to the higher amount of water incorporated in the recipe; it also decreased with increasing levels of DF. Protein and starch contents were lower in bread containing DF in comparison to the control, and the values decreased with increasing levels of DF. This was again to be expected due to the initial formulations involving the replacement of flour with various levels of DF.

Table 3.4. ANOVA table summarising chemical composition of DF enriched bread (the values represent means of all values at a given treatment level)

Sample	Dry matter (%)	Protein content (%)	Starch content (%)	Available carbohydrate content (%)	TDF (%)
Control	*54.5±0.28*	*8.6±0.03*	*39.8±0.21*	*40.1±0.23*	*1.4±0.39*
Effect of the type of dietary fibre					
Pea fibre	53.7^{b}	7.4^{a}	37.4^{b}	37.9^{b}	4.7^{b}
Inulin	57.6^{a}	7.6^{a}	41.1^{a}	41.6^{a}	5.3^{a}
Guar gum	$49.5^{c,d}$	$6.6^{b,c}$	$34.5^{c,d}$	35.0^{c}	4.5^{b}
Bamboo fibre	52.9^{b}	6.9^{b}	38.1^{b}	38.5^{b}	4.6^{b}
Hi Sol	47.3^{d}	6.2^{c}	33.5^{d}	33.9^{c}	2.8
Xanthan gum[XX]	$51.0^{b,c,d}$	$6.8^{b,c}$	$34.7^{b,c,d}$	$35.2^{b,c}$	$4.7^{a,b}$
Locust bean gum	$51.2^{b,c}$	6.9^{b}	$36.2^{b,c}$	$36.8^{b,c}$	4.5^{b}
Significance	***	***	***	***	***
SEM	0.71	0.10	0.62	0.63	0.12
Effect of the level of DF addition					
2.5%	53.9^{A}	7.6^{A}	39.7^{A}	40.2^{A}	2.5
5.0%	$52.4^{A,B}$	7.1^{B}	37.4^{B}	37.8^{B}	4.0^{C}
7.5%	$51.0^{B,C}$	6.6^{C}	35.4^{C}	35.9^{C}	5.1^{B}
10.0%	50.1^{C}	6.3	33.6^{C}	34.0^{C}	6.3^{A}
Significance	***	***	***	***	***
SEM	0.59	0.08	0.47	0.47	0.10

- within the same column, the values with the same letter are not significantly different
- *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant.
- [XX] formulations with 7.5% and 10% xanthan gum were not included in the analysis

Photographs captured for each type of bread produced are presented in Appendix 3.5. The breads made from formulations containing 7.5 or 10% xanthan gum were excluded from the experiment due to their very poor quality (Appendix 3.5). The effect of DF supplementation of bread quality characteristics is summarised in ANOVA Table 3.5 and illustrated in Figures 3.4 and 3.5. Except for crust thickness, all the other attributes describing bread quality were significantly affected by the DF addition ($p<0.001$, Table 3.5).

Generally, loaf volume, specific volume and height decreased with increasing levels of DF. This general trend appears to be related with the results obtained for dough extensibility suggesting that increasing levels of DF increasingly limits the dough to freely extend and to form thin, smooth gluten layers. Thus, the final products tend to present poorer characteristics in comparison to the control. It is important to note, however, that this is the overall effect, and as Figure 3.4 shows, different DF led to different results.

Figure 3.4. Bread specific volume

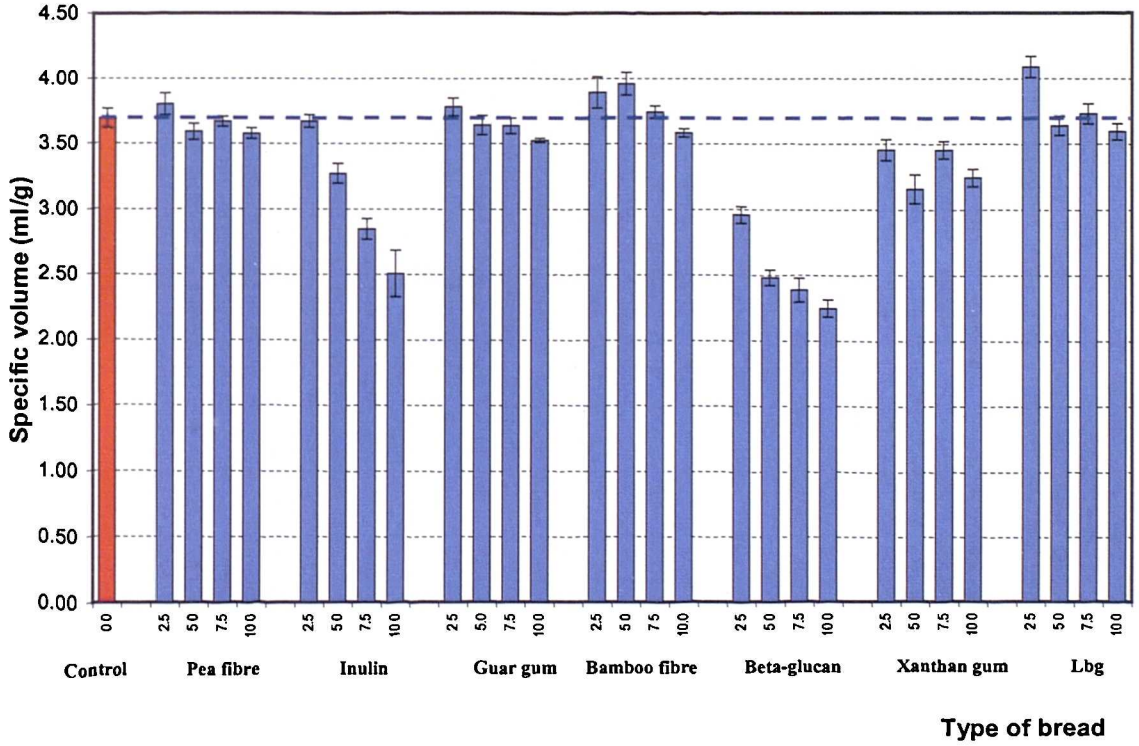


Table 3.5. ANOVA table summarising quality characteristics of DF enriched bread (the values represent means of all values at a given treatment level)

Sample	Loaf volume (ml)	Loaf height (mm)	Loaf specific volume (ml/g)	Crust thickness (mm)	Crumb lightness, Y (%)	Crust lightness, Y (%)
Control	*1477±25.1*	*112±1.3*	*3.7±0.07*	*1.92±0.14*	*38.2±0.39*	*30.6±1.52*
Effect of the type of dietary fibre						
Pea fibre	1462^{a}	106^{c}	3.6^{a}	1.97	$38.1^{a,b}$	$31.2^{c,d}$
Inulin	1243^{b}	96^{d}	3.0^{c}	1.96	$39.1^{a,b}$	27.9^{d}
Guar gum	1459^{a}	$109^{b,c}$	3.6^{a}	1.98	42.0^{a}	37.6^{a}
Bamboo fibre	1498^{a}	$111^{a,b}$	3.8^{a}	1.95	36.0^{b}	$32.7^{b,c}$
Hi Sol	979^{c}	75^{e}	2.5^{d}	2.02	25.9^{c}	28.7^{d}
Xanthan gum[XX]	1324^{b}	$109^{b,c}$	3.3^{b}	1.99	$41.2^{a,b}$	$33.8^{a,b,c}$
Locust bean gum	1488^{a}	115^{a}	3.8^{a}	1.97	37.3^{b}	$35.6^{a,b}$
Significance	***	***	***	NS	***	***
SEM	19.0	1.1	0.05	0.02	0.99	0.83
Effect of the level of DF addition						
2.5%	1462^{a}	111^{A}	3.7^{A}	1.98	39.1^{a}	33.0
5.0%	1350^{b}	106^{B}	3.4^{B}	1.98	36.9^{a}	33.0
7.5%	1329^{b}	101^{C}	3.4^{B}	2.00	36.8^{a}	32.3
10.0%	1262^{c}	94	3.2^{C}	1.96	35.7^{b}	31.5
Significance	***	***	***	NS	*	NS
SEM	14.4	0.9	0.04	0.01	0.75	0.63

- within the same column, the values with the same letter are not significantly different: *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant;
- [XX] formulations with 7.5% and 10% xanthan gum were not included in the analysis

The type of DF used in the formulation modified significantly bread quality characteristics ($p<0.001$, Table 3.5). The breads containing bamboo fibre, LBG, pea fibre or guar gum, showed volumes, specific volumes and heights similar to the control indicating that the quality of the final product was not deleteriously affected by the addition of these DF (Table 3.5 and Figure 3.4).

In contrast, the use of inulin, β-glucan (as HiSol) and xanthan resulted in bread with poorer quality than the control bread. The corresponding loaf volume, specific volume and height were significantly lower than of the control bread suggesting a closer, more compact crumb structure; the effect was directly related to the level of the DF used (Figure 3.4). Bread quality (in terms of height and volume) as affected by DF could be also visualised in the photographic images presented in Appendix 3.5.

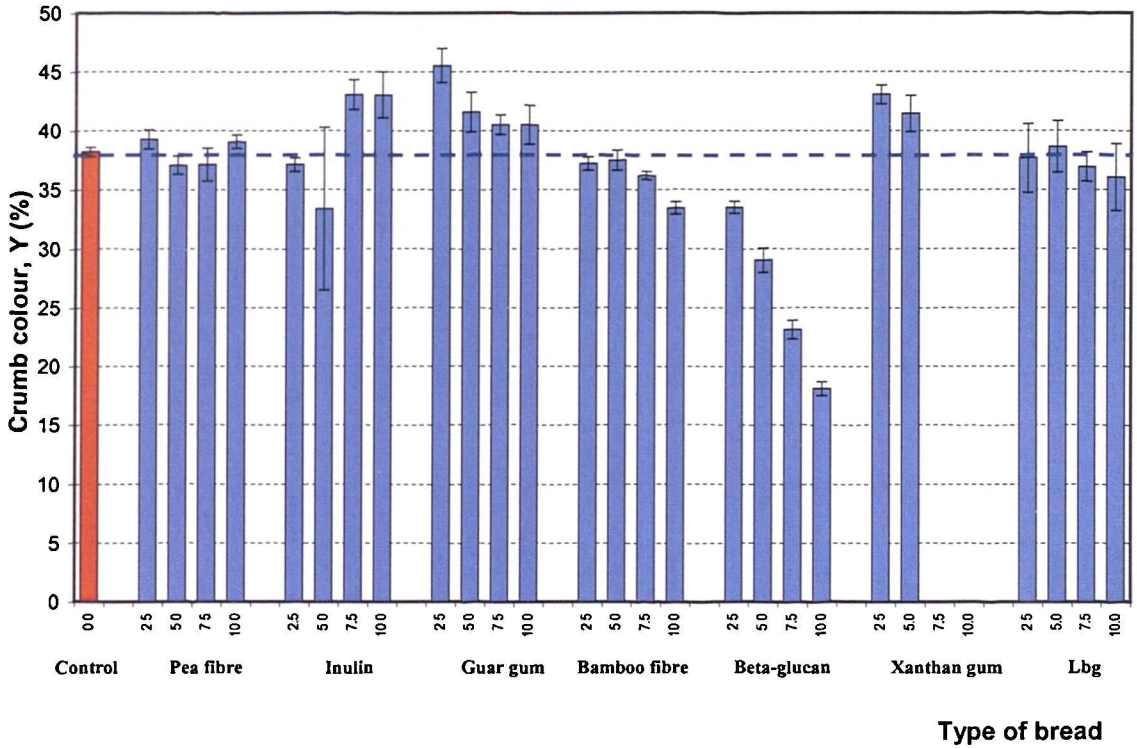
The observation made for the use of inulin in bread formulation is in agreement with earlier reports indicating a decrease in bread volumes and specific volumes when more than 3% inulin was used in the formulation (Wang et al., 2002a; O'Brien et al., 2003). Similarly, previous studies on the use of β-glucan in bread, have reported a decrease in bread volume (Keagy et al., 200; Knuckles et al., 1997). Nevertheless, the decrease in volumes/specific volumes of bread containing β-glucan as HiSol can not be associated only with β-glucan addition, but also with the quantity and quality of the proteins present in the formulation, since part of the wheat flour was replaced by HiSol.

In contradiction to the present findings, xanthan gum was previously reported to increase bread volume and specific volume (Rosell et al., 2001). The reason for the difference observed might be related to the low level of xanthan gum (0.5% on flour weight) used in the aforementioned study in comparison to 2.5 to 10% used in the present experiment.

Moreover, previous research on the use of pea fibre into breads has shown a deleterious effect on bread volume/specific volume (20.7% volume reduction) (Wang et al., 2002a). This was not reproduced by the present experiment although the levels of pea fibre used were higher than the 3% used in the above mentioned study.

The most interesting and positive results on bread quality characteristics were associated with the use of bamboo fibre, LBG, pea fibre and guar gum. In the light of previous reports indicating poor quality bread as a result of DF addition in the formulation, the present observations become even more exciting and encouraging since they reveal that there is a good potential to develop DF enriched bread. However, sensory analysis needs to be carried out before making any conclusive statements.

Figure 3.5. Bread crumb lightness (Y values)



Statistical analysis indicate that crumb lightness was significantly affected by both type of fibre used ($p<0.001$) and the level of DF used ($p<0.05$). Increasing levels of DF added to the formulation appear to decrease crumb lightness; however a statistical significant

decrease was observed only at 10% level of DF addition (Table 3.5). This indicates that crumb structure was affected by DF addition (also shown by the specific volume results). Increasing levels of DF resulted in bread crumb with non-uniform and larger gas cells typically giving a darker appearance (lower percentage reflectance). Regarding the type of DF used, it appears that only the use of guar gum or HiSol resulted in a crumb with lightness significantly different from the control bread. Bread containing guar gum appeared to have a lighter crumb, suggesting that at the levels used guar gum improves crumb structure. Conversely, bread containing β-glucan as HiSol had a significantly darker crumb which was due on one hand to the darker pigmentation of the β-glucan enriched flour (HiSol) used as an ingredient, and on the other hand to the detrimental effect HiSol had on the crumb structure (Table 3.5).

Crust lightness was significantly affected by the type of DF used ($p<0.001$ with the same extremes: lighter crust for the guar gum containing bread and darker crust for the bread with HiSol), but not by the level of DF ($p>0.05$).

3.3.3 Influence of DF on bread textural characteristics during storage.

Besides a general loss of aroma and flavour, the main changes associated with staling of bread are an increase in firmness and decrease in springiness of the bread crumb, both of which contribute to the depreciation of the final product quality. The change in crumb firmness is mainly associated with moisture migration within bread loaf (from crumb to crust), moisture redistribution between the flour components (starch, gluten, pentosans) and starch retrogradation/recrystallisation.

Crumb firmness and springiness were assessed during 4 days of storage using Texture Analysis and the results obtained are summarised in ANOVA Table 3.6. Three main

effects are evident. The first of these was expected and is the progressive firming ($p<0.001$) and decreasing springiness ($p<0.001$) of all the breads assessed with increasing storage time indicating that the changes associated with the physical characteristics of staling took place for all treatments. The second aspect of interest is that increasing levels of DF used lead to increasingly firmer ($p<0.001$) and decreasingly springy ($p<0.001$) bread crumb. This trend is not surprising if we consider the results obtained for the bread specific volume. Higher levels of DF resulted in lower specific volumes, which would correspond to a closer, denser crumb structure and hence firmer crumb.

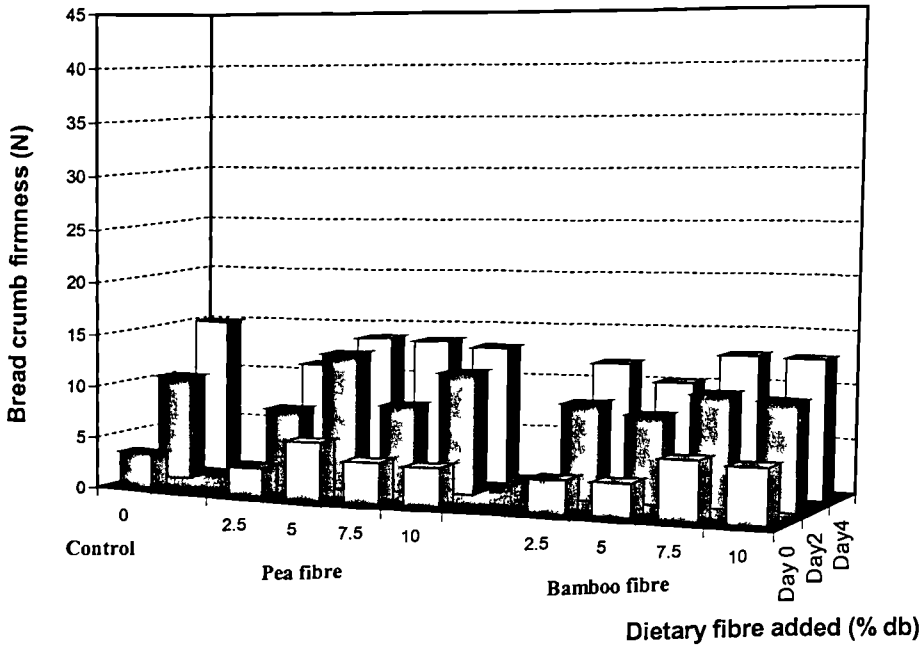
Table 3.6. ANOVA table summarising the textural attributes of bread crumb (the values represent means of all values at a given treatment level)

Sample	Firmness (N)	Springiness (%)
Control	*10.3±0.42*	*59.2±2.85*
Effect of the type of dietary fibre		
Pea fibre	9.0^{c}	56.9^{b}
Inulin	21.6^{a}	52.1^{c}
Guar gum	$7.0^{c,d}$	56.6^{b}
Bamboo fibre	$8.4^{c,d}$	57.0^{b}
Hi Sol	14.8^{b}	50.5^{c}
Xanthan gum^{XX}	$8.3^{c,d}$	61.7^{a}
Locust bean gum	5.9^{d}	57.1^{b}
Significance	***	***
SEM	0.64	0.52
Effect of the level of DF addition		
2.5%	8.9^{A}	57.3^{A}
5.0%	$9.7^{A,B}$	57.0^{A}
7.5%	$11.3^{B,C}$	55.8^{A}
10.0%	12.8^{C}	53.8^{B}
Significance	***	***
SEM	0.48	0.39
Effect of the storage time		
Day 0	4.5^{X}	64.0^{X}
Day 2	11.7^{Y}	54.1^{Y}
Day 4	15.9^{Z}	50.0^{Z}
Significance	***	***
SEM	0.44	0.36

- within the same column, the values with the same letter are not significantly different;
- *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant
- ^{XX} formulations with 7.5% and 10% xanthan gum were not included in the analysis

Most interesting, however, was the effect of the type of DF on the textural attributes of bread crumb (Table 3.6 and Figures 3.6 and 3.7). Both crumb firmness and springiness were significantly affected by the type of DF used ($p<0.001$). Amongst the DF used, inulin and β-glucan (as HiSol) had a negative influence on bread crumb resulting in increased overall firmness and decreased overall springiness in comparison to the control and all the other breads containing various types of DF (Table 3.6 and Figure 3.7).

Figure 3.6. Evolution during storage of the firmness of bread containing insoluble DF as assessed with the Texture Analyser



These results are in agreement with those for bread specific volume (Table 3.5). Inclusion of either inulin or β-glucan in bread formulation led to a decrease in loaf specific volume, indicating denser crumb structure and hence higher firmness in comparison to control bread. Nevertheless, it is worthwhile to note that although the overall specific volume of bread containing β-glucan was significantly lower than of that containing inulin, the overall firmness of the bread containing inulin was the highest. This appears to be due to higher rates of bread firming observed for the bread containing inulin (Figures 3.7 and 3.8)

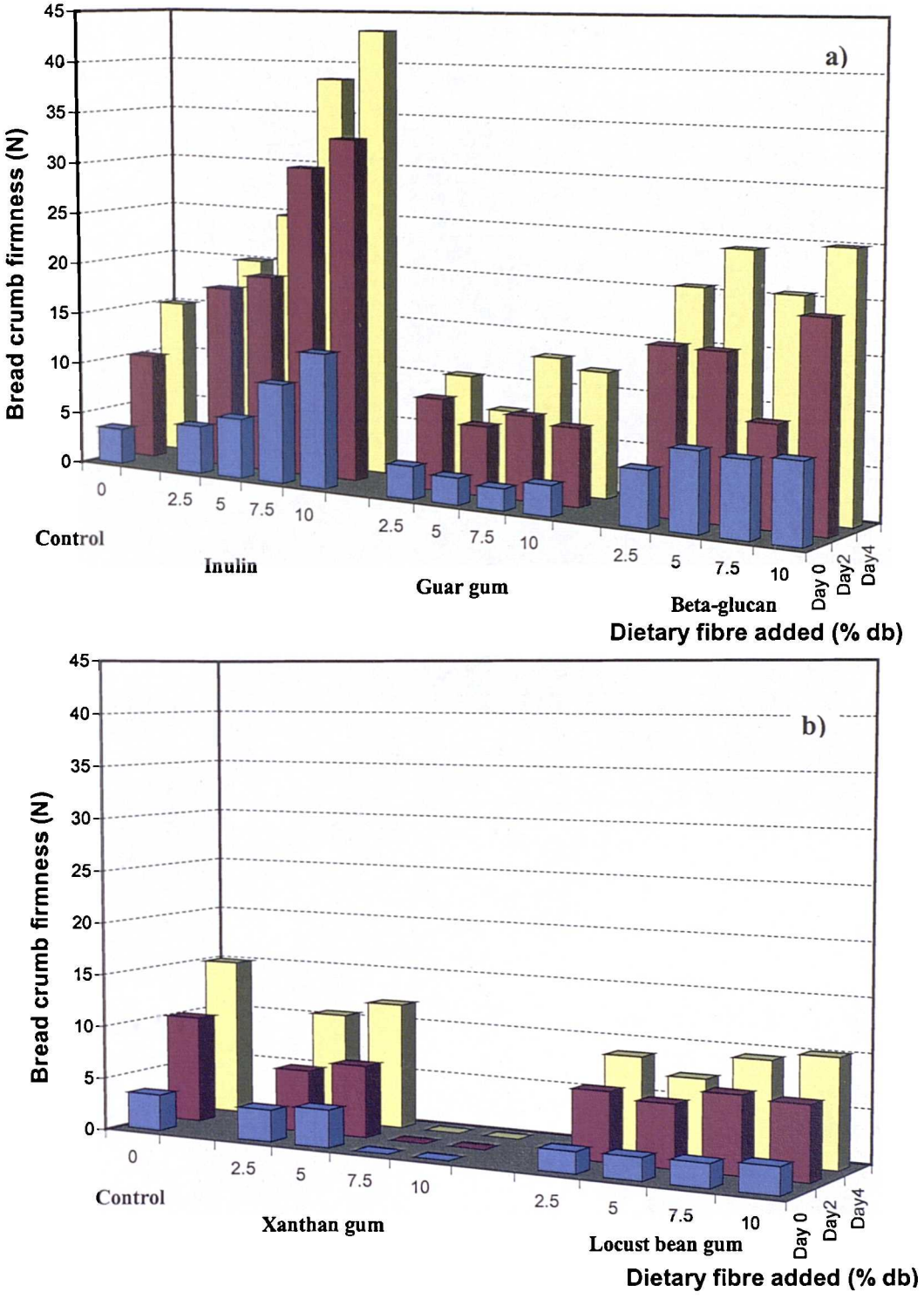
in comparison to β-glucan containing bread or with any other type of bread and probably partially related to the initial bread formulation which necessitated overall the lowest amount of water (Tables 3.2 and 3.4). Similar results indicating increased crumb firmness in bread containing inulin (at levels higher than 3%) were previously reported by Wang et al. (2002a) and O'Brien et al. (2003).

All the other types of fibres resulted in either similar (e.g. pea fibre) or improved (e.g. guar gum, xanthan gum, LBG, bamboo fibre) textural characteristics in comparison to the control: decreased overall crumb firmness and increased overall crumb springiness (Table 3.5 and Figures 3.6 and 3.7). In the case of insoluble DF (pea fibre and bamboo fibre) the slight decrease in overall firmness appears to be due mainly to decreased rates of firming (calculated as the ratio between crumb firmness at the assessment day and crumb firmness at the day of bread production - day 0) during the storage period (Figures 3.6 and 3.8). A similar effect related to the use of pea fibre (at 3% addition level) was reported by Wang et al. (2002a) but no results were previously available for higher levels of pea fibre.

The best results in terms of crumb textural attributes and their evolution during storage were obtained for the formulations containing LBG, followed by guar gum and xanthan gum (Table 3.5). Statistical analysis showed however that breads containing guar gum, xanthan gum, LBG or bamboo fibre were not significantly different in terms of crumb firmness. Figure 3.7 suggests that these effects are due on one hand to the production of a bread with initially softer crumb than the control, and on the other hand by reduced rates of crumb firming (Figure 3.8). The results illustrated in Figure 3.8 suggest that the most effective DF in reducing the bread firming rate were bamboo fibre, β-glucan (as HiSol), xanthan gum and pea fibre. LBG and guar gum appeared to have a smaller effect on the rate of crumb firming, but they were very effective in reducing the initial firmness of the

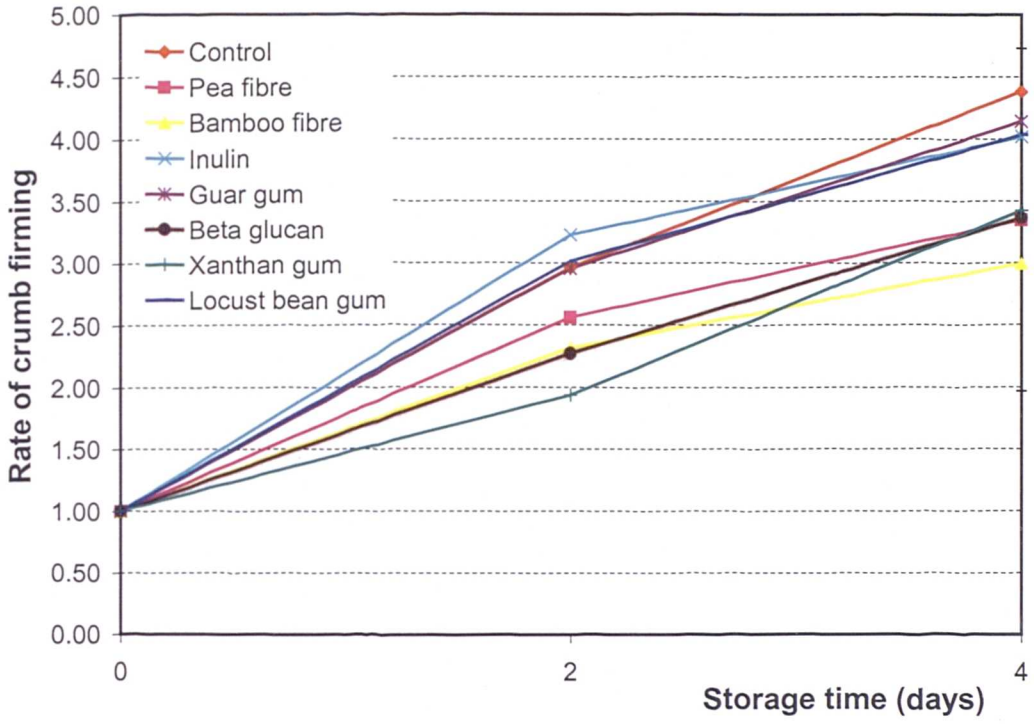
crumb. Hence, bread containing these DF were still softer than the control even after 4 days of storage (Figure 3.7).

Figure 3.7. Evolution during storage of the firmness of bread containing soluble DF as assessed with the Texture Analyser a) firmness of bread containing inulin, guar gum and β-glucan; b) firmness of bread containing xanthan gum and LBG



Previous research studies on the effect of DF on bread staling indicated similar effects of certain DF (hydroxypropylmethylcellulose, LBG, guar gum) on retarding crumb firming, when used at levels ranging from 0.1 to 1% (Guarda et al., 2004; Barcenas et al., 2004; Ribbota et al., 2004; Davidou et al., 1996). The effect was found to be dependent on the specific DF (Guarda et al., 2004), and the proposed explanation related to potential changes that may occur in the amorphous part of the crumb due to the presence of DF which potentially inhibit starch-gluten interactions and water redistribution between gluten and starch, or slows down the retrogradation of starch (Davidou et al., 1996).

Figure 3.8. Effect of DFs on the rate of bread firming



3.3.4 Influence of DF on bread digestibility *in vitro*

The parameters thought to be representative for monitoring bread digestibility and which were evaluated by the *in vitro* procedure are presented in the ANOVA Table 3.7 and illustrated in Figures 3.9-3.12. Statistical analysis indicated that all these parameters related to the digestibility of DF enriched bread were significantly affected by both the types and the level of DF used in bread formulation ($p<0.001$; Table 3.7), and this is in agreement with the effects seen in pasta (Chapter 2). Taking into account the proportion of starch digested at various digestion intervals (150, 180, and 300 min), the statistical analysis indicated that on average, bread samples containing 7.5% or 10% DF in the formulation were significantly less digestible than bread containing 2.5 or 5% DF.

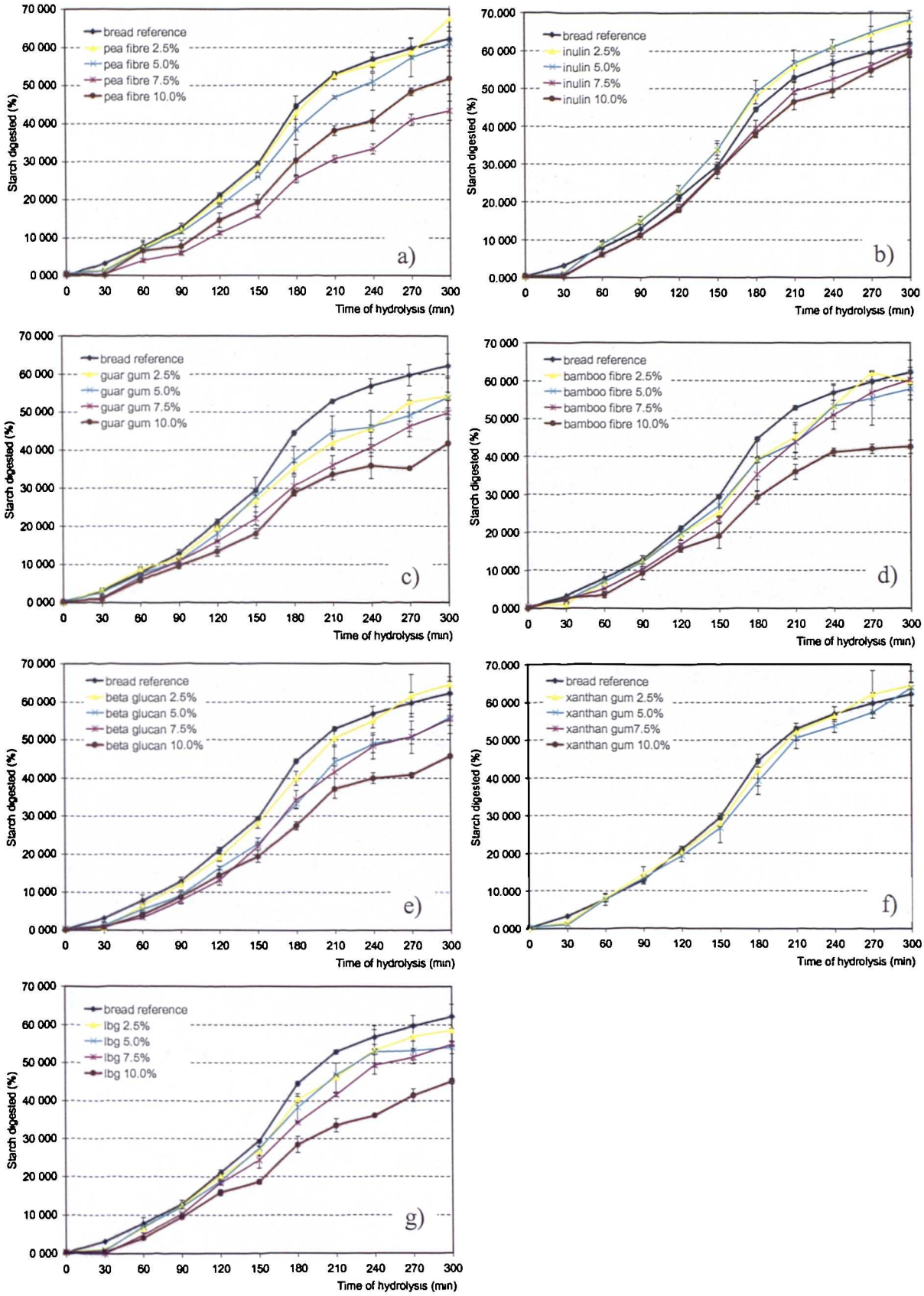
Although the reference (control) white bread was not included in the ANOVA - General Linear Model analysis, comparison of the results presented in the Table 3.7 (mean values and the corresponding standard error of the mean - SEM) appear to indicate that overall, the addition of 2.5 or 5% DF did not significantly affect starch hydrolysis rates in comparison to the control. However, at 7.5 or 10% addition levels, DF seem to significantly reduce bread digestibility. This is also evident from Figure 3.9 presenting the *in vitro* bread hydrolysis curves for all the types of DF and levels of addition used.

Table 3.7. ANOVA table summarising the digestion characteristics of DF enriched bread and its thermal characteristics (the values represent means of all values at a given treatment level)

Sample	Enthalpy (J/g)	Starch digested after 150 min(%)	Starch digested after 180 min(%)	Starch digested after 300 min(%)	Hydrolysis Index	Predicted glycaemic (GI cf. Eq. 2.5)	Predicted glycaemic (GI cf. Eq. 2.6)
Control	*0*	*29.3±0.4*	*44.5±0.6*	*62.2±3.2*	*100*	*96.3±5.7*	*94.4±1.6*
Effect of the type of DF							
Pea fibre	0	22.3^{b}	34.1^{b}	$55.9^{b,c}$	$79.2^{a,b}$	90.6^{b}	$76.5^{a,b}$
Inulin	0	31.0^{a}	43.6^{a}	64.1^{a}	89.9^{a}	116.9^{a}	85.7^{a}
Guar gum	0	23.5^{b}	32.9^{b}	49.8^{c}	76.8^{b}	90.5^{b}	74.4^{b}
Bamboo fibre	0	23.6^{b}	35.6^{b}	$55.0^{b,c}$	76.1^{b}	89.8^{b}	73.8^{b}
β-glucan (as Hi Sol)	0	23.1^{b}	33.7^{b}	$55.4^{b,c}$	67.6^{b}	86.9^{b}	66.5^{b}
Xanthan gum[XX]	0	23.9^{b}	36.3^{b}	$59.4^{a,b}$	$78.5^{a,b}$	89.9^{b}	$75.8^{a,b}$
Locust bean gum	0	25.1^{b}	36.2^{b}	$53.1^{b,c}$	74.9^{b}	92.0^{b}	72.8^{b}
SEM		0.85	1.14	1.61	2.7	2.62	2.34
Significance	NS	***	***	***	***	***	***
Effect of the level of DF addition							
2.5%	0	28.6^{A}	41.6^{A}	62.4^{A}	92.5^{A}	101.8^{A}	87.9^{A}
5.0%	0	27.3^{A}	39.2^{A}	$59.2^{A,B}$	88.4^{A}	$97.6^{A,B}$	84.4^{A}
7.5%	0	22.5^{B}	33.2^{B}	54.6^{B}	71.2^{B}	89.8^{B}	69.6^{B}
10.0%	0	20.3^{B}	30.4^{B}	48.3^{C}	58.3^{C}	85.9^{C}	58.4^{C}
SEM		0.72	0.96	1.22	2.29	2.21	1.97
Significance	NS	***	***	***	***	***	***

- within the same column, the values with the same letter are not significantly different; *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant
- [XX] formulations with 7.5% and 10% xanthan gum were not included in the analysis

Figure 3.9. Proportion of starch digested during *in vitro* digestion of DF enriched bread: a) pea fibre; b) inulin; c) guar gum; d) bamboo fibre; e) β-glucan; f) xanthan gum; g) LBG.



The type of DF used affected significantly the rate of starch digestion ($p<0.001$; Table 3.7). After 150 and 180 minof *in vitro* the highest rate of starch hydrolysis was found in bread containing inulin, while all the other breads (containing either pea fibre, bamboo fibre, guar gum, xanthan gum, LBG, or β-glucan) appeared to be similar in terms of starch digestibility ($p>0.05$). Moreover, inulin containing breads seem to have comparable digestibility to that of the white reference bread, whilst all the other DF enriched fibres were digested at slower rates (Table 3.5). At the end of digestion time (300 min), however, the overall digestibility of both inulin and xanthan gum containing breads were similar ($p>0.05$) and no different from the control, while a more distinctive difference was noticed in relation to all the other types of DF.

Bread digestibility was shown to be reduced by the use of the following DF in the order: inulin≥control≥xanthan gum≥pea fibre ≥ β-glucan≥ bamboo fibre ≥ LBG ≥ guar gum. Amongst these, only inulin and xanthan gum appeared to yield similar results to the control bread in terms of starch digestibility, while all the other DFs were effective in reducing starch hydrolysis rates. One-way Anova was used to compare the effect of each individual DF and addition levels on starch digestibility against the white reference bread and the results are shown in Figure 3.10. This illustrated that inulin and xanthan gum had no effect on starch digestibility, while all the other DF reduced digestibility rates, but only when used at levels generally of 7.5% or higher.

Figure 3.10. Proportion of starch digested from DF enriched bread at various *in vitro* digestion times (*for simplicity purposes the comparisons were made against the control; values with the same symbol as the control are not significantly different - $p>0.05$)

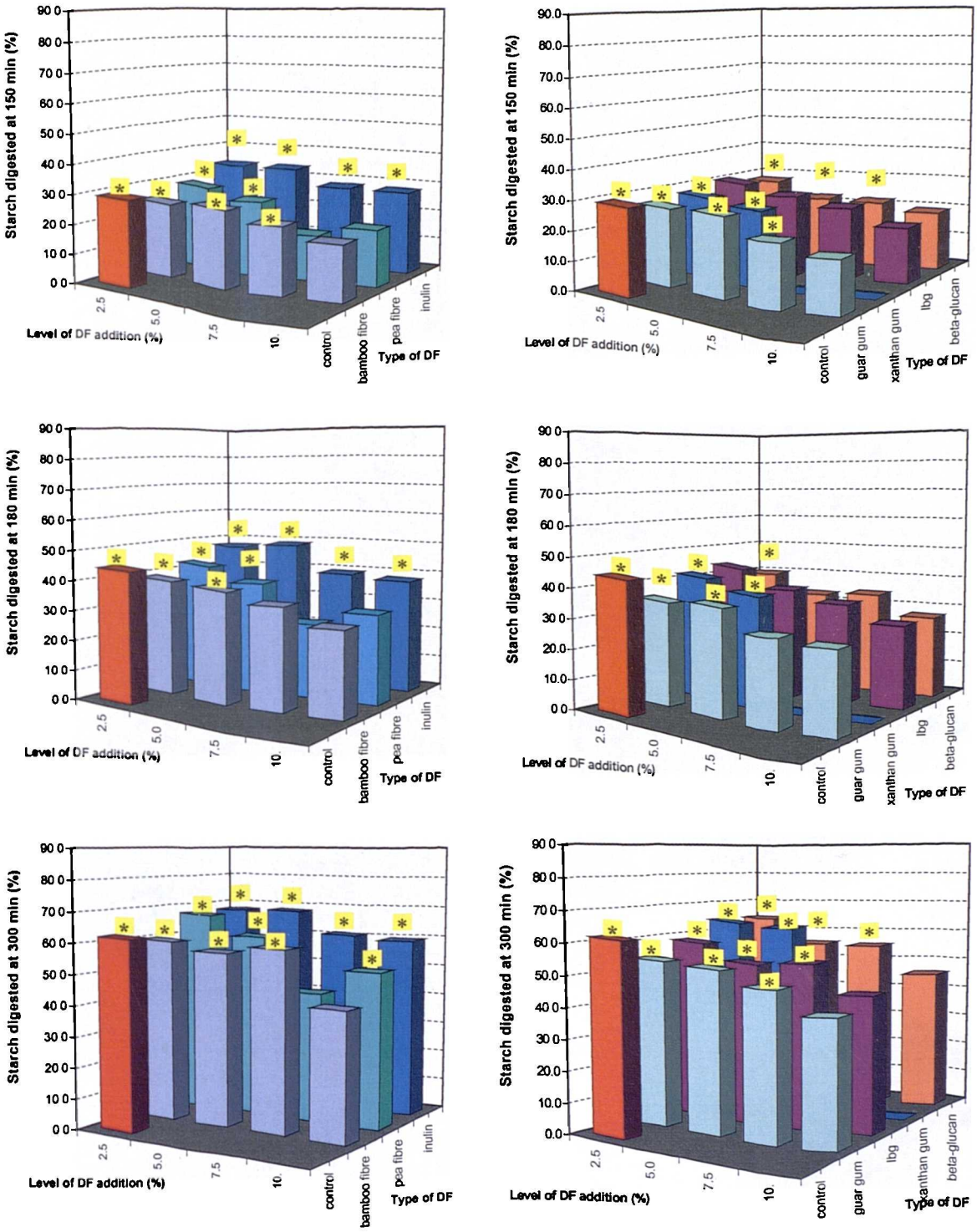
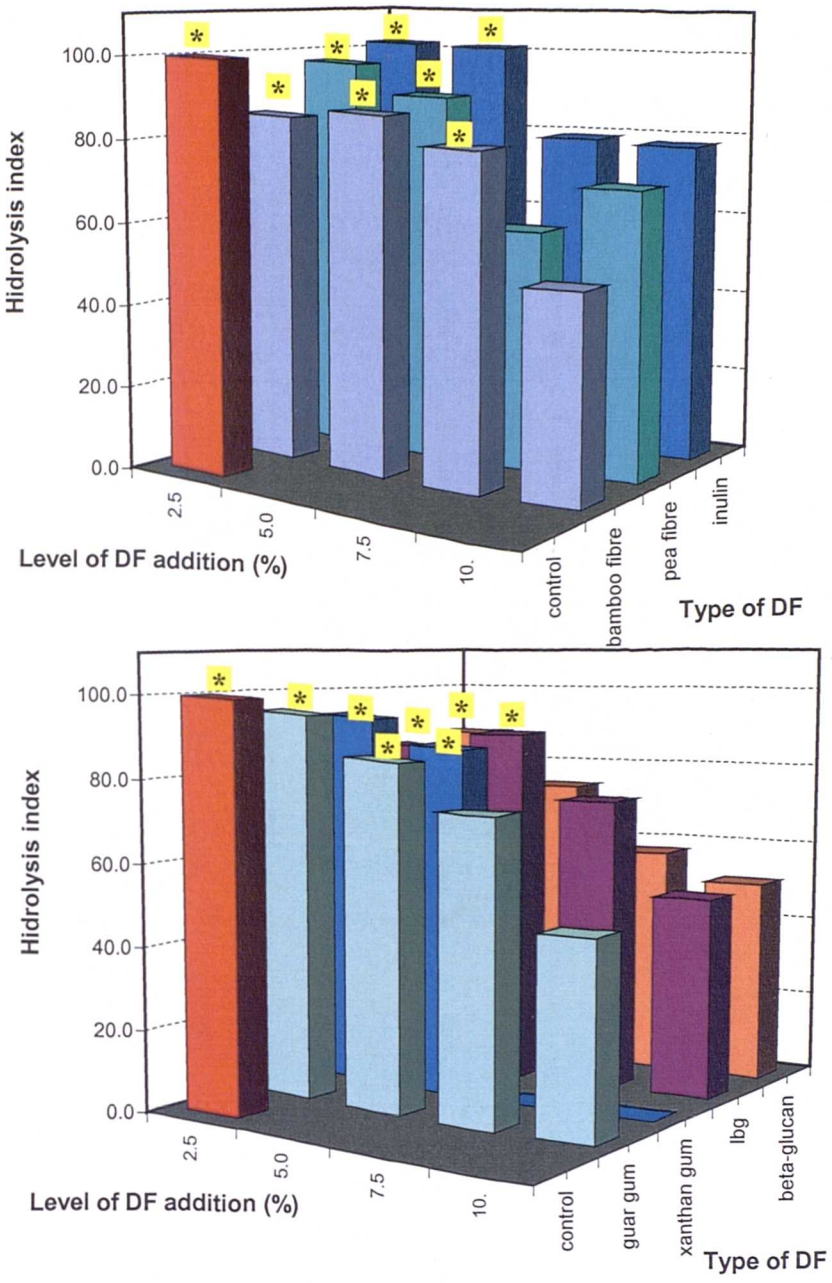


Figure 3.11. Hydrolysis indexes of DF enriched bread (*based on RSR values) (*for simplicity purposes the comparisons were made only against the control; values wearing the same symbol as the control are not significantly different - $p>0.05$)

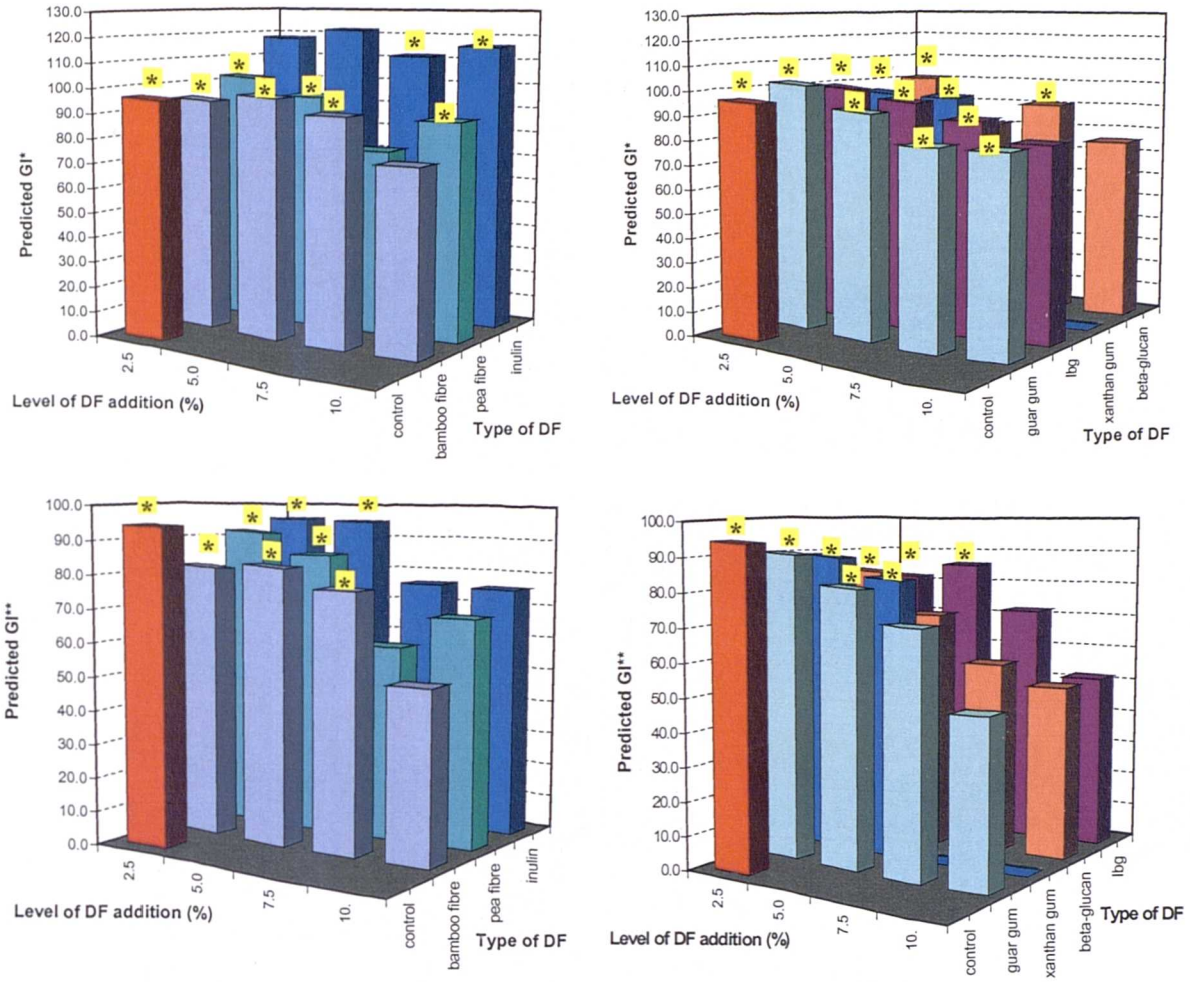


The HI values were significantly affected by both type and level of DF used ($p<0.001$ in both cases, Table 3.7 and Figure 3.11), and this is agreement with the proportion of starch digested at various times. Again, statistical analysis showed that a significant decrease in HI was obtained only when DFs were used at levels higher than 7.5%. The ANOVA Table

3.7 indicates that all types of DF led to lower average HIs in comparison to the control white bread (ranging from 89.9 to 67.6 and corresponding to a decrease in HI of 10.1-32.4%), with decreasing HI values (and thus bread digestibility) in the following order: (inulin ≥ pea fibre ≥ xanthan gum)>(guar gum ≥ bamboo fibre ≥ LBG ≥ β-glucan). It is interesting, and at the same time encouraging to note that this series (type of DF *vs* their effect of DF on bread digestibility) is similar to what was found for pasta products (Chapter 2). As found for pasta it appears that generally soluble DF produced similar HI values. In addition, bamboo fibre, although insoluble, had comparable effects on bread digestibility with soluble DFs and this confirms what was previously found in Chapter 2. However, in this experiment xanthan gum appeared to have no effect on starch digestibility. At first sight this outcome seems to contradict the results from the *in vitro* digestion of pasta enriched with xanthan gum. Bearing in mind that bread samples which used 7.5% and 10% xanthan gum in the formulation were eliminated from the experiment due to their very poor quality, it is understandable why the average values for the parameters expressing the digestion of bread containing xanthan gum were not significantly different from those of control bread or bread with pea fibre or inulin.

The predicted GIs for all types of bread were calculated using both equation 2.5 and 2.6 presented in Chapter 2 and are presented in Table 3.7 and Figure 3.12. Equation 2.6 uses the HI values ($GI_{predicted}$**=0.862HI+8.189), the predicted GI in this case being directly proportional to the HI values. Therefore it is not surprising that the statistical analysis indicated the same effects and trends for this predicted GI** as for HI. The $GI_{predicted}$** was significantly decreased by increasing levels of DF ($p<0.001$; Table 3.7, Figure 3.12) and also generally by incorporation of DF in bread formulation ($p<0.001$) (the strongest effects being related to the use of guar gum, LBG, bamboo fibre or β-glucan).

Figure 3.12. Predicted GIs of DF enriched bread (*for simplicity purposes the comparisons were made only against the control; values with the same symbol as the control are not significantly different - $p>0.05$)



A predicted GI for each type of bread and DF level was also calculated based not only on the amount of sugars released but also on the chemical composition of bread products and the diffusibility of the sugars through dialysis tubings (Equation 2.5, Chapter 2). The values are presented in Table 3.7 and illustrated in Figure 3.12. Statistical analysis showed that levels of DF of 7.5% or higher led to a significant decrease ($p<0.001$) of the predicted GI*. This conclusion also arose when the predicted GI* values were compared using One-

Way ANOVA (Figure 3.12). The DF used lead generally to similar average predicted GI*s ($p>0.05$) and similar to the GI* of control bread; the exception was made by the breads containing inulin, which had the highest average predicted GI* value. It is important to note that although the predicted GI* values calculated using equation 2.5 were higher than when equation 2.6 was used, the two GI series were relatively well correlated ($R^2=0.74$). Moreover, the predicted GI values for control bread were within the range of GI reported for this type of bread (Table 1.4, Chapter 1) regardless of the equation used.

3.3.5 Influence of DF on bread microstructure

Figures 3.13 and 3.14 present SEM micrographs of bread containing DF (at 2.5% and 10%) before and after *in vitro* digestion. Figure 3.13 shows the microstructure of WWB and breads containing insoluble DF and inulin (inulin was added here because its incorporation in bread led to results closer to the insoluble DF rather than soluble DF). As previously discussed in Chapter 2, the crumb of WWB has an open, continuous structure with visible starch granules, well swollen and elongated parallel to the pore surface (Figure 3.13 a). Following *in vitro* digestion, the appearance of the WWB crumb completely changed: the network that forms the crumb appears discontinuous presenting massive holes, and no intact starch granules were visible indicating a high proportion of starch having been hydrolysed (Figure 3.13 b).

Bread containing pea fibre, inulin or bamboo fibre appeared to have a similar crumb structure in which starch granules seem to be less swollen and also less fused with starch granules from their proximate vicinity (Figures 3.13 c,e,g,i,k,m) than in control bread; this may be a consequence of restricted water available for starch swelling due to the competition for water between starch and DF. In addition, the integrity and the continuity of the starch-gluten network appeared to be altered by DF addition. This is most obvious

for samples containing inulin (Figures 3.13 g,i) but it can also be noticed in samples containing 10% pea or bamboo fibre (Figures 3.13 e,m). This observation is consistent with data showing a reduced gas retention capacity and thus reduced loaf volume (especially for samples containing inulin), which is in accordance with the results presented in paragraph 3.3.2. Additionally, these samples exhibited a crumb which seemed rougher and appeared to contain thicker cell walls, compared to the WWB; this trend appeared to accentuate as the level of DF increases. Bread with 2.5% bamboo fibre (Figure 3.13 k) showed a crumb microstructure similar to WWB (although with starch granules still less swollen) which would suggest similar quality characteristics. As expected, after 300 min of *in vitro* digestion the structure of all breads containing DF changed, presenting large pores/discontinuities due to the enzymatic action (Figures 3.13 d,f,h,j,l,n). However, in samples containing pea fibre or bamboo fibre partly digested starch granules were still visible (especially at 10% addition level), while no starch granules appear to be present at the end of *in vitro* digestion in bread containing inulin. These observations confirm what was found from the starch hydrolysis curves (paragraph 3.3.4), and give a visible record of the events related to starch degradation during the *in vitro* digestion procedure.

Figure 3.13. SEM micrographs (x 500) for bread containing insoluble DF and inulin before and after 300 min *in vitro* digestion: a) bread control; b) bread control - digested; c) pea fibre 2.5%; d) pea fibre 2.5% - digested; e) pea fibre 10%; f) pea fibre 10% - digested; g) inulin 2.5%; h) inulin 2.5% - digested; i) inulin 10%; j) inulin 10% - digested; k) bamboo fibre 2.5%; l) bamboo fibre 2.5% - digested; m) bamboo fibre 10%; n) bamboo fibre 10% - digested (S - starch granules)

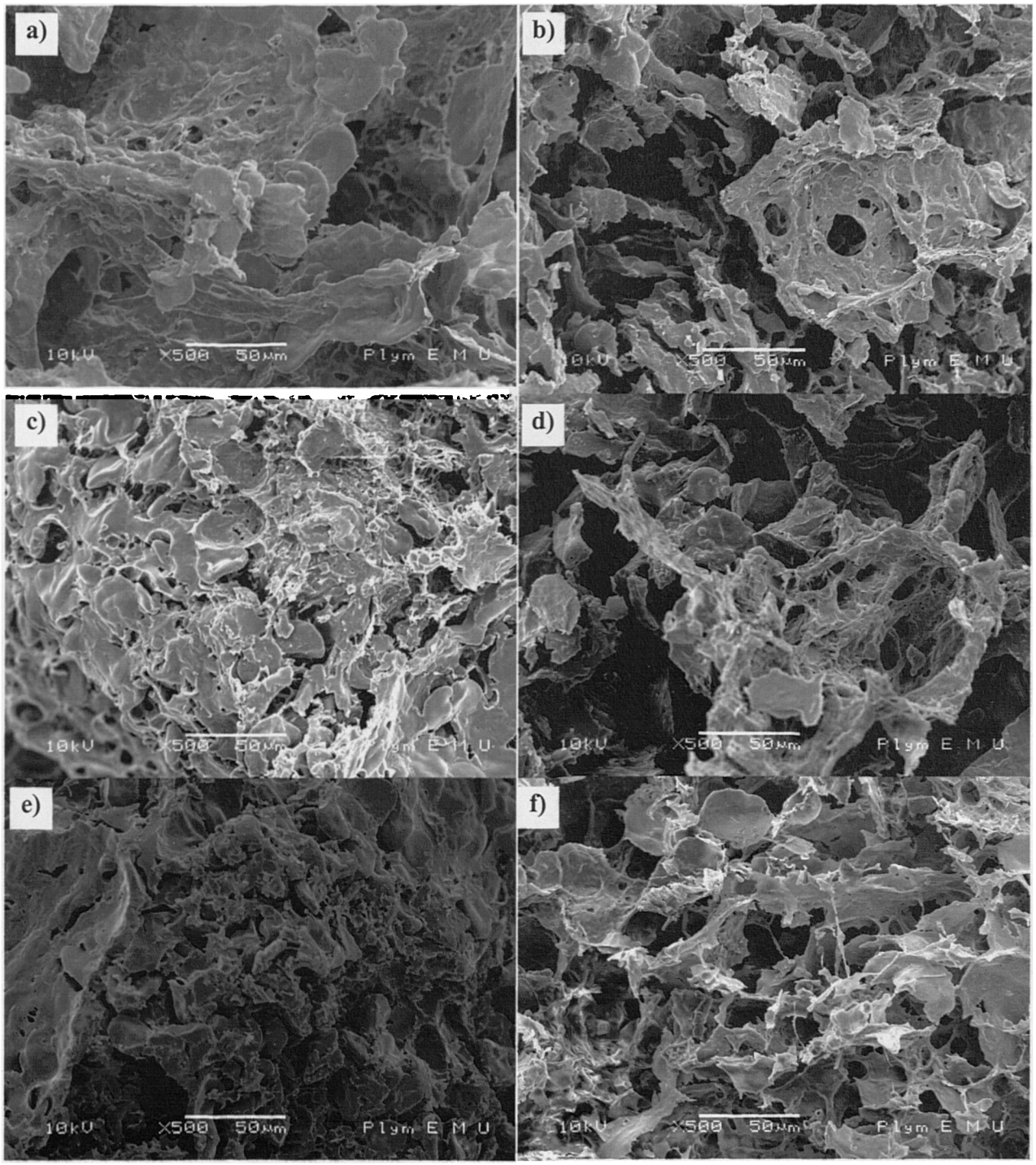


Figure 3.13. (continued)

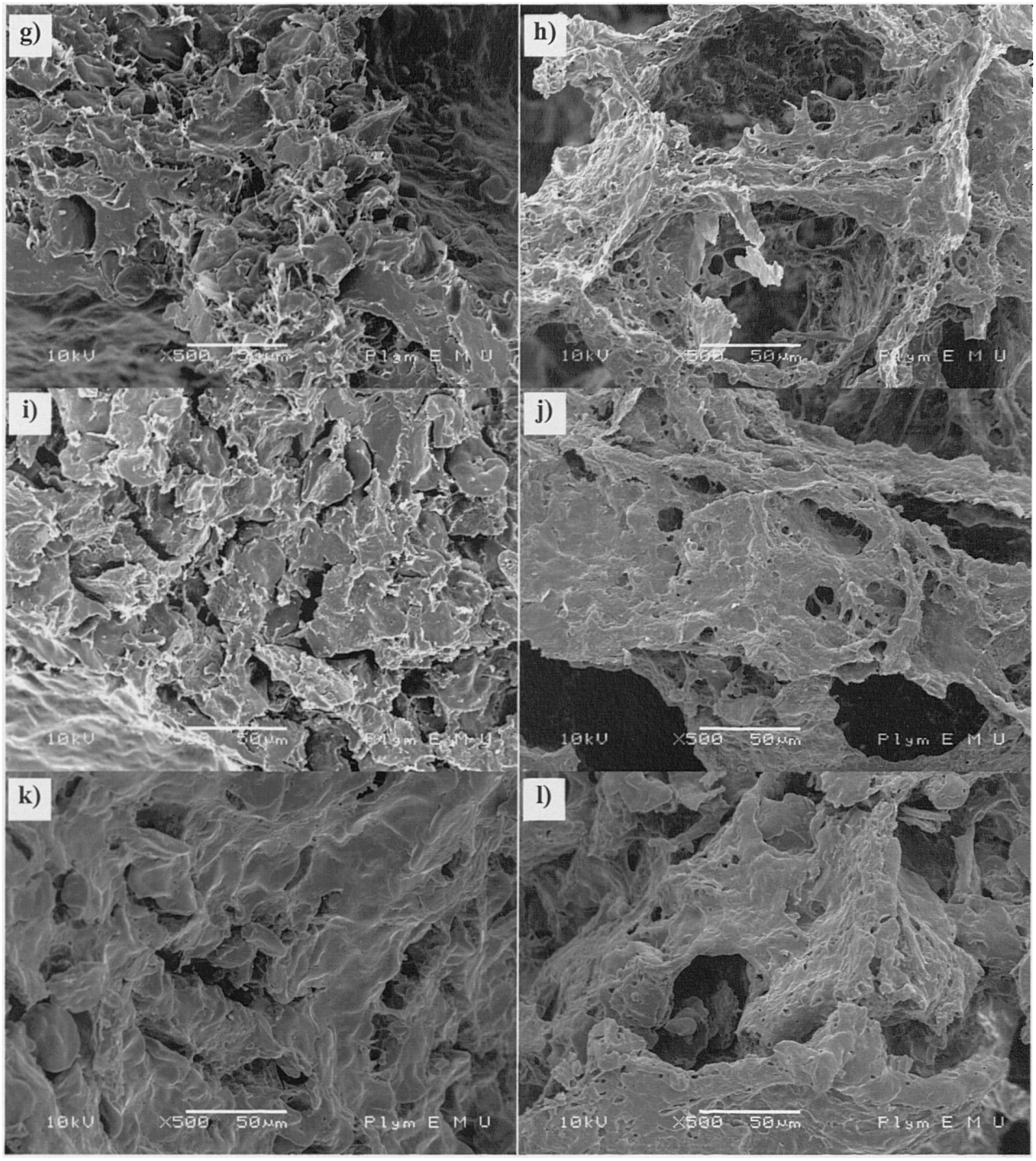
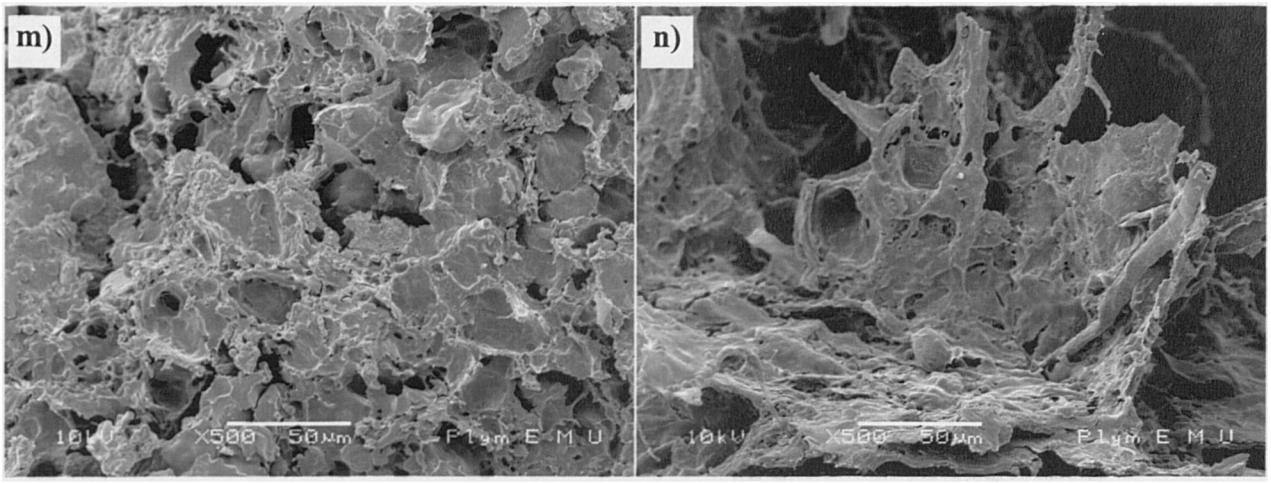


Figure 3.13. (continued)



The SEM micrographs of breads (un-digested and digested) containing soluble DF are presented in Figure 3.14. As previously observed with insoluble DF and inulin, the structure of bread crumb containing soluble DF shows starch granules less swollen than in WWB (Figure 3.14 a,c,e,g,i,k,m,o), the potential explanation being again the competition for water between starch and DF, leading to a reduced amount available for starch to fully swell. In contrast to what was found for the insoluble DF enriched breads, in these cases the starch granules are part of a continuous network composed of gluten, starch and soluble DF (which forms the cell walls). Moreover, (and similar to what was found in pasta products containing soluble DF), while maintaining their shape and remaining relatively distinct from one another, they appear to be coated in a mucilaginous looking layer (especially in samples where 10% soluble DFs were added in the formulation) formed probably from proteins and most likely soluble DF as previously reported by Brennan et al. (1996). This is the most obvious difference between these breads and bread containing either insoluble DFs or inulin. Another difference, in comparison to the bread containing insoluble DF, is the surface of the cell walls/pores which is much smoother and also continuous, indicating a better gas retention capacity, with direct

positive effects on bread volume. The exception was the bread containing β-glucan (for both 2.5% and 10% addition) which was characterised by discontinuities in the cell walls and also by rougher surfaces, which is compatible with poor gas retention capacity and hence, diminished bread volume. These observations confirm the results obtained for bread volume and specific volume (section 3.3.2).

The digested samples showed altered structures due to enzymatic attack (Figures 3.14 b,d,f,h,j,l,n,p); discontinuities of the network forming the crumb appeared, but it is worth observing that the extent of the structure damage was less striking than in breads containing insoluble DF or inulin. Of equal importance was the presence of undamaged or partially damaged starch granules in all the digested samples. In samples containing low levels of DF, small starch granules embedded in the soluble DF-gluten matrix are still visible (Figure 3.14 b,f,j,n). In comparison, digested bread samples containing high levels of soluble DF exhibit relatively high proportions of both large and small starch granules (either intact or partially damaged - Figure 3.14 d,h,l) embedded in the mucilaginous matrix, which is compatible with reduced rates of starch hydrolysis in comparison to WWB or bread containing insoluble DF. This observation is especially noticeable for samples containing guar gum or locust bean gum (at 10%) and confirms what was reported in paragraph 3.3.4 with regard to bread digestion parameters (HI and predicted GI).

Figure 3.14. SEM micrographs (x 500) for bread containing soluble DF before and after 300 min *in vitro* digestion: a) guar gum 2.5%; b) guar gum 2.5% - digested; c) guar gum 10%; d) guar gum 10% - digested; e) β-glucan (as HiSol) 2.5%; f) β-glucan (as HiSol) 2.5% - digested; g) β-glucan (as HiSol) 10%; h) β-glucan (as HiSol) 10% - digested; i) LBG 2.5%; j) LBG 2.5% - digested; k) LBG 10%; l) LBG 10% - digested; m) xanthan gum 2.5%; n) xanthan gum 2.5% - digested; o) xanthan gum 5%; p) xanthan gum 5% - digested

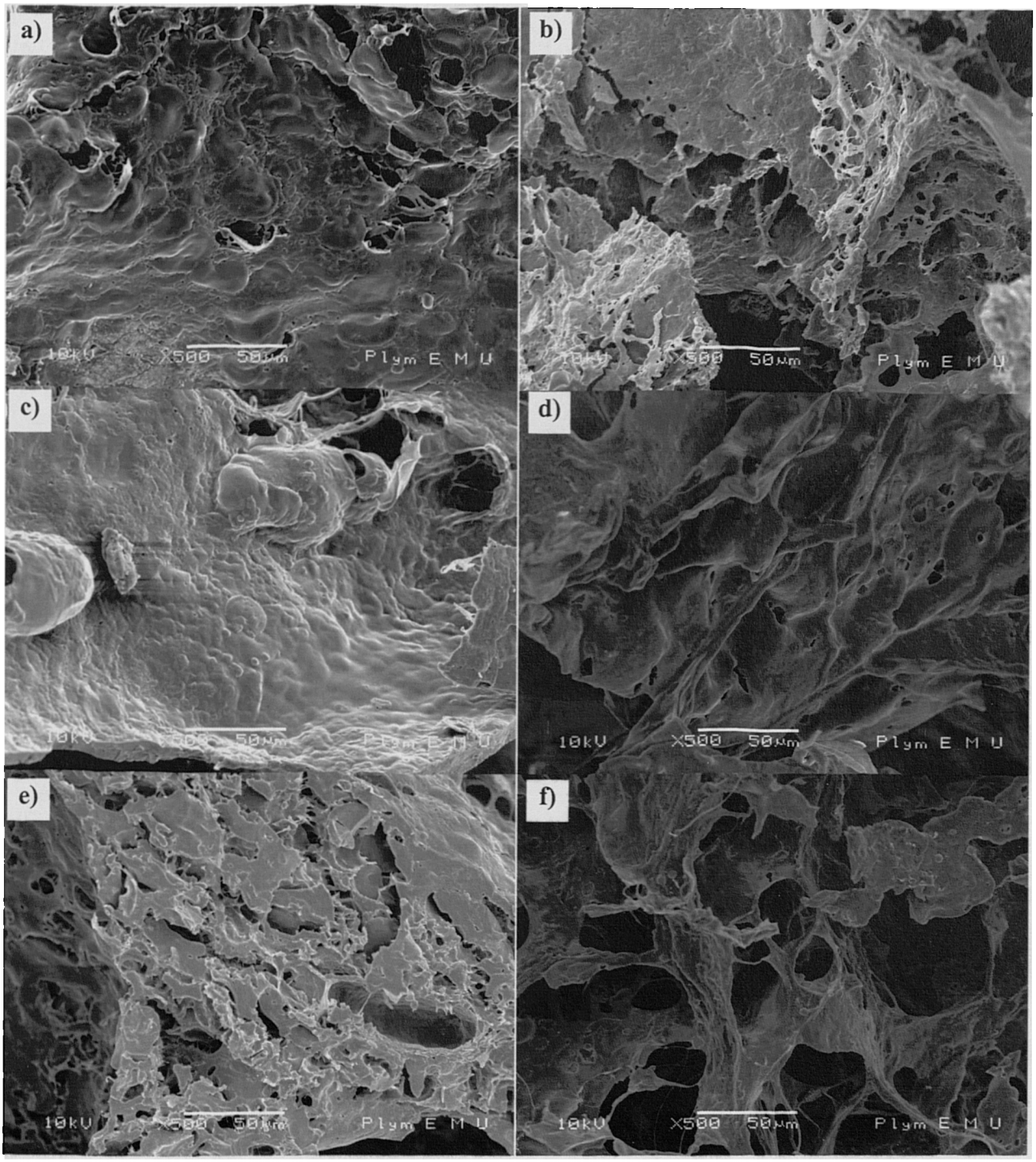


Figure 3.14. (continued)

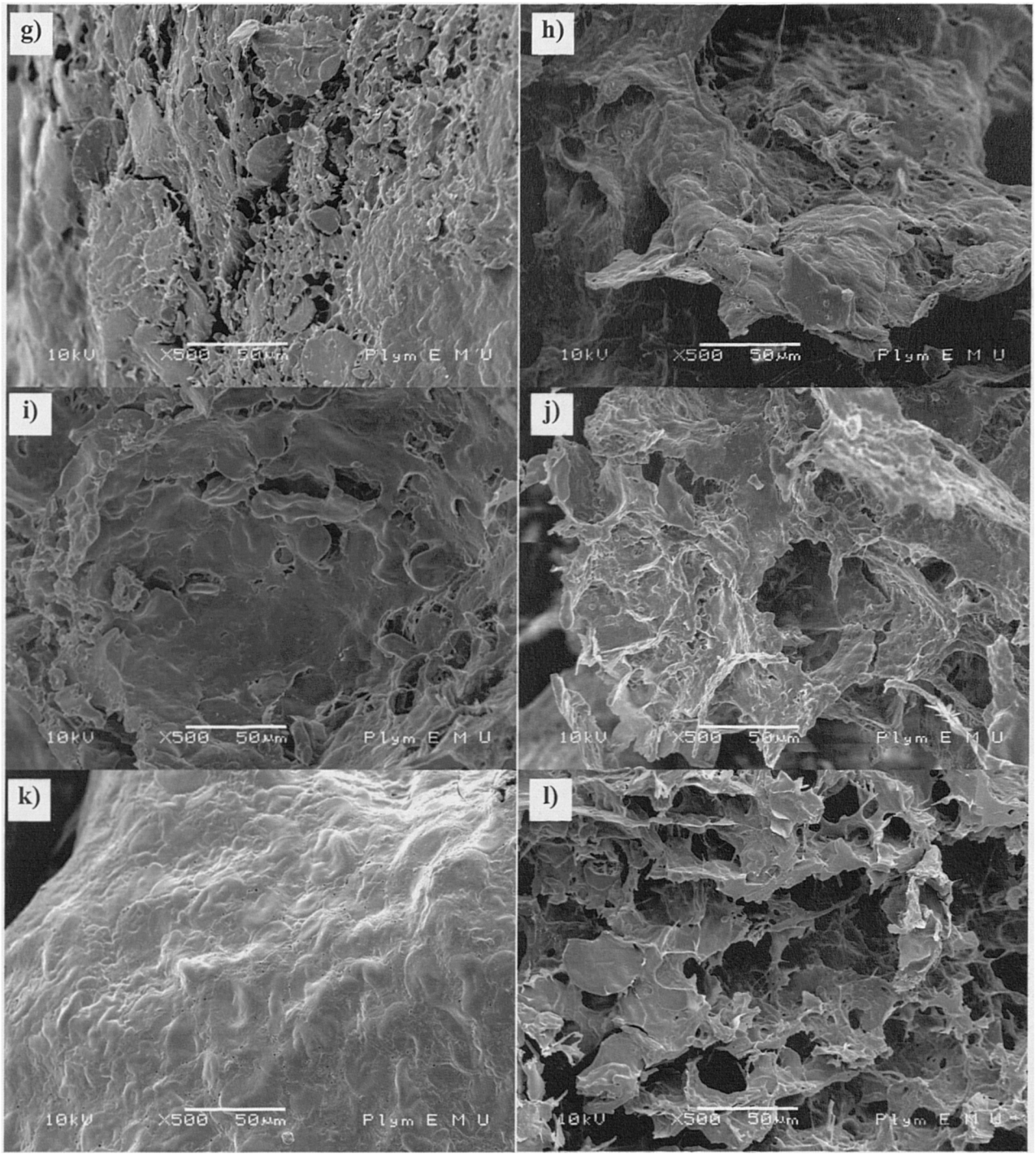
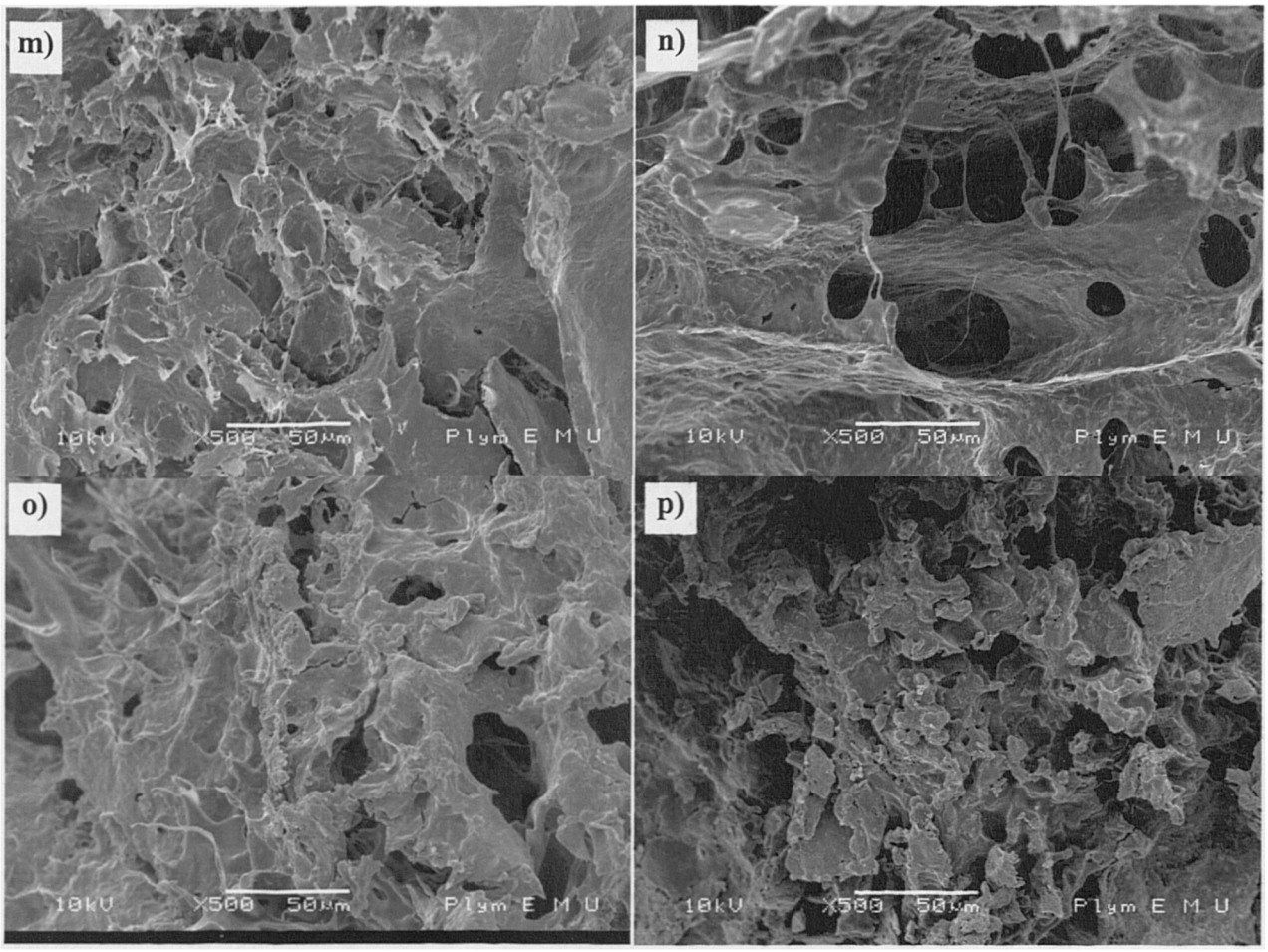


Figure 3.14. (continued)



It is important to point out that the results obtained from the *in vitro* bread digestibility parallels what was previously found for pasta: starch digestibility was decreased in products containing DFs in comparison to the control. The effect was more noticeable when soluble DFs were included in the formulation (exceptions were made by inulin which resulted in digestibility parameters comparable to the control, and bamboo fibre which resulted in a bread digestibility comparable to those containing soluble DF); increasing levels of DF resulted in decreased bread digestibility. Moreover, the relationship between the type of DFs and their effect on starch digestibility in bread was similar to what was found in pasta. Thus, these

results confirm that the effect of DF on starch digestibility is real and it manifests independent of the product's internal structure (porous as well as in compact structure).

Similar to what was found for DF enriched pasta, incomplete starch gelatinisation due to the presence of DF in the formulation can be definitely ruled out as a cause for slower rates of starch hydrolysis in DF enriched breads. This is supported by the DSC results that showed no endothermic peak between 20 and 100°C, indicating complete gelatinisation of starch during baking. Based on the *in vitro* digestibility results and microstructure information gathered using SEM, there is little reason to believe that the mechanisms involved in retarded rates of starch hydrolysis due to the presence of DF in the bread products are different from the ones suggested for pasta products.

As previously found in pasta, the swelling of starch granules in DF enriched bread (as observed in the SEM micrographs) was lower than in control bread. Moreover, in comparison with the WWB (control) where the starch granules were highly swollen and fused into the starch granules from the immediate vicinity, forming a continuous network, in the DF enriched bread the granules appeared to be distinct within the protein-DF matrix, and to maintain their integrity. This can be explained on one hand by the competition for water between starch and DF, resulting in less water left available for starch to swell, and on the other hand by thermodynamic incompatibility between the biopolymers (starch, DF, proteins) existent in bread formulation. As previously discussed in Chapter 2, when present in the formulation, DF may lead to phase separation accompanied (in the case of soluble DF) by encapsulation of starchy phase (Tolstoguzov, 2003) with DF enriched phase. This in turn may interfere with granule swelling and thus reducing the leaching of amylose from the starch granule. In WWB (control) the starch granules are highly swollen, elongated, and previous studies have found amylose rich zones outside starch granules along the

starch-protein interface (Hug-Iten et al., 1999). Taking into consideration that increased starch swelling is generally associated with increased porosity of the granules, increased amorphous material, and ultimately with an increased rate and extent of amylolysis, it becomes evident that the lesser extent of starch hydrolysis observed in DF enriched breads in comparison to WWB may be partially explained by reduced starch swelling.

The other possible explanation may rely on the formation of a physical barrier by the DF, protecting the starch granule against α-amylase attack. Soluble DFs were seen to form a mucilaginous coating around starch granules (Figure 3.14), while insoluble DFs appeared to increase the thickness of the gas cell walls (Figure 3.13). Both appeared to be related to decreased rates of starch hydrolysis.

Nevertheless, it is interesting to observe that in pasta products low levels of DFs were effective in reducing the rate of starch hydrolysis in comparison to the control (the addition of DFs in proportions as low as 2.5% had a significant effect - Table 2.9). In contrast, for DF enriched bread a percentage of 7.5 or higher of DFs is needed in order to obtain a statistically significant effect (Table 3.7), and this may be due to the product structure. In comparison to pasta, bread has a porous, open structure, with higher surface area, making the starch more exposed to enzymatic action. Therefore higher levels of DFs are needed to achieve sufficient 'barrier building' and decrease in starch swelling which would significantly impact on bread digestibility.

As also observed in Chapter 2, the differences obtained in bread digestibility within the same category of DF (soluble or insoluble), may be explained by other potential mechanisms such as inhibition of α-amylase (Slaughter et al., 2002), or absorption of sugars by DF (Ou et al., 2001). However, further experiments will be needed to confirm these for the range of DF used in the present study.

The results presented so far in this chapter indicate that there are several DFs that may be used successfully in production of high DF low GI bread, which more importantly show also good quality, and improved storage properties in comparison to the WWB. The best results were obtained with the use of guar gum, LBG and bamboo fibre which significantly improved crumb softness (Table 3.6) and loaf specific volume (Table 3.5) in comparison to the WWB; moreover, even at high level of addition (10%) these DFs produced acceptable quality products. Since for guar gum there are several published research papers, which relate its use in bread with sensory attributes and *in vivo* digestibility, it felt logical to use for the next stage (*in vivo* study and sensory assessment) formulations using LBG and bamboo fibre, and incorporated at the highest level utilised in the present study (10%).

3.3.6 Influence of DF on bread GI as determined *in vivo*

The mean incremental blood glucose response curves as obtained after the ingestion of different test meals (isoenergetic meals) are presented in Figure 3.15 and the corresponding GI values calculated at 90 min and 120 min are presented in Table 3.8.

The graphs presented in Figure 3.15 reveal that bread containing LBG or bamboo fibre produced lower glucose responses than WWB, with LBG appearing to be the most effective. Statistical analysis indicated that the blood glucose levels following the ingestion of the test meal containing DF enriched bread were significantly lower than when WWB was ingested at 15 and 30 min ($p<0.05$). Paired t-test showed no statistical differences in the increments of blood glucose levels were observed between the meals containing LBG or bamboo fibre ($p>0.05$).

Figure 3.15. Postprandial blood glucose response in healthy subjects following ingestion of breakfast test meals (n=12 and for the same sampling point, means sharing the same letter are not significantly different)

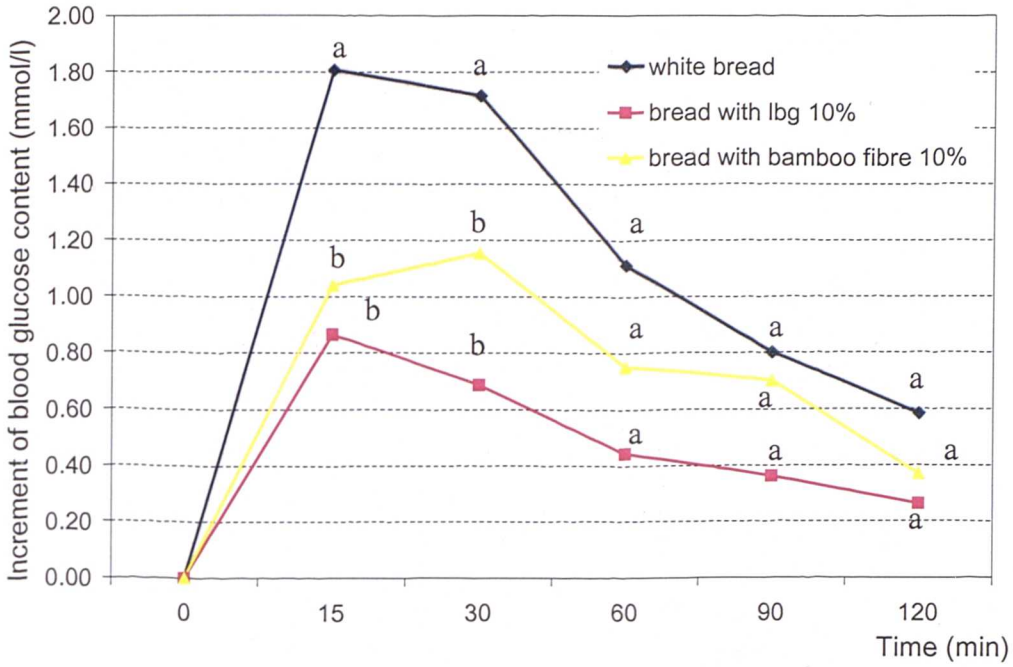


Table 3.8. Calculated GI and related SA for the test meals and predicted GIs for breads

Bread product	**GI_{90min}**	**GI_{120min}**	**$GI_{predicted}$** (eq. 2.5)	**$GI_{predicted}$** (eq. 2.6)	**SA_{120min}**
White reference bread	$100^{a,A}$	$100^{a,A}$	96.3^{a}	94.4^{a}	$100^{a,A}$
Bread with LBG (10%)	$35.8\pm12.9^{b,C}$	$42.9\pm14.8^{b,C}$	81.9 ± 1.4^{b}	51.6 ± 2.3^{b}	$129.0\pm38.6^{a,A}$
Bread with bamboo fibre (10%)	$56.6\pm13.5^{b,B}$	$68.7\pm10.9^{b,B}$	74.8 ± 2.6^{b}	51.7 ± 5.5^{b}	$120.1\pm25.3^{a,A}$

- values in the tables represent means (n=12) ± SEM
- within the same column, values sharing the same letter are not significantly different
- lower cases are related to the results from the paired t-test, while upper cases are related to non-parametric Wilcoxon test for paired observations

According to the paired t-test, the calculated GIs of test meals containing either LBG or bamboo fibre were significantly lower than those for WWB ($p<0.05$, Table 3.8) regardless of the point at which they were calculated (using IAUC at 90 min or 120 min). However,

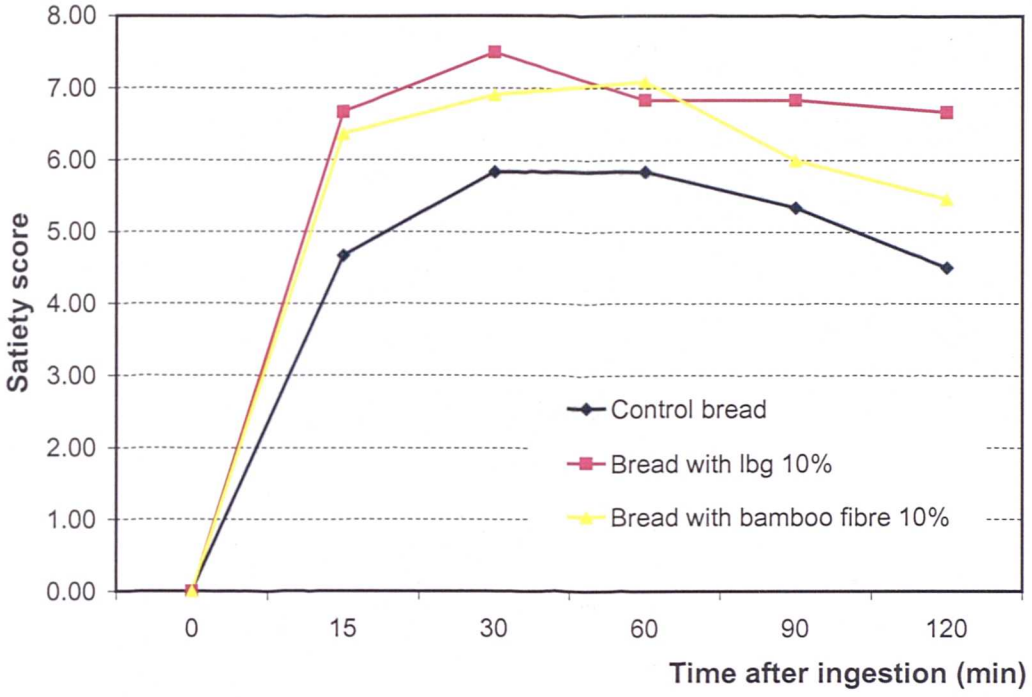
when compared against each other, the GIs of the test meals containing LBG or bamboo fibre were not significantly different ($p>0.05$), although the mean values indicate that LBG appears to be more effective in reducing the calculated GI (Table 3.8). Nevertheless, when the results were analysed using the non-parametric Wilcoxon test for paired observations the GI's of all test meals were significantly different from the control and from each other, regardless of the IAUC used (90 min or 120 min) ($p<0.001$, Table 3.8). The analysis of the increments in glucose responses using repeated measures design revealed that all the type of bread ingested, the sampling time and the subjects significantly affected the raise in the blood glucose ($p<0.001$). Moreover, similar with that found using the Wilcoxon test, all test meals were significantly different from the control and from each other ($p<0.05$). These findings are interesting and extremely important because they confirm the results found during the *in vitro* digestion of bread, which indicated a significant decrease of the predicted GIs of bread containing LBG or bamboo fibre (at 10% addition) in comparison to the WWB (Figure 3.12). Even more exciting are the results obtained for the bread containing bamboo fibre (an insoluble DF), showing decreased GI values (*in vivo* as well as *in vitro*) in comparison to WWB, and similar to LBG (a soluble DF) enriched bread. It is worth to point out that this kind of effect was previously thought to be mainly related to the use of soluble DFs in food formulations. The *in vivo* results proved that the selected DFs (LBG and bamboo fibre) flatten the glycaemic response when they are part of the meal and that the *in vitro* digestion method represents an useful tool in predicting the GI of the test meals and also in ranking various products according to their potential glycaemic response.

3.3.7 Influence of DF on satiety

The mean incremental satiety scores obtained after the ingestion of the test meals are presented in Figure 3.16 and the corresponding satiety areas (SA) are shown in Table 3.8.

These results indicate a slight increase in satiety after ingestion of DF enriched meals in comparison to WWB. The mean SA values for LBG and bamboo enriched meals were 129.0 and 120.1 respectively, both higher than for WWB (100). However statistical analysis (paired t-test, Wilcoxon test, and repeated measures design) indicated no significant difference ($p>0.05$). The lack of significance may be related to the number of subjects used in this study. Probably many more subjects are needed to take part in the study in order to obtain a definitive conclusion in this respect.

Figure 3.16. Mean satiety scores evaluated in healthy subjects following ingestion of breakfast test meals (n=12)



3.3.8 Influence of DF on bread sensory attributes

The mean hedonic scores as produced by the assessors for the control and the two DF enriched breads are illustrated in Figure 3.17 and presented in Table 3.9.

Figure 3.17. Mean hedonic scores for WWB and DF enriched bread as evaluated by the assessors (n=12)

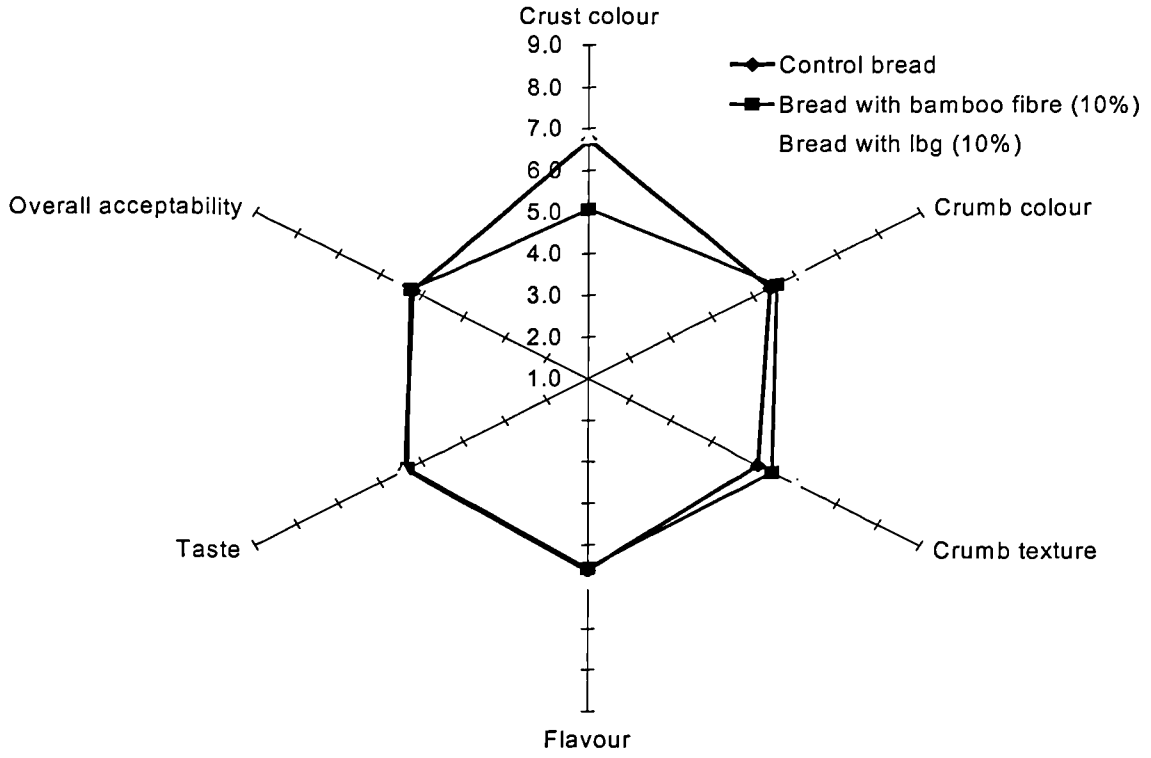


Table 3.9. Sensory characteristics of WWB and DF enriched bread

Bread product	Crust colour	Crumb colour	Crumb texture	Flavour	Taste	Overall acceptability
White reference bread	6.7±0.3[a]	5.4±0.3[a]	5.1±0.4[a]	5.6±0.4[a]	5.4±0.4[a]	5.2±0.4[a]
Bread with LBG (10%)	6.8±0.3[a]	6.2±0.3[a]	6.0±0.4[a]	5.9±0.4[a]	5.4±0.4[a]	5.6±0.4[a]
Bread with bamboo fibre (10%)	5.1±0.3[b]	5.6±0.3[a]	5.4±0.4[a]	5.6±0.4[a]	5.3±0.4[a]	5.3±0.4[a]

- values in the tables represent means (n=12) ± SEM
- within the same column, values sharing the same letter are not significantly different (p>0.05)

The results reveal that all the products were acceptable from a sensory point of view, each receiving scores higher than 5 for each attribute assessed. Moreover, statistical analysis demonstrated that generally the sensory attributes of breads containing LBG or bamboo fibre were not significantly different from the control (p>0.05, Table 3.9). The only

exception was made by the crust colour of bamboo fibre enriched bread, which received a lower score than the WWB or LBG enriched bread ($p<0.05$).

These results confirm what was reported early in this chapter with regard to the quality characteristics of these breads. Both objective evaluation (bread specific volume, crumb colour and texture, crust colour) and sensory assessment of bread quality indicated that LBG and bamboo fibre (even at 10% addition level) do not diminish the palatability of the final product, conferring sensory qualities similar to WWB.

3.4 Conclusions

Encouraged by the results presented in Chapter 2, this study aimed to evaluate the effects of DFs (the same range and level as in Chapter 2) on dough characteristics and on the quality and nutritional attributes of DF enriched bread.

The results indicate that the type and level of DF added in the formulation altered dough textural properties and consequently bread quality attributes. However, except for β-glucan, xanthan gum and inulin (used at high levels), the use of DF led to the production of products with quality characteristics comparable to WWB. Moreover some DFs (LBG and bamboo fibre) appeared to improve the textural characteristics of the crumb and its storage properties, and what is even more important - sensory analysis performed on selected samples revealed that the palatability of the product was similar to the WWB.

Equally significant are the outcomes from the *in vitro* digestibility of DF enriched bread, which confirmed the results reported in the previous chapter: DFs significantly reduced the rate of starch hydrolysis in comparison with WWB, making possible the reduction of bread

GI. The mechanisms involved are thought to be similar to those discussed in Chapter 2: the formation of a physical barrier between the starch and α-amylase, reduced rate of starch swelling and possibly the inhibition of α-amylase by DF. A pilot *in vivo* experiment on healthy volunteers was also performed using as test meals selected bread samples and the results were in accordance to those from the *in vitro* study. This result is of importance when considering the use of *in vitro* methods for the determination of GI of foods, and justify the *in vitro* studies conducted both in this chapter and in previous one.

This study showed that the use of DFs offers a possible and suitable way to tailor low GI breads and to improve the nutritional quality of the bread as far as GI and DF content are concerned. Thus the physiological benefits of high DF intake are complemented by the metabolic merits of a low GI diet. In addition it was demonstrated that certain DFs do not diminish the quality and sensory acceptability of the final products; this offers a significant clinical potential in the development of new food products of considerable therapeutic use (e.g. the management of diabetes).

Chapter 4. Dietary fibre and dairy products. - The effect of dietary fibre on the physico-structural properties of low fat dairy products: curd and yoghurt

4.1 Introduction

Many health organisations consider that the level of fat consumption is too high, which in long term has negative implications for the public health. The Surgeon General's Report on Nutrition and Health (Anon, 1988) stated that high intake of total dietary fat is associated with increased risk for developing obesity, some types of cancer, CVD and possibly gallbladder disease. A recent WHO report recommended that the level of total fat intake should be between 15-30% of the energy, of which saturated fatty acids should account for less than 10% energy (WHO, 2003). Such reports started to modify eating patterns: reducing dietary fat becoming a major dietary goal for many consumers. Due to a general nutritional recommendation to reduce calories and blood serum cholesterol level, and with encouragement from health groups, the public is increasingly choosing foods low in fat. Within this general trend, there has been an increased market interest during recent years for low fat dairy products, increasingly popular being low fat skim milk, low fat yoghurt and reduced fat cheese (Kentor, 1990). However producing such foods is not a straightforward task.

The presence of fat in dairy products has considerable impact on their physical properties, rheological and textural characteristics, and microbiological stability (Rosenberg, 1992; Olson and Johnson, 1990; Chronakis, 1997). In addition, fat influences other product characteristics such as handling, stability, appearance, flavour, and mouthfeel (Olson and Johnson, 1990; Clark, 1994). For example low fat yoghurts are poorly acceptable mainly due to rheological characteristics and increased syneresis, while the main problem associated with low fat cheese is related to changes in texture (becoming rubbery and hard) and flavour. While most consumers are aware of the health benefits of low fat diets, they

are not prepared to sacrifice in exchange the taste, texture and aroma they enjoy in dairy products (McIlveen and Armstrong, 1995). Thus, the goal of the food industry is to respond to consumer demand and to offer an increasing variety of low fat choices, in which the attributes the consumers desires are not impaired.

A reduction in fat content can be achieved by replacing it with water and several ingredients to control this water and to provide the functionality of the missing fat. Although it is complex to create a characteristic total identical to fat, biopolymer combinations such as milk proteins, starch maltodextrins, DFs, emulsifiers and flavouring agents could be used (Clark, 1994). The challenges are to identify the fat replacer that works best for a given product.

DFs belongs to the category of carbohydrate-based fat replacers, which have been used safely for many years as thickeners and stabilisers especially in sauces and dressing formulations. They are effective fat replacers in many food systems, including heat applications. Limited research has focussed on the use of specific soluble DF in dairy products; several papers have reported the utilisation of carrageenan, gellan gum or guar gum in cheese production in relation to the texture and sensory attributes of the end product (Kailasapathy, 1996; Kailasapathy, 1998; McMahon et al., 1996). This is also true for DF applications in yoghurt, with only few papers published in this area (Fernández-Garcia and McGregor, 1997; Tamime et al., 1994; Keogh and O'Kennedy, 1998).

Therefore this study aimed to contribute to the understanding of the behaviour of certain DFs in milk formulations designated to be used in cheese or yoghurt production. Based on published studies (Fernández-Garcia and McGregor, 1997) and preliminary work on our laboratory (results not shown) indicating that the inclusion of insoluble DFs in milk formulations does not result in good quality products, the decision was made to concentrate

on the use of soluble DFs. Barley β-glucan, inulin and partially hydrolysed guar gum (PHGG) were chosen to be investigated for their effects on milk coagulation, and the final products (i.e. curd or yoghurt) texture and rheological properties. A substantial part of the work focused on gathering information about the structure of the products, which is particularly important to better control the texture and stability of the final dairy foods.

4.2 Materials and methods

4.2.1 Stage 1. The effects of DF on milk coagulation and curd characteristics

4.2.1.1 Materials

Food grade barley β-glucan (Glucagel™, 86% β–glucan) was obtained from PolyCell Technologies LLC, USA, inulin was Frutafit-HD (Calleva Ltd., UK), and partially hydrolysed guar gum (PHGG) (Sunfibre, Selectchemie AG, Switzerland). The DFs characteristics as provided by the suppliers are presented in Table 4.1. Commercial pepsin rennet (Stamix 50L, Chr. Hansen, UK) was used according to the manufacturers instructions (40 IMCU/litre of milk). Pasteurised and standardised milk batches (3.2% fat and 0.1% fat) were used for the tests.

4.2.1.2 Methods

4.2.1.2.1 Chemical analysis

Fat content of the milk was analysed following the Gerber (butyrometric) method (Anon., 1989) and the protein content following the Kjeldhal method (AOAC Official method 991.22 - AOAC, 1998).

4.2.1.2.2 Milk coagulation

The changes in the rheological properties of milk samples during coagulation were monitored using a controlled stress rheometer (AR1000, TA Instruments, UK) with a cone and plate geometry (40mm diameter, 2°, stainless steel). Ranges of tests were carried out to determine the linear viscoelastic range for the samples. From these tests, experimental conditions were set at low amplitude oscillation mode (maximum strain 0.02), and at a frequency of 1Hz; the gap between the plate and the base was 50μm and tests were performed at 32^{0}C for 1h (300 data acquisition points).

Table 4.1. Characteristics of DFs used in the formulations of dairy products.

	β-glucan (Glucagel™)	**Inulin (Frutafit HD)**	**Partially hydrolysed guar gum (Sunfibre)**
Chemical composition			
Dry mater content, %	>95	95.0	>95
Carbohydrates, % db		99.0	
of which:			
▪ Total dietary fibre, %db	86	95.0	80
Protein, %	<1	0.0	<0.1
Ash, %	<1	<0.1	<1.5
Physico-chemical aspects			
Particle size		10<80%<85μm	
Dispersability	Good	Good	Good
pH	7.2	Neutral	5.2-7.0
Colour	Cream white	White	White
Taste	Neutral	Neutral, slightly sweet	Neutral

The DFs were dispersed in milk at 60^{0}C for 5 min using a high-speed mixer (model SL2T, Silverson Machines Ltd., UK) operating at 8000 rpm following the formulations shown in Table 4.2. Samples containing DF were stored at 5^{0}C overnight to promote their hydration.

Prior to coagulation, milk was heated to 40^{0}C in a water bath and held for 10 min to restore cold ageing effects (i.e. to restore the ionic balance) (O' Callaghan et al., 1999b), and then cooled to the renneting temperature (32^{0}C). Rennet was added to the milk and mixed thoroughly before rheological determinations. The coagulation tests were run in triplicates.

4.2.1.2.3 Curd manufacture

Curd was made from standardised skimmed (0.1% fat), semi-skimmed (1.0% fat) and full fat (3.2%) milk with added DFs using the formulations shown in Table 4.2. DFs were dispersed in milk as previously described for milk coagulation, and subsequently used for curd manufacture consisting of pasteurisation (60°C/30 min), cooling at 32°C, coagulation at 32^{0}C/1h and whey separation. This protocol was used for the preparation of samples for curd yield, rheological, texture and microstructure characteristics.

Table 4.2. Milk - DF formulations used for the experiments on milk coagulation/curd characteristics

Milk used	β-glucan		Inulin (%, w/w)	PHGG (%, w/w)
	(%, w/w)	Code		
Full fat (3.2% fat)	0 (control)	FF_co	0	0
	0.5	FF_0.5%G	2	2
	1.0	FF_1%G	4	4
	1.5	FF_1.5%G	6	6
	2.0	FF_2%G	-	-
Semi-skimmed (1% fat)	0 (control)	SS_co	0	0
	0.5	SS_0.5%G	2	2
	1.0	SS_1%G	4	4
	1.5	SS_1.5%G	6	6
	2.0	SS_2%G	-	-
Skimmed milk (0.1% fat)	0 (control)	SM_co	0	0
	0.5	SM_0.5%G	2	2
	1.0	SM_1%G	4	4
	1.5	SM_1.5%G	6	6
	2.0	SM_2%G	-	-

4.2.1.2.4 Curd yield

The yield of fresh curd was determined as of Macheboeuf et al. (1993). Milk samples (40±1g) were placed into centrifuge tubes, rennet was added, the mixture was then vortexed for 30s and held undisturbed in a water bath at 32°C for 1h. The coagulum formed was cut, rested for 10 min, and centrifuged at 2000g for 10 min at 20^0C. The expelled whey was decanted, and the remaining curd was weighed. Fresh curd yield was determined as g of retained curd *per* 100 g milk. The test was run in quadruplicates.

Decanted whey samples were analysed in triplicate for protein content following the Kjeldhal method (AOAC Official method 991.22 - AOAC, 1998); protein loss was calculated as the percentage of the initial amount of proteins contained in milk (Lopez-Fandino and Olano, 1998). The separated curd samples were used for rheological, textural and microstructural investigations.

4.2.1.2.5 Curd rheological characteristics

Rheological properties of curd samples were investigated using a controlled stress rheometer (AR1000, TA Instruments, UK). Samples were prepared as for the curd yield determination. Tests were performed on slices of 1.7 ± 0.2mm thickness and 15mm diameter obtained using a sharp blade, and a 15mm diameter corer. The geometry used was plate and plate with serrated platens (15mm diameter, 1.5mm gap). The measurements were conducted in triplicate, and within the linear viscoelastic range using a frequency sweep mode with the frequency ranging from 1 to 10 Hz at a maximum strain 0.02.

4.2.1.2.6 Curd texture characteristics

Firmness of curd samples was determined using a Texture Analyser (model TA.XT2, Stable Micro Systems, UK) fitted with a load cell of 5 kg, and using a cylinder probe

(probe P/4 - 4mm diameter). The test settings were: test speed - 1mm/sec, distance - 10mm, trigger force - 5g, and a rate for data acquisition of 200pps. The peak force of the curves obtained for each of the samples was related to curd firmness. A typical curve is presented in Appendix 4.1. Samples tested were prepared as previously described for curd manufacture and curd yield determination, and the measurements were made on curd slices (30mm diameter and 20mm thickness); for each sample 10 replicate measurements were performed.

4.2.1.2.7 Curd microstructure

The microstructure of the curd samples was investigated using Cryo - Scanning Electron Microscopy (Cryo SEM) under a high vacuum (model JSM6100 JEOL Ltd., Akishima, Japan). Curd samples (1 x 1 x 5 mm) were cut out and placed on a cryostat sample holder and immersed in liquid nitrogen for 30s. Immediately after freezing the samples were transferred into the cryo-SEM, and fractured with a blunt blade. Ice was sublimed by raising the sample temperature to -80°C, and finally the sample was gold coated (Taneya et al., 1992) and examined. Representative micrographs for each treatment taken at 1000x magnification are presented.

4.2.1.2.8 Statistical analysis

Results from all the tests were calculated as means ± SD. Analysis of variance (one way ANOVA and GLM) followed by Tukey's test of Minitab 13.1 software (Minitab Inc., USA) were used for statistical analysis. Data sets for formulations containing β-glucan were analysed separately from the ones containing inulin and PHGG due to the different levels of addition used. Therefore they will be presented in separate tables/figures.

4.2.2 Stage 2. The effects of DF on yoghurt characteristics

4.2.2.1 Materials

The DFs used for yoghurt manufacture were the same as for curd manufacture (paragraph 4.2.1, Table 4.1), and the concentrated frozen starter culture used consisted of *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophillus* (1:1) (CH-3 Chr Hansen A/S, Hørsholm, Denmark). Skimmed milk powder (SMP) was also used in certain formulations as it will be explained later in this chapter. Pasteurised, standardised, and homogenised milk batches (3.2% fat and 0.1% fat) were used for the tests.

4.2.2.2 Methods

4.2.2.2.1 Manufacture of yoghurt

Yoghurts were manufactured using standardised, homogenised milk, to which the DFs were added as described in paragraph 4.2.1.2.2. The formulations used for yoghurt manufacture are presented in Table 4.3. For comparison purposes, low fat yoghurts with increased level of total solids (2% and 6% SMP) were also produced

Table 4.3. Milk - DF formulations used for the experiments on yoghurt characteristics

Milk used	β-glucan		Inulin (%, w/w)	PHGG (%, w/w)
	(%, w/w)	Code		
Full fat (3.2% fat)	0 (control)	FF_co	0	0
Skimmed milk (0.1% fat)	0 (control)	SM_co	0	0
	0.5	SM_0.5%G	2	2
	1.0	SM_1%G	4	4
	1.5	SM_1.5%G	6	6
	2.0	SM_2%G	-	-
	2.5	SM_2%G	-	-

The mixtures were then heated at 95°C for 10 min, cooled to 44°C, inoculated with 0.04% of the frozen starter culture and incubated at 44°C to a final pH of 4.2. The coagulum was then broken and stirred yoghurt was stored at 5°C for two days prior to testing. Duplicate trials were conducted.

4.2.2.2.2 Yoghurt syneresis

To investigate susceptibility to syneresis, samples of 30-40 g were centrifuged at 222g for 10 min at 4°C. After centrifugation the supernatant was poured off, weighed and recorded as syneresis (%) (Keogh and O'Kennedy, 1998). The measurements were performed in quadruplicate.

4.2.2.2.3 Rheological properties of the yoghurt

Apparent viscosity of the samples was measured using a Brookfield DVIII Rheometer (Brookfield Engineering Laboratories Inc., USA) and a cylinder in cylinder geometry (probe SC4-21). All the measurements were done at a temperature of 5°C and 0.5 rpm. Yoghurt was gently stirred for 20s before testing and triplicate measurements were conducted.

Rheological properties of yoghurt samples were also investigated using a controlled stress rheometer (AR1000, TA Instruments, UK). Measurements were carried out on shear mode at 5°C, using a cone and plate geometry (the gap between the plate and the base was of 50μm). A shear rate sweep test was used with the shear rate ranging from 10^{-2} to 20 s^{-1}. A frequency sweep test was also performed (with the frequency ranging from 1 to 20 Hz at a maximum strain of 4.06E-03, and an amplitude of 1.42E-04. Triplicate measurements were performed for each sample.

4.2.2.2.4 Textural properties of the yoghurt

Textural characteristics of the yoghurt were determined using a TA.XT2 Texture Analyser (Stable Micro Systems, UK) (load cell of 5kg), fitted with a back extrusion cell (A/BE) - 35mm and the tests run at the following settings: test speed: 1mm/sec., post test speed: 1mm/sec, distance: 25mm, rate for data acquisition: 200pps. A typical curve is presented in Appendix 4.2, where the peak force gives an indication of product firmness, and the negative region of the graph gives an indication of product consistency/resistance to flow off the disk. Five replicate measurements were performed for each sample.

4.2.2.2.5 Sensory analysis of yoghurt

Descriptive sensory analysis was performed under normal light in clear plastic pots in the sensory laboratory at the University of Plymouth. A panel consisting of 14 semi-trained panellists was used for the evaluation. In five training sessions the panellists were trained in the products and descriptors were chosen on the basis of consensus among panellists, followed by testing and scoring yoghurt products available on the market to cover a range of consistencies (from full fat Greek yoghurt to low fat stirred yoghurt). A total of 14 descriptors were used for the assessment of product appearance, texture, taste and overall acceptability. Test samples, identified by a three digit code, were presented to the panellists in a randomised order, immediately after being removed from the fridge (4°C). Testing was conducted on duplicate, each panellist being asked to score each attribute on a seven points scale. The form used by each panellist is presented in the Appendix 4.3.

4.2.2.2.6 Yoghurt microstructure

The microstructure of the yoghurt samples was investigated using Cryo - Scanning Electron Microscopy following the procedure described in paragraph 4.2.1.2.7. Representative micrographs for each treatment taken at 1000x magnification are presented.

4.2.2.2.7 Statistical analysis

Results from all the tests were calculated as means ± SD. Analysis of variance (one way ANOVA and GLM) followed by Tukey's test of Minitab 13.1 software (Minitab Inc., USA) was used for statistical analysis. Data from the experiments involving β-glucan were analysed using One-way Anova, while GLM was used to analyse the data from the experiments involving inulin and PHGG.

4.3 Results and discussions

4.3.1 Stage 1. The effects of DF on milk coagulation and curd characteristics

4.3.1.1 Milk coagulation

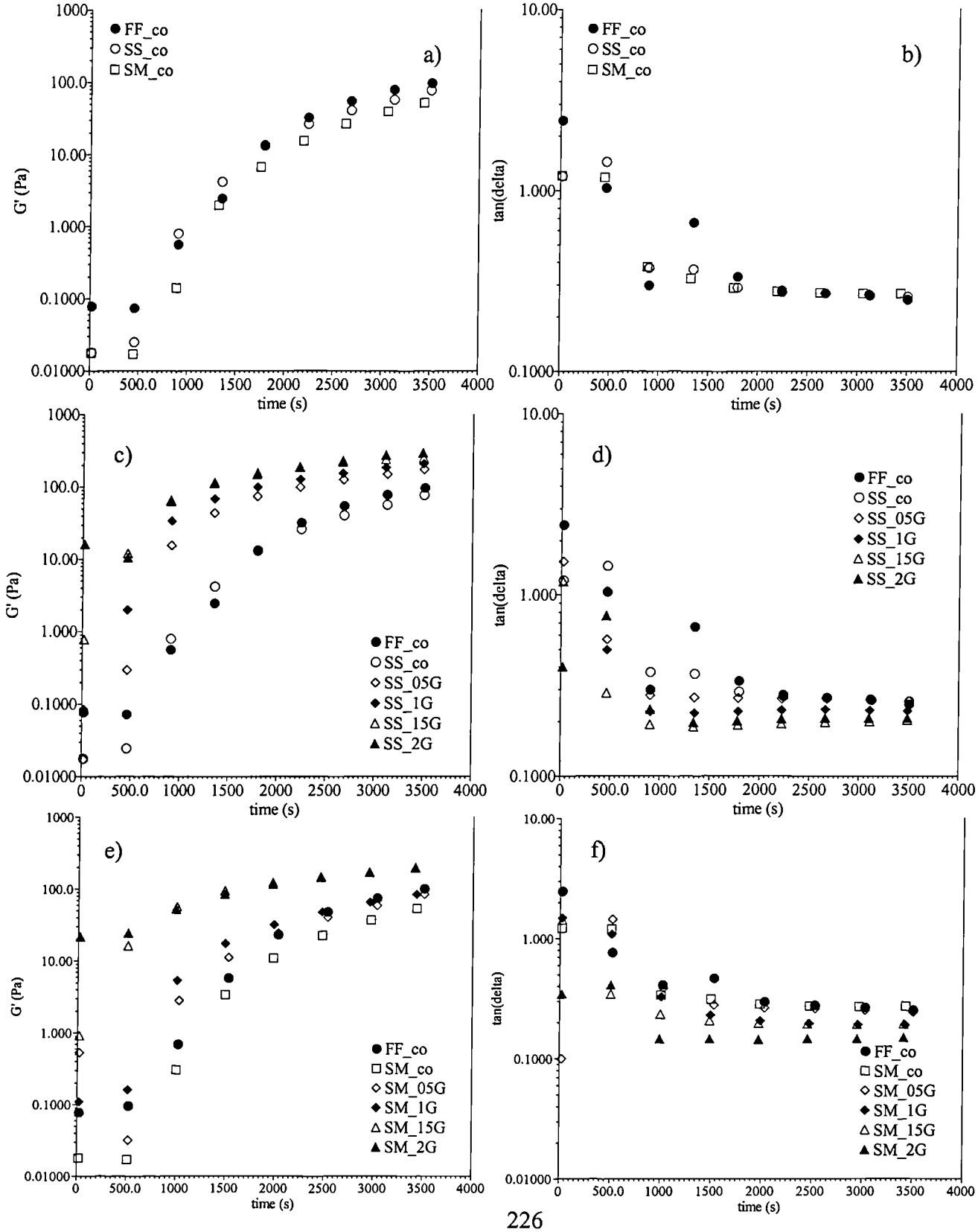
Cheese manufacture involves a stage in which a gel is formed (coagulum) by the aggregation of casein micelles during a proteolytic phase catalysed by rennet. Whey is expelled from the coagulum (syneresis) resulting in a curd, which may be further processed into cheese. During milk coagulation a steady aggregation of the rennet treated casein micelles occurs with chains of micelles forming, linking together into a loose network and then extending to become a more differentiated structure. During this process the linkages between the casein micelles strengthen, initially joined by association, followed by casein micelles contraction causing eventually partial fusion (Lopes da Silva and Rao, 1999; Green, 1993). This theory suggests a progressive firming of coagulum due to the increase in both the number and the strength of linkages between casein micelles. One way of monitoring these complex interactions is by rheological measurements using a controlled stress rheometer. Small amplitude oscillation tests result in minimal modification of

internal structure caused by shear, thus enabling the identification of structural changes due to casein micelles aggregation (Lopes da Silva and Rao, 1999).

The evolution of storage modulus (G') and tangent of the phase angle (tanδ =G"/G') during milk coagulation for formulations containing β-glucan are presented in Figure 4.1 (for each β-glucan and fat levels). The coagulation curves for the formulations containing inulin and PHGG followed a similar development pattern, and therefore are not presented here. The first stage of the coagulation process is dominated by the viscous behaviour of the system as indicated by the G" values which are initially higher than those G'. As expected, both G' (Figures 4.1 a,c,e) and G" increase in time reflecting gradual development of the casein network structure, however G' rises more sharply intercepting and then exceeding G" values. This behaviour was observed for most of the milk samples regardless of the type of DF used or of the fat/DF levels, and is consistent with known rheological behaviour during transitions from liquid to gel (Lopes da Silva and Rao, 1999).

Previous research has estimated the point at which gelation occurs during milk coagulation as either the time at which the value of G' equals G" (the storage modulus and loss modulus curves cross each other, or tanδ=1) (Lopes da Silva and Rao, 1999), or by assigning a threshold G' value (Lopes da Silva and Rao, 1999). Studies conducted on milk coagulation (O'Callaghan et al., 1999a) have defined this gelation point as the time at which G' reached 0.2 Pa. In addition, an optimum cutting time for coagulum was determined as the point where G' reached 20 Pa (O'Callaghan et al., 1999a).

Figure 4.1. Development of G' and tanδ with time (log scale) a) G' for milk samples containing 3.2, 1 and 0.1% fat and 0% β-glucan; b) tanδ for milk samples containing 3.2, 1 and 0.1% fat and 0% β-glucan; c) G' for milk samples containing 1% fat and β-glucan d) tanδ for milk samples containing 1% fat and β-glucan; e) G' for milk samples containing 0.1% fat and β-glucan; f) tanδ for milk samples containing 0.1% fat and β-glucan [(◊) - 0.5% β-glucan ; (♦) - 1% β-glucan; (Δ) - 1.5% β-glucan, and (▲)- 2% β-glucan]



The results obtained in this experiment for coagulation and optimum cutting times are presented in Tables 4.4 and 4.5. For the formulations containing β-glucan, statistical analysis indicated that each parameter was significantly affected by both β-glucan addition and fat levels in milk ($p<0.05$ - Table 4.4). Decreasing the fat content of milk lengthened the coagulation process, as shown by increased values for coagulation and optimum cutting times (Table 4.4). Additionally, as the fat content of milk decreased, the G' values of the resulting coagulum decreased as well, suggesting the formation of a weaker gel, with a reduced elastic behaviour (Figure 4.1a). These results agree with previous research (Green, 1993) suggesting that fat limits (but not inhibits) casein network movement, whilst also supporting the casein strands to form a more elastic structure. However, the values of tanδ for coagulum after 1h coagulation were similar, regardless of the milk fat content (Figure 4.1b).

Increased levels of β-glucan in milk significantly decreased ($p<0.05$) both the coagulation time and coagulum cutting time (Table 4.4). This trend was observed for the milk containing 1% fat (SS) as well as for the milk containing 0.1% fat (SM). For certain levels of β-glucan in milk, the coagulation time could not be estimated using these experimental settings. For example, when 1.5% or 2% β-glucan was added to the milk, it was found that the initial values of G' were higher than the threshold value of 0.2 Pa and therefore this parameter seemed unsuitable in these cases to estimate the start of coagulation. Similarly, the use of the cross over calculation to identify the gelation point was not appropriate for the milk sample containing 0.1% fat and 2% β-glucan (SM_2%G). In this case the two curves (G' and G") did not have an intersection point, since the initial values of G' were higher than G" values. Such observations suggest the existence of a polymer network before the proteolitic phase due to rennet started.

Moreover, the inclusion of β-glucan in milk formulation affected the final rheological behaviour of the resulting coagulum (Figures 4.1 c,d,e,f). The higher the level of β-glucan used, the higher the values for G' and G" of coagulum were obtained.

Table 4.4. Values for the coagulation time and curd optimum cutting time for formulations containing β-glucan

Milk samples	Coagulation time (s)		Optimum cutting time (s)
	cross over point (G'=G")	Time at which G' = 0.2 Pa	Time at which G'=20 Pa
FF_co	467± 78 ^{b,c}	679± 91 ^{a,b}	1924± 185 ^{a}
SS_co	568± 47 ^{a,b}	699± 02 ^{a,b}	2046± 09 ^{a}
SS_0.5%G	376± 94 ^{b,c}	463± 33 ^{b,c}	1023± 50 ^{b}
SS_1%G	328± 57 ^{b,c}	288± 66 ^{c,d}	801± 81 ^{b}
SS_1.5%G	260± 41 ^{c,d}	NA	527± 08 ^{b,c}
SS_2%G	333± 96 ^{b,c}	149± 64 ^{d,e}	532± 64 ^{b,c}
SM_co	705± 99 ^{a}	788± 97 ^{a}	2099± 205 ^{a}
SM_0.5%G	532± 36 ^{a,b}	690± 11 ^{a,b,c}	1917± 08 ^{a}
SM_1%G	552± 30 ^{a,b}	516± 59 ^{b,c}	1784± 233 ^{a}
SM_1.5%G	366± 32 ^{b,c}	NA	638± 116 ^{b,c}
SM_2%G	NA	NA	189± 81 ^{c}

* means±SD, n=3

**within the same column, the values followed by the same letter are not significantly different (p>0.05)

In the case of inulin and PHGG addition, the coagulation tests were performed only on the basis of their mixture with skimmed milk (0.1% fat), alongside the controls. The results are presented in Table 4.5 and they indicate that inulin did not influence the rate of milk coagulation, regardless of the level of addition, the values for coagulation time and cutting times being not significantly different from the SM_co (p>0.05, Table 4.5). PHGG however, when in combination with 0.1% fat milk, led to decreased values for all these parameters in comparison to SM_co, but this was not related to various levels of PHGG

used ($p<0.05$, Table 4.5). Nevertheless, it appears that the formulations based on skimmed milk and PHGG had coagulation characteristics similar to those of FF_co ($p>0.05$, Table 4.5).

Table 4.5. Values for the coagulation time and curd optimum cutting time for formulations containing inulin and PHGG

Milk samples		Coagulation time, s		Optimum cutting time, s
		cross over point (G'=G")	Time at which G' = 0.2 Pa	Time at which G'=20 Pa
FF-co		451±101[b]	654±89[b]	1889±205[b]
SM-co		832±97[a]	989±110[a]	2541±211[a]
SM-inulin	2	812±111[a]	954±98[a]	2418±198[a]
	4	850±101[a]	1026±63[a]	2781±233[a]
	6	798±99[a]	1014±96[a]	2286±187[a]
SM-PHGG	2	465±87[b]	691±71[b]	1712±173[b]
	4	392±94[b]	621±85[b]	1699±165[b]
	6	351±92[b]	495±113[b]	1632±204[b]

* means±SD, n=3

**within the same column, the values followed by the same letter are not significantly different ($p>0.05$)

These results could be explained by considering the potential interactions between proteins and polysaccharides in aqueous solutions. As discussed in several review articles three equilibrium situations could be possible: miscibility, thermodynamic incompatibility, and complex coacervation (Dickinson, 2003; Syrbe et al., 1998). At relatively high polymer concentration either thermodynamic incompatibility or coacervation could occur, depending on whether the interaction between protein-polysaccharide is repulsive or attractive (Dickinson, 2003). Since the DFs included in this study are non-ionic polysaccharides with a different structural organisation than that of casein, the most plausible situation is thermodynamic incompatibility between the two polymers (DF and casein), which is basically a volume exclusion effect. Molecules of different polymers

cannot occupy the same volume in the bulk of their mixed solutions when there is no specific interaction between them (Zasypkin et al., 1997). Thermodynamically incompatible polymers in a mixed aqueous solution show a preference to be surrounded by their own type; they concentrate each other in solution. In other words, their mixture separates into liquid phases, which further leads to an increase of their concentration in the different phases (Tolstoguzov, 2003). As a result of the excluded volume effect, the rate of gelation and elasticity modulus of mixed gels can be higher and the minimum concentration for gelation can be lower (Zasypkin et al., 1997).

This theory offers a possible explanation of the results obtained for the formulations containing β-glucan or PHGG. Thus, when added to milk, β-glucan/PHGG may promote self association of casein which explains the decreased values for coagulation and coagulum cutting times, and also the increase in strength of the gels formed as shown by higher G' values. Similarly, casein appears to promote the association of β-glucan, which results (at high concentrations of β-glucan) in the formation of a gel network reinforcing the casein network. This was suggested initially by the rheological properties of 0.1% fat milk containing 2% β-glucan, which showed gel-like behaviour before the proteolysis started, and later by the increased G' and decreased tanδ values with increased levels of β-glucan. This was also observed for mixtures containing PHGG, but in a lesser extent; although PHGG was used at significantly higher concentrations than β-glucan no gel like behaviour was observed before the proteolysis started. However, the final G' values were higher than of SM-co, but not significantly affected by the level of PHGG (within the range used).

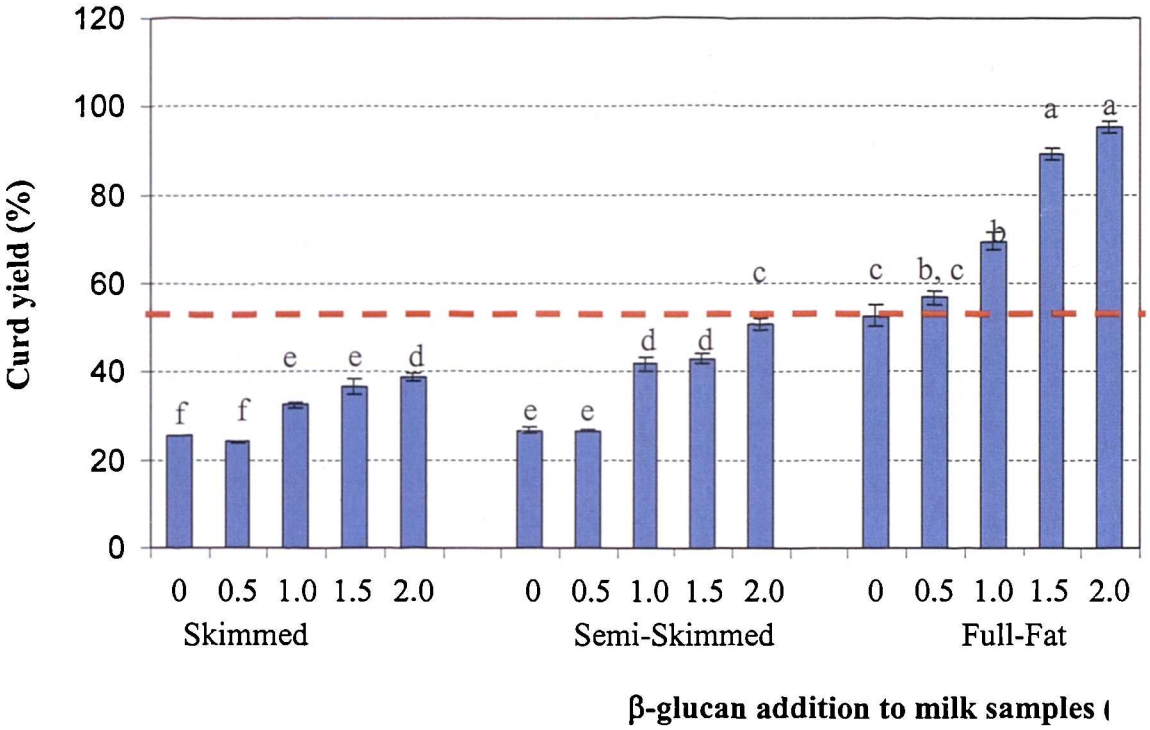
Different results were obtained for the milk formulations containing inulin; they indicate that inulin had no significant effect on the rate of milk coagulation (Table 4.5) showing

coagulation times and optimum coagulum cutting times similar to those of SM_co ($p>0.05$). Moreover, SM_co and coagulum containing inulin had similar rheological properties at the end of the coagulation time (results not shown).

4.3.1.2 Curd yield

The results for the yield of fresh curd containing β-glucan are presented in Figure 4.2 and summarised in ANOVA Table 4.6, while the results for the formulations containing inulin and PHGG are shown in Figure 4.3 and ANOVA Table 4.7.

Figure 4.2. Curd yield for formulations containing β-glucan [means ± SD; the columns labelled with the same letter are not significantly different ($p>0.05$)]



Statistical analysis of the data related to formulations containing β-glucan showed that yield values were significantly affected by the amount of fat, β-glucan and the interaction between the two ($p<0.001$, Table 4.6). Generally, increasing levels of fat resulted in increasing curd yield ($p<0.001$, Table 4.6). The yield value for the curd made from full fat

milk (52.7%±2.5) was found to be significantly higher ($p<0.05$), than the yield values for curds obtained from semi-skimmed (25.5%±0.1) and skimmed milk (26.7%±0.5) (Figure 4.2). These results are in agreement with previous studies which have suggested that the milk fat globules limit casein aggregation and act as 'plugs', blocking the flow of whey through channels in the curd, thus preventing shrinkage of the casein matrix (Green, 1993; Fox et al., 2000b). As such, the rate of whey loss from the structure by physical means is reduced, leading to increased curd yield (Green, 1993; Fox et al., 2000b).

Table 4.6. ANOVA table summarising the attributes of curds containing β-glucan (the values represent means at a given treatment level)

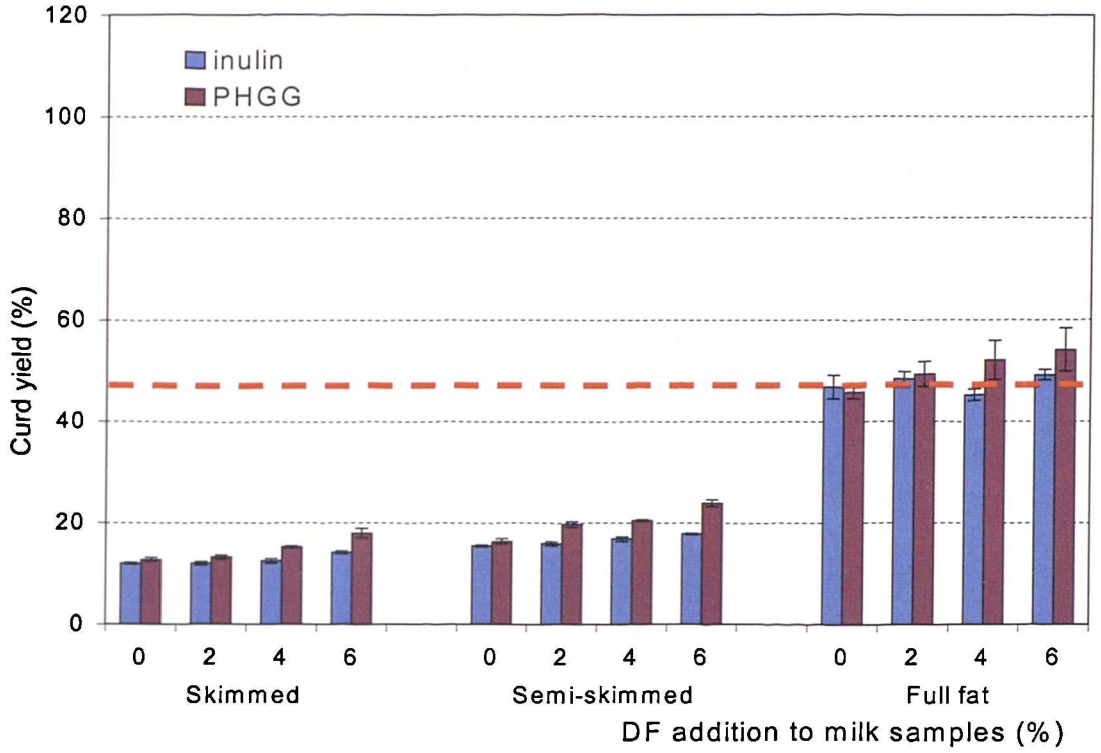
Sample	Curd yield (%)	Protein lost (%)	Curd firmness (N)
Effect of the level of fat in milk			
0.1%	29.1^{c}	15.2^{a}	0.68^{a}
1.0%	39.3^{b}	13.1^{b}	0.29^{b}
3.2%	71.6^{a}	6.4^{c}	0.07^{c}
Significance	***	***	***
SEM	0.76	0.28	0.02
Effect of the level of β-glucan addition			
0%	34.7^{D}	15.1^{A}	0.64^{A}
0.5%	36.0^{D}	14.0^{A}	0.54^{B}
1%	44.6^{C}	11.5^{B}	0.32^{C}
1.5%	55.0^{B}	9.5^{C}	0.14^{D}
2%	63.0^{A}	7.8^{D}	0.09^{D}
Significance	***	***	***
SEM	0.97	0.36	0.02
Effect of the interaction level of fat*level of β-glucan			
Significance	***	***	***

-within the same column, the values with the same letter are not significantly different;
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$

Addition of β-glucan in the milk formulations also resulted in higher curd yield values (Figure 4.2 and Table 4.6); this was observed in all the samples regardless of the amount of fat in milk, and the effect was significant for levels of β-glucan greater than 1% (Figure 4.2, Table 4.6). These results are not surprising since β-glucan has a high water holding

capacity, therefore contributing to higher whey retention within the curd structure, and consequently to higher yield values. It is important to note that yield values for SS_2%G and FF_co curds were similar ($p>0.05$). Moreover, yield results for SM_2%G, SS_1%G and SS_1.5%G curd resembled the yield value of FF_co curd (Figure 4.2).

Figure 4.3. Curd yield for formulations containing inulin and PHGG (means ± SD)



In the case of the formulations containing inulin or PHGG, statistical analysis indicated that the curd yield was significantly influenced by level of fat in milk, type of DF, and level of addition (for all $p<0.001$, Table 4.7). The effect of the milk fat level is not surprising and reiterates what was previously found during the experiment involving β-glucan. PHGG was shown to lead to increase curd yield in comparison to inulin, which appeared to have no effect on the curd yield values (Figure 4.3). The yield results for curds containing inulin at various levels are not surprising if the observations from the milk coagulation tests are taken into account (no effect on the coagulation rates was assigned to inulin). The explanation may be related to the low MW of inulin (DP between 2 to 60) leading probably to a large proportion of the inulin to be lost in the whey during curd

syneresis. Thus no significant amount of extra water was retained within the curd structure with implications for curd yield values comparable to the controls (Figure 4.3).

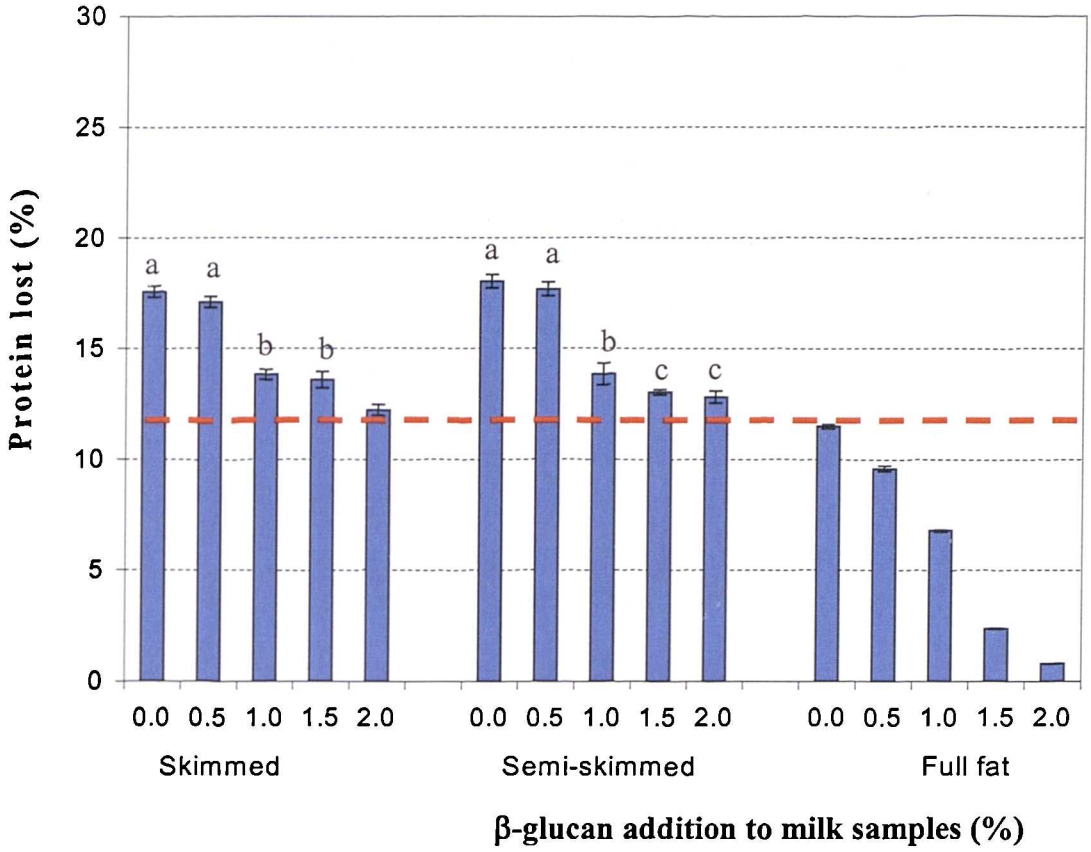
Table 4.7. ANOVA table summarising the attributes of curds containing inulin and PHGG (the values represent means at a given treatment level)

Sample	Curd yield (%)	Protein lost (%)	Curd firmness (N)
Effect of the level of fat in milk			
0.1%	13.7^{c}	18.4^{a}	2.3^{a}
1.0%	18.5^{b}	16.9^{a}	1.0^{b}
3.2%	48.9^{a}	10.9^{b}	0.1^{c}
Significance	***	***	***
SEM	0.45	0.58	0.10
Effect of the type of dietary fibre			
Inulin	25.5^{B}	17.2^{A}	1.23
PHGG	28.6^{A}	13.6^{B}	1.03
Significance	***	***	NS
SEM	0.37	0.58	0.08
Effect of the level of DF addition			
0%	25.4^{Y}	16.8	1.4^{X}
2%	26.4^{Y}	16.2	1.2^{X}
4%	27.0^{Y}	13.8	1.1^{X}
6%	29.5^{X}	14.7	0.8^{Y}
Significance	***	NS	*
SEM	0.52	0.82	0.11

- within the same column, the values with the same letter are not significantly different
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

Incorporation of PHGG in milk formulations led to trends similar to what was observed for β-glucan (higher yield values in comparison to the controls and increasing values with increasing levels of PHGG - Figure 4.3). The explanation may be again related to the ability of PHGG to hold water, and to retain it within the curd structure. It is interesting to note however that although the levels of addition for PHGG were far higher than for β-glucan, the effects on curd yield were not as spectacular (e.g. skimmed and semi-skimmed milk containing 6% PHGG led to curd yield values representing approximately 50% that of FF_co).

Figure 4.4. Amount of protein lost in whey for formulations containing β-glucan (means ± SD; the columns labelled with the same letter are not significantly different (p>0.05))

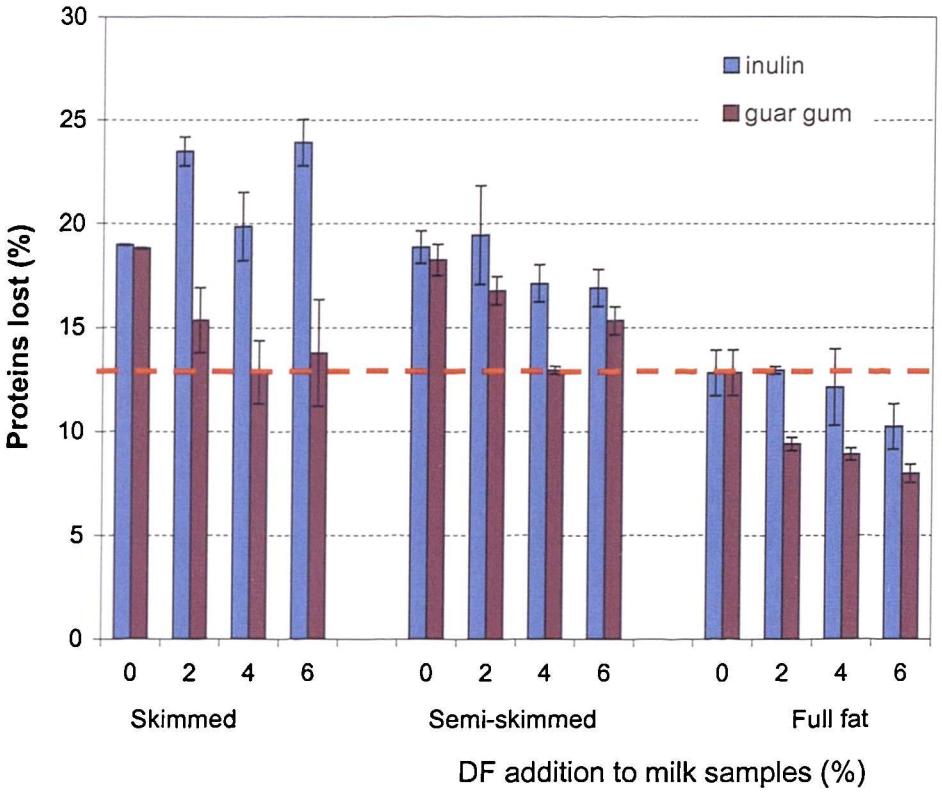


The amount of protein lost in whey for each curd sample was calculated as the percentage of the initial amount of proteins contained in milk, and for the formulations containing β-glucan the results are presented in Figure 4.4 and summarised in ANOVA Table 4.6. Statistical analysis suggests that protein losses in whey decreased significantly ($p<0.001$, Table 4.6) with both increasing levels of fat, and increasing levels of β-glucan in the formulations. These results are related to the curd yield values (Figure 4.2) and also with the rheological parameters shown by various coagulums (Figure 4.1). Generally, high percentages of protein loss during whey separation correspond to low curd yield values, and also to weak coagulums formed during renetting (indicated by low G' values).

The decreased percentage of proteins lost in whey in relation to increasing amounts of β-glucan added into formulation may be due to the higher proportion of serum retained

within the structure. As a consequence, the amount of proteins (whey proteins, glyco-macropeptide, and part of caseins) potentially washed away during syneresis is reduced, and a higher percentage of the proteins remains within the structure also contributing to increased curd yield.

Figure 4.5. Amount of protein lost in whey for formulations containing inulin and PHGG



The results for protein lost in curd formulations containing inulin and PHGG are illustrated in Figure 4.5 and summarised in Table 4.7. Similar to what was found during the experiments with β-glucan, statistical analysis showed that increasing levels of fat significantly reduced the amount of protein lost in the separated whey ($p<0.001$, Table 4.7); in contrast, however, the level of DF addition appeared to have no significant effect on protein loss ($p>0.05$, Table 4.7). It is worthwhile to note that Figure 4.5 indicates that there is a trend of reduced amount of protein lost with increasing levels of PHGG, while no such trend could be observed for formulations containing inulin. This difference on the effects of PHGG *vs* inulin on protein lost was also indicated by statistical analysis, which

showed significantly higher protein losses in formulations containing inulin ($p<0.001$, Table 4.7). As observed for β-glucan, these observations are in agreement with the results on the curd yield. The explanation is similar too: high curd yields are related to high amounts of protein and water retained within the curd structure; since inulin had no effect on the curd yield, it is to be expected that it will also show no effect on the amount of protein lost; PHGG, however led to an increase in curd yield and decrease in protein loss, and these are related, as explained earlier.

4.3.1.3 Curd rheological and textural properties

The rheological attributes (storage modulus G' and tanδ) of curds containing β-glucan as recorded during frequency sweep tests are presented in Figure 4.6a-h and their values indicate that the viscoelastic behaviour of fresh curd was affected by both β-glucan and fat concentrations in milk. Generally, the values of G' and tanδ for curd decreased with increasing levels of fat and β-glucan in the milk used for processing.

Figures 4.6a and 4.6b illustrate that for curds without β-glucan, the values of both G' and tanδ increased as fat levels decreased, suggesting significant changes in their viscoelastic properties. The curd made from full fat milk was found to be characterised by significantly reduced elastic component (smaller G' values) when compared with its low fat counterparts (SM-co and SS_co). This is not surprising when considered in relation to previous results on curd yield. Generally, low curd yield values are directly related to the small amount of serum retained within curds structure, and thus to high concentration of protein. Although the protein content of the curd samples was not determined for this study, it is plausible to associate low curd yield values with high percentage of protein in the curd, and *vice versa*.

Based on this assumption, and taking into consideration that for gels the G' at the plateau region appears to vary as a power function of the concentration of the gelling agent (the power exponent for gels of many biopolymers was found to be close to 2) (Aguilera and Stanley, 1999), then the differences found between the G' of FF_co, SM_co and SS_co curds may be partly explained by the differences in the protein content of the curds tested. Further work would need to be conducted in order to verify this assumption.

Figure 4.6. Evolution of G' and tanδ for curd samples (log-log scale) a) G' for curd made from milk containing different levels of fat and 0% β-glucan; b) tanδ for curd made from milk containing different levels of fat and 0% β- glucan; c) G' for curd samples made from milk containing 1% fat and β-glucan; d) tanδ for curd samples made from milk containing 1% fat and β-glucan; e) G' for curd samples made from milk containing 0.1% fat and β-glucan; f) tanδ for curd samples made from milk containing 0.1% fat and β-glucan; g) G' for curd samples made from milk containing 3.2% fat and β-glucan; h) tanδ for curd samples made from milk containing 3.2% fat and β-glucan [(◊) - 0.5% β-glucan ; (♦) - 1% β-glucan; (Δ) - 1.5% β-glucan, and (▲)- 2% β-glucan]

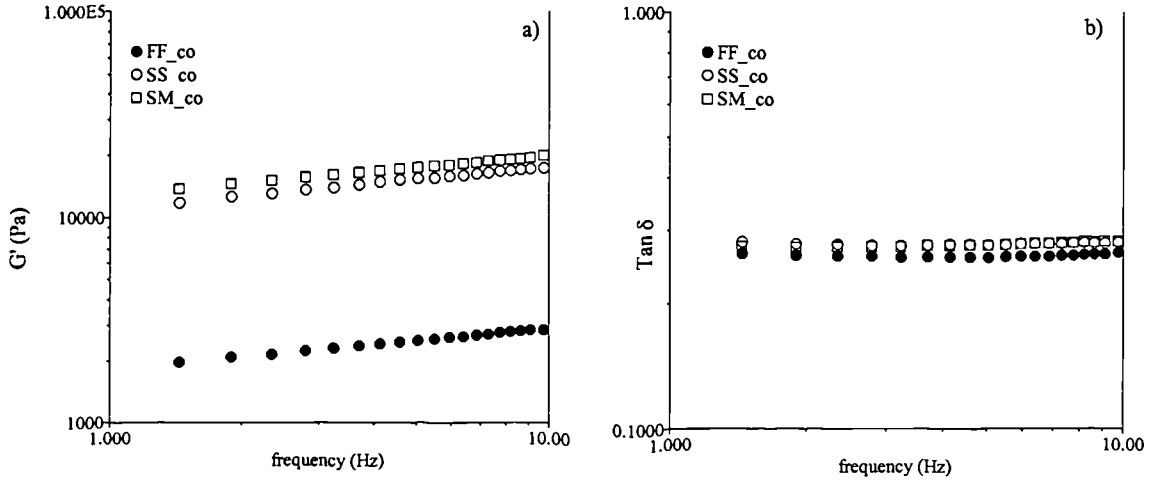
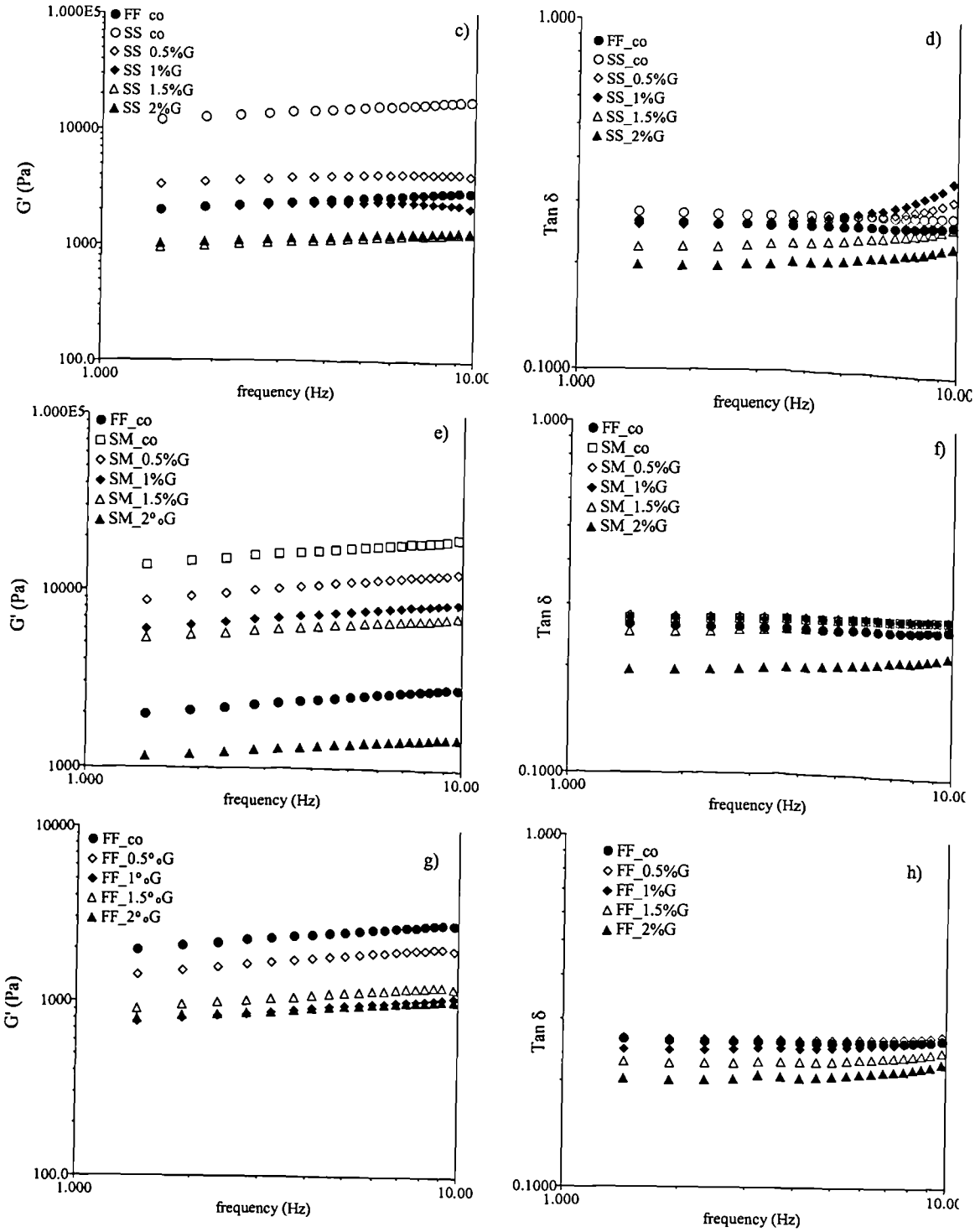


Figure 4.6. (continued)

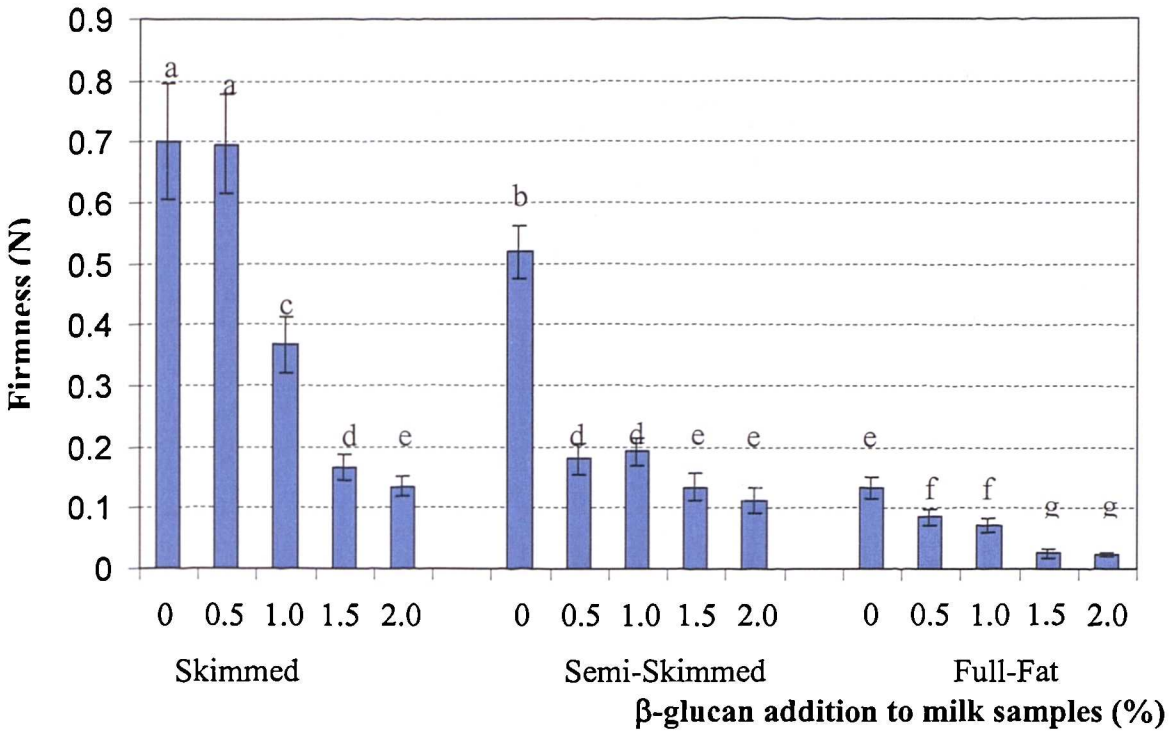


The evolution of G' and tanδ for the curds made from milk samples containing β-glucan is presented in Figures 4.6 c-h. The graphs indicate that irrespective of the amount of fat in the milk (0.1%, 1% or 3.2%), values for curd G' and tanδ decreased with increasing levels of β-glucan added to the milk. The observed trend for the G' values of curds is again not

unexpected if regarded in relation to the already mentioned results for curd yield. High levels of β-glucan in milk were associated with high curd yield values (due mainly to whey retention within the curd structure by β-glucan). This in turn appeared to be related to smaller protein concentration in the curd, and hence lower elastic component and structure strength of the curds (illustrated by smaller G' values). Interestingly, the use of increasing concentration of β-glucan (1.5 and 2%) resulted in a significant decrease of tanδ in comparison to the controls. The significant decrease of the tanδ in these cases is due to an even greater reduction in the G" alongside the reduction in G', suggesting that increasing levels of β-glucan in the curd intensifies its gel-like behaviour. These results may be explained again by the possible segregative interactions between casein and β-glucan, which facilitates the association of β-glucan into a gel network alongside with the casein network.

From the point of view of product acceptability, it is relevant to note that when β-glucan is added at concentrations of 1.0% to 1% fat milk or 1.0, 1.5% to 0.1% fat milk, the rheological behaviour of the resulting curds becomes closer, and possibly similar to that of the full fat curd (Figures 4.6 c-h). These results suggest a complex β-glucan-caseins-fat interaction in the formation of curd structure starting during milk coagulation and continuing during syneresis, and also the existence of an optimum level of β-glucan to be added to milk according to its fat content. As such, the results clearly illustrate the potential use of β-glucan for obtaining low fat curd/cheese products with similar rheological characteristics to their full fat counterparts.

Figure 4.7. Firmness of curd samples containing β-glucan [means ± SD; the columns labelled with the same letter are not significantly different (p>0.05)]



The texture data showing that curd firmness decreased significantly ($p<0.001$) with increasing levels of fat and β-glucan in the milk (Figure 4.7, Table 4.6) support these conclusions. This trend observed for curd firmness is related to the results obtained for curd yield and storage modulus (G') and supports previous research which has suggested that high percentage of whey retained within the structure makes the resulting curd less firm and more susceptible to fracture upon compression (Fox et al., 2000a). Thus, higher moisture content of curds was associated with weaker structures indicated by their smaller values for the storage modulus - G' (Figure 4.6) and firmness (Figure 4.7). A similar effect has been previously reported for carrageenan and carboximethylcellulose which, when added to milk, generated a softer texture for the cheese (Kanombirira and Kailasapathy, 1995; Elneshawy et al., 1986).

Table 4.8. ANOVA table summarising the rheological attributes of curds containing inulin and PHGG (the values represent means at a given treatment level)

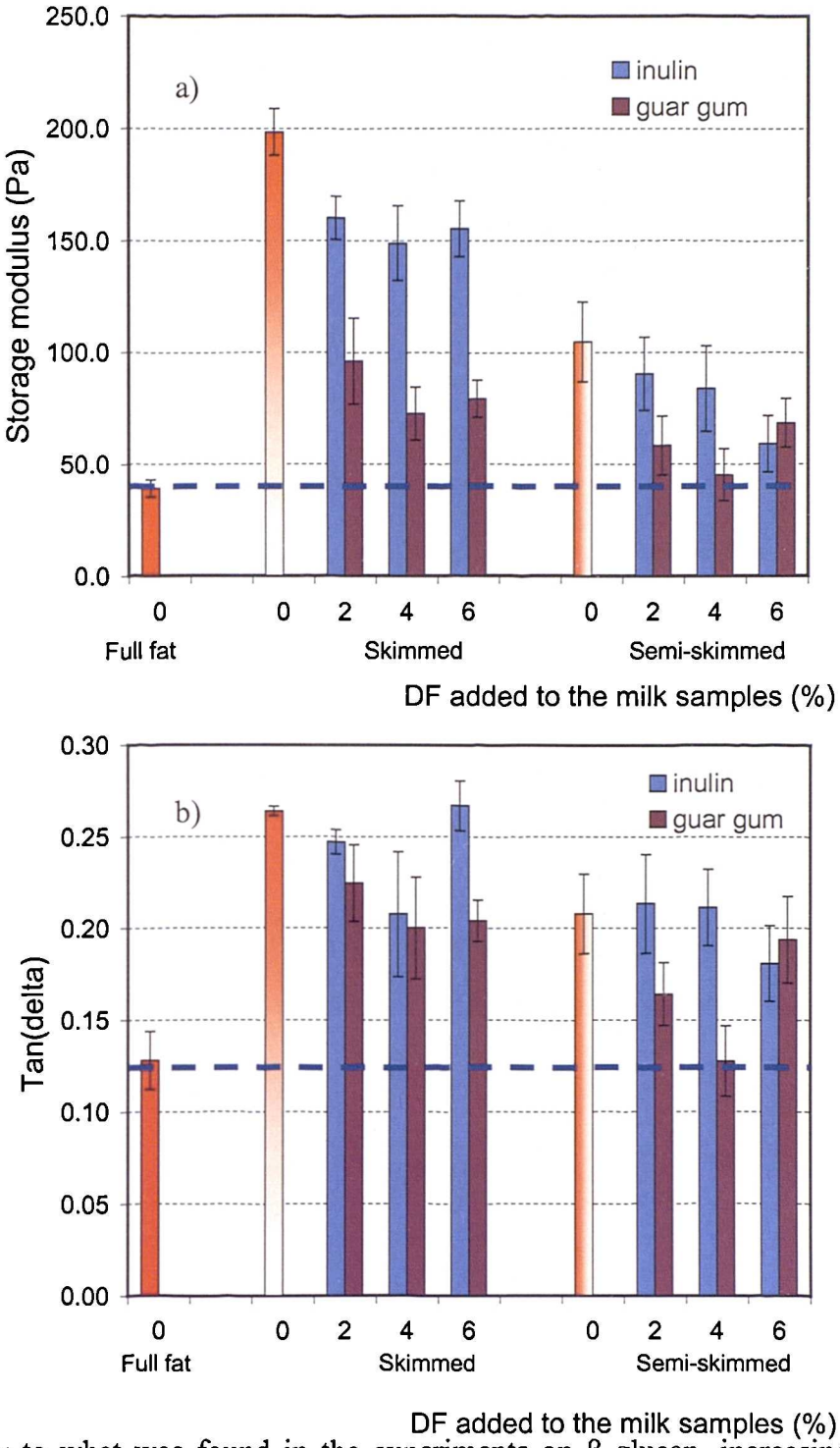
Sample	Storage modulus (Pa)	Tanδ
FF_co	*39.1±3.1*	*0.13±0.01*
Effect of the level of fat in milk		
0.1%	142.7	0.24
1.0%	76.73	0.18
Significance	***	*
Effect of the type of dietary fibre		
Inulin	126.3	0.22
PHGG	93.1	0.20
Significance	*	NS
Effect of the level of DF addition		
0%	159.8^{a}	0.25
2%	101.1^{b}	0.20
4%	87.5^{b}	0.17
6%	90.4^{b}	0.22
Significance	***	NS

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

*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

The results on rheological and textural attributes of curds containing inulin and PHGG are summarised in ANOVA Tables 4.8 and 4.7 and illustrated in Figures 4.8 and 4.9. Statistical analysis indicates in these cases that curd storage modulus was significantly affected by the fat level ($p<0.001$), type of DF used ($p<0.05$), and the level of DF used ($p<0.001$).

Figure 4.8. Rheological parameters [G' (a) and tan(delta) (b) at 6.4Hz] of curds containing inulin and PHGG



Similar to what was found in the experiments on β-glucan, increasing levels of fat and increasing levels of DF produced curds with decreasing G' (Table 4.8); as illustrated in Figure 4.8a), this is particularly obvious in the samples containing PHGG. These observations are in agreement with the results on curd yield. As discussed earlier in this

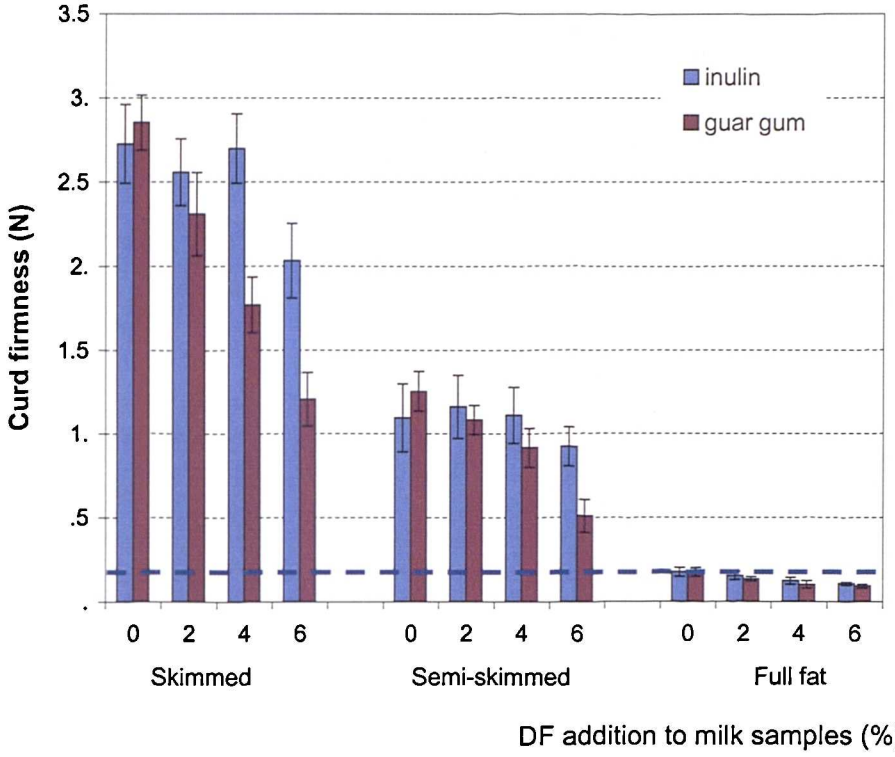
chapter, increasing levels of fat and DF lead to increasing curd yields; this in turn is equivalent to a higher proportion of whey being retained within the structure, and thus lower protein content which directly influenced curd G' (lower G' values). Although for a particular fat content, both inulin and PHGG appeared to lead to curds with G' values lower than the controls (Figure 4.8a), PHGG was demonstrated to be more effective in comparison to inulin ($p<0.001$, Table 4.8).

Tanδ values for curd samples showed a similar trend to that shown by samples containing β-glucan: decreasing average values with increasing levels of fat and DF and with the use of PHGG as opposed to inulin (Table 4.8). Nevertheless, it is important to note that statistical analysis found no significant effect of DF (type or level) on tanδ values ($p>0.05$) and this is due mainly to the results obtained for the samples containing inulin. Figure 4.8b clearly illustrates this trend, and also indicates that PHGG decreased significantly tanδ in comparison to the controls (SS_co and SM_co); this translates into the fact that samples containing PHGG exhibit more gel-like behaviour. Equally important, Figure 4.8 suggests that in milk formulations based on 1% fat, the addition of 4% PHGG makes possible the production of a curd with rheological characteristics (G' and tanδ) comparable to FF_co.

The effects of PHGG and inulin on curd rheological characteristics could be explained (as for β-glucan) by the possible thermodynamic incompatibility between casein and polysaccharides. This would promote concentration and association of polysaccharides and would result for PHGG in the formation of a gel network alongside the casein network, which on one hand would retain more water within the structure (lower G' values), but will also reinforce gel-like behaviour of the curd (lower tanδ). Although this is envisaged to be true and to happen for the inulin containing samples, the effects are not significant possibly due to an important loss of inulin in the whey, during syneresis, related to the small chain

length (DP=2-60) of inulin. Higher levels of inulin addition would potentially be needed in order to obtain a significant effect, but this approach would be difficult to justify economically.

Figure 4.9. Firmness of curd samples containing inulin and PHGG (means ± SD)



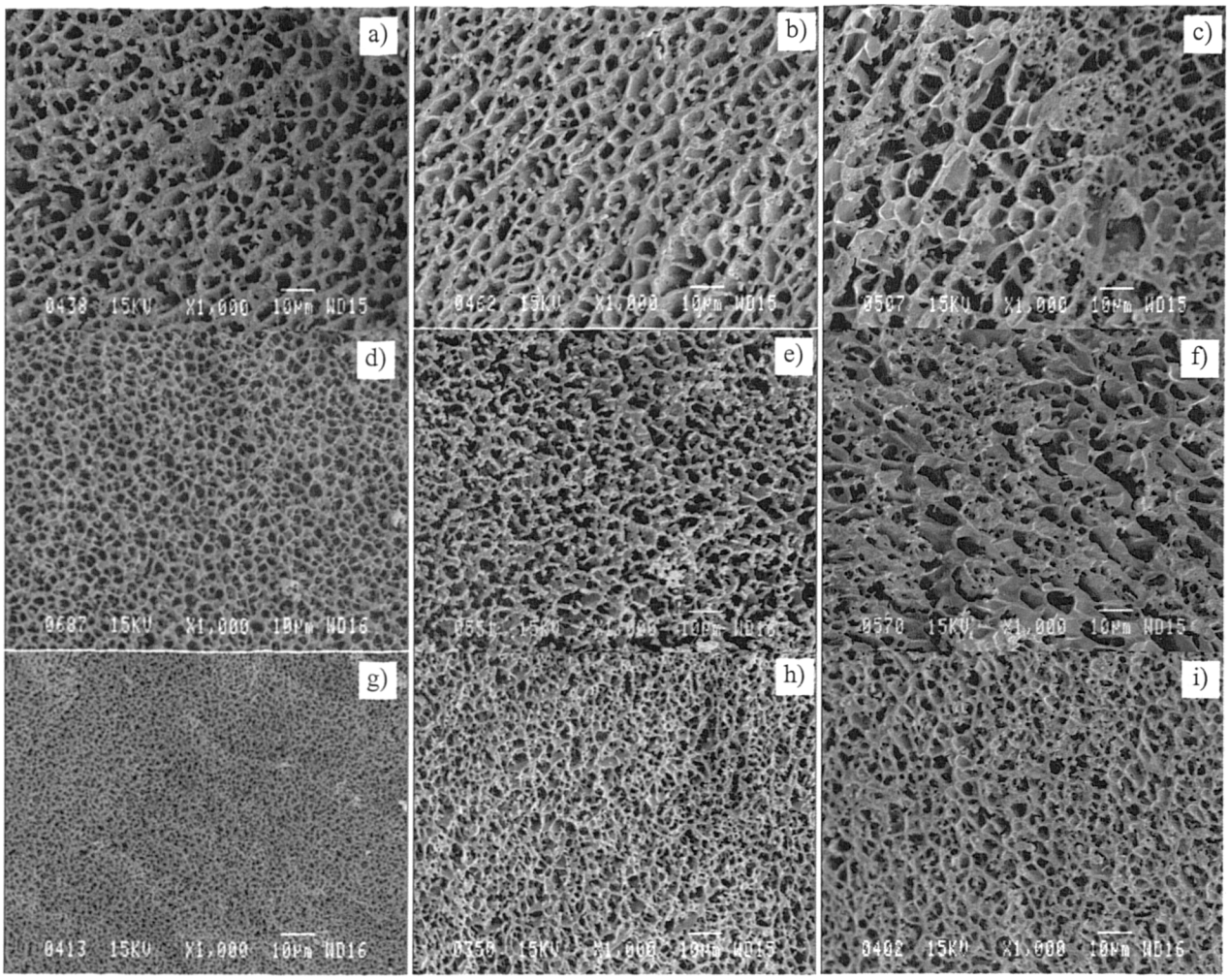
In agreement with the outcomes from the rheological tests are the results on curd firmness (assessed by Texture Analysis) presented in Table 4.7 and Figure 4.9. They clearly indicate that increasing levels of fat and DF decreased the firmness of the resulting curd ($p<0.001$, Table 4.7), while no significant difference was found between the samples containing inulin in comparison to those containing PHGG ($p>0.05$). Nevertheless, Figure 4.9 indicates that at addition levels higher than 4%, PHGG produced softer curd in comparison to inulin.

4.3.1.4 Curd microstructure

The micro and macro-structure of food products are major determinants of their rheological and textural behaviour. Cryo SEM was used to investigate the structure of

curds made from milk containing different levels of fat and DFs in order to better understand their rheological behaviour and textural characteristics.

Figure 4.10. SEM micrographs (x 1000) for curd containing β-glucan: a) FF_co; b) FF_1%G; c) FF_2%G; d) SS_co; e) SS_1%G; f) SS_2%G g) SM_co; h) SM_1%G; h) SM_2%G

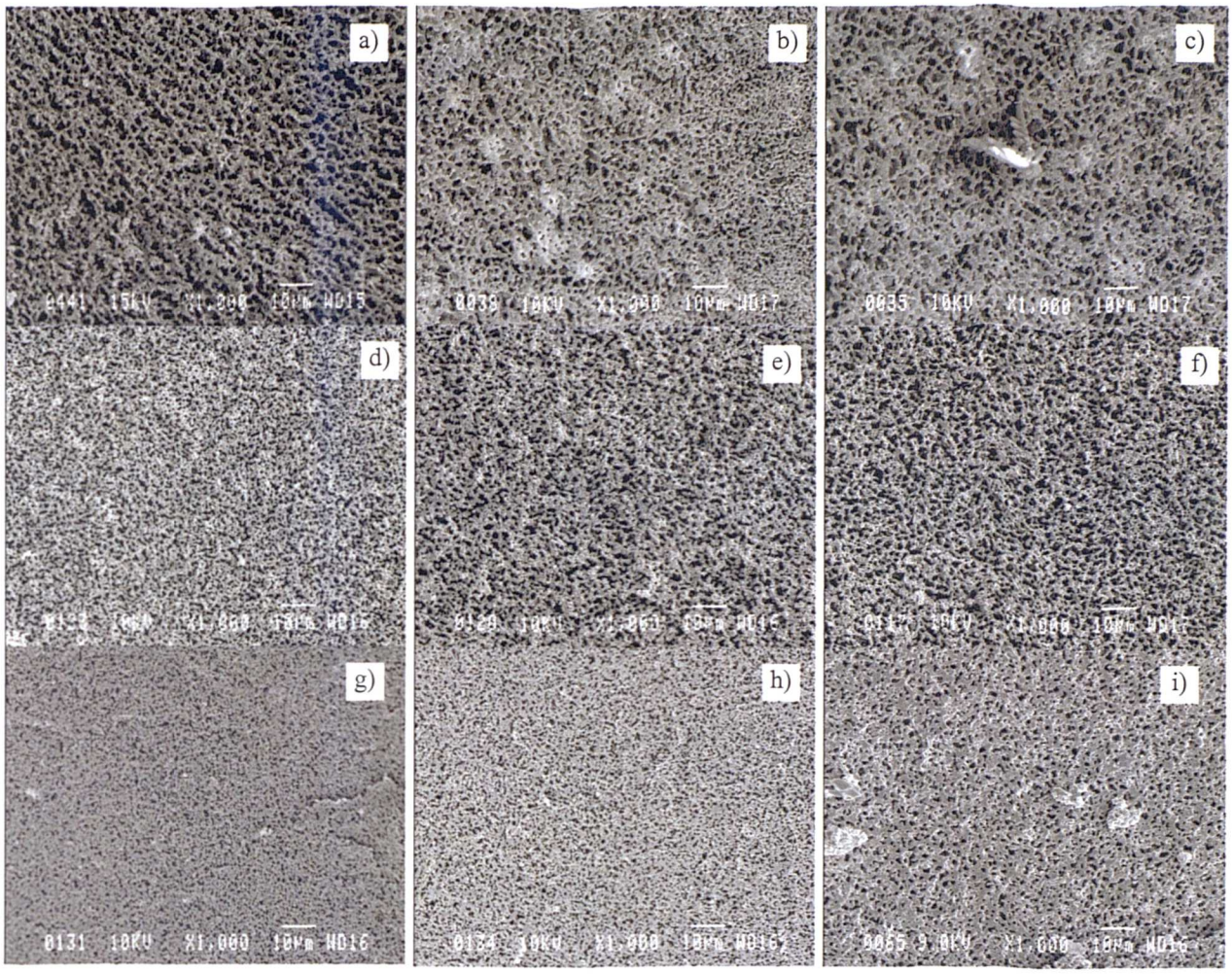


Representative micrographs of the curds containing β-glucan, inulin and PHGG are presented in Figures 4.10, 4.11 and 4.12 respectively. Within the curd made from full fat milk (Figures 4.10a, 4.11a), the network formed by the casein was composed of relatively large pores entrapping higher amount of whey when compared to the matrix of curds made from 1% fat (Figures 4.10d, 4.11d) and 0.1% fat (Figures 4.10g, 4.11g) milk respectively.

Low fat curds (Figures 4.10d, 4.11d, 4.10g and 4.11g) were characterised by a denser, closer knit matrix with small interstices retaining reduced amounts of whey. The cryo-SEM micrographs clearly illustrate that the lower the level of fat in the initial milk, the denser the structure of the resulting curd, and consequently the firmer the curd would be expected to be. As such, the micrographs support the results obtained for curd rheological properties (lowering the fat content resulted in curd with higher G' - Figures 4.6a and 4.8) and firmness (milks with decreasing levels of fat produced curds with increasing values for firmness - Figures 4.7 and 4.9).

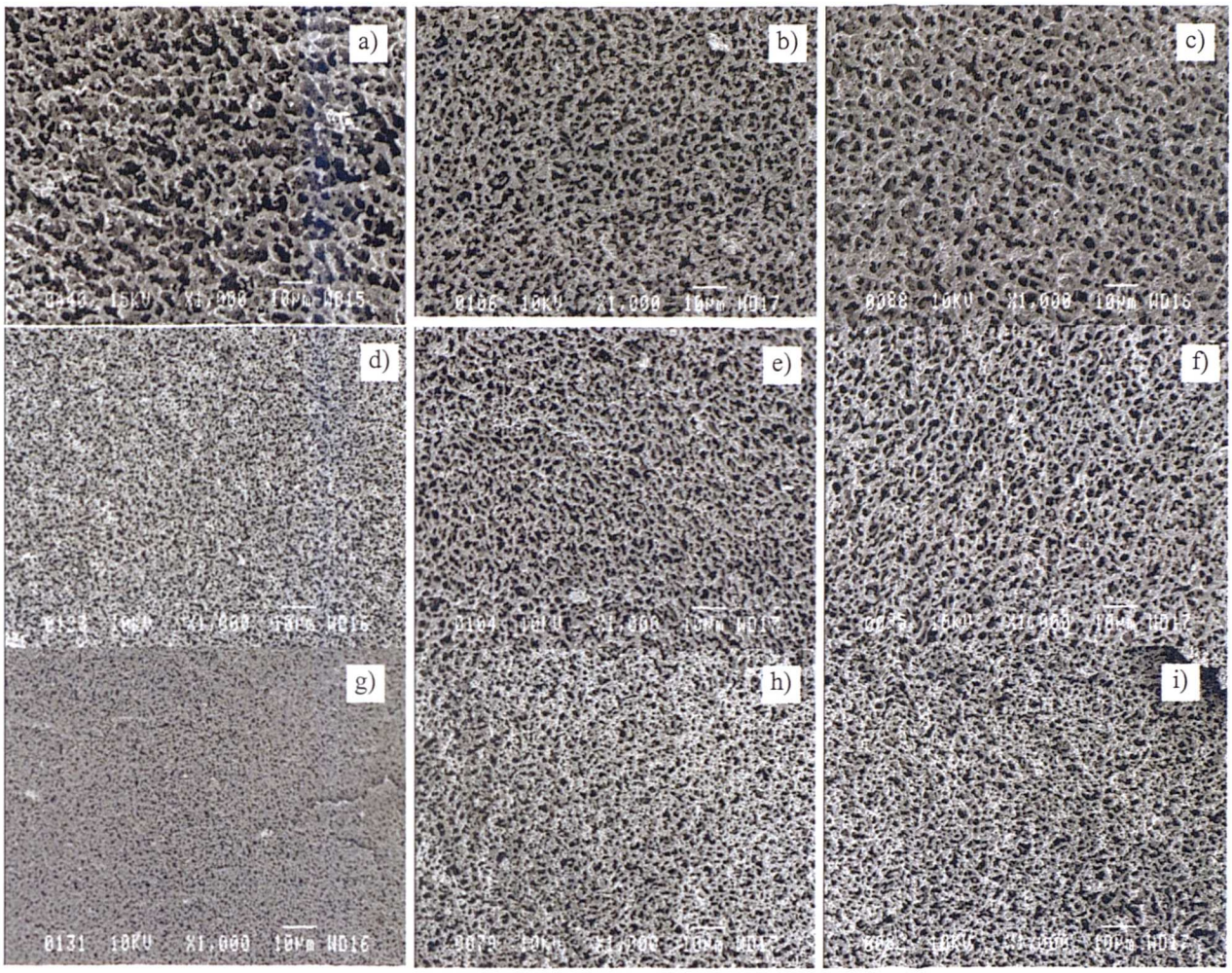
Addition of β-glucan into the milk formulations altered the structure of the resulting curds (Figures 4.10 b,c,e,f,h,i). Increasing levels of β-glucans resulted in increasing pores sizes and in the formation of a more open network. Such a matrix appeared to entrap higher quantities of whey as the amount of β-glucan increased. Micrographs of curd samples obtained from full fat milk with added β-glucan 1% (Figure 4.10b) and 2% (Figure 4.10c) show structures becoming more open, with larger interstices, retaining higher amounts of serum. Similar trends were observed for low fat curds (Figures 4.10e,f,h,i). Thus, higher amounts of β-glucan additions and higher levels of fat result in looser curd structures. These structures appear to support the results for yield, rheological and textural parameters, and may again be explained by the water binding capacity of β-glucan. The structures of curds made from full fat milk (Figure 4.10a), 1% fat milk and 1% β–glucan (Figure 4.10e) and from milk with 0.1% fat and 2% β-glucan (Figure 4.10i) are similar in terms of pore size and matrix conformation. These indicate that the incorporation of β-glucan to low fat milk formulation could result in curds with structural configuration similar to that of full fat curd.

Figure 4.11. SEM micrographs (x 1000) for curd containing inulin: a) FF_co; b) FF_2%inulin; c) FF_6%inulin; d) SS_co; e) SS_2%inulin; f) SS_6%inulin; g) SM_co ; h) SM_2%inulin; i) SM_6%inulin



The micrographs acquired during the experiments on curds containing inulin or PHGG are presented in Figures 4.11 and 4.12 respectively. In comparison to the control samples obtained during the experiment with □-glucan (Figure 4.10a,d,g), the structure of the controls produced this time was closer, denser, with smaller spaces between the casein matrix (Figures 4.11a,d,g and 4.12a,d,g), indicating firmer products; these observations were particularly obvious for SS_co and SM_co and they confirm the results from texture testing. It is possible that these differences may be due to seasonal differences in milk quality (different protein ratios and ion values), with effect on its coagulation characteristics.

Figure 4.12. SEM micrographs (x 1000) for curd containing PHGG: a) FF_co; b) FF_2%PHGG; c) FF_6%PHGG; d) SS_co; e) SS_2%PHGG; f) SS_6%PHGG; g) SM_co; h) SM_2%PHGG; i) SM_6%PHGG



Nevertheless, similar to what was observed for the curds containing β-glucan, Figures 4.11 and 4.12 indicate that there was a tendency towards more open structures, with larger interstices as the level of DF used increases. This was more noticeable within milk formulations based on 1% and 0.1% fat (Figures 4.11 d-i and Figures 4.12 d-i) and especially when PHGG was used (Figures 4.12 e,f,h,i). PHGG 'opened' the curd structure, at the highest level of addition and in low fat formulations producing curd structures (Figures 4.12 f,i) with an appearance closer to the FF_co (Figure 4.12a) rather than SS_co (Figure 4.12 d) or SM_co (Figure 4.12 g). An analogous trend was observed for the curd containing inulin; the extent of the effect was however not as pronounced as with PHGG.

Overall, it is encouraging that these micrographs are in agreement with the results obtained for curd yield, rheological and textural curd characteristics discussed earlier. Thus, increasing levels of DF increased the level of moisture in the product, and an increased ratio of water to protein made the curd softer (water acting as a plasticiser of the protein matrix, making it more pliable).

The cryo-SEM images of curd samples, together with the information gained from the rheological and textural characterisation of DF – fat – casein networks illustrate that the inclusion of certain DFs have positive effects in the production of low fat dairy products. Additionally, it was demonstrated that it is possible to use these DFs in low fat milk formulations as a fat replacer to create low fat curd structures that resemble those of the full fat products. This was shown especially when β-glucan or PHGG were used in milk formulations. This manipulation of protein-fat-DF interactions opens the opportunity to control the structure, and as a result the texture and rheological properties of dairy products. However, further work needs to be conducted to determine the optimum levels of DF addition in low fat milk formulation, which corresponds to acceptable rheological, textural and sensory quality attributes.

4.3.2 Stage 2. The effects of DF on yoghurt characteristics

4.3.2.1 Yoghurt syneresis

Fermented milk products have been consumed since 2,000 BC, but they became very popular only recently when scientific research had documented the belief that they confer

health benefits. As a consequence they are now produced throughout the world, and amongst them yoghurt remain the most popular.

Yoghurt and cheese productions are somehow similar since they both involve the formation of a gel (coagulum) during milk coagulation. However, the coagulation mechanisms are different: while for cheese milk coagulation is most commonly achieved by enzymatic action (as seen for curd), in the case of yoghurt the gel is formed as a result of acidification of milk by starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophillus.* During acidification, colloidal calcium phosphate dissociates from the casein micelles, and aggregation occurs as the isoelectric point (pH=4.6) of casein is approached. As result a continuous three-dimensional network is formed by casein micelles linked together in clusters, chains and strands (Kalab et al., 1983). This network has pores where the aqueous phase (whey) is confined; the entire structure is relatively fragile and can expel whey relatively easy. These, together with seasonal variations in milk quality explain the main problems associated with yoghurt (especially low fat formulations) and which imparts its acceptability: expulsion of serum (syneresis) and variations in rheological/textural attributes (Keogh and O'Kennedy, 1998).

The syneresis results for the yoghurts are illustrated in Figure 4.13 and also summarised in the ANOVA Table 4.9 (for samples containing inulin and PHGG). As expected, the yoghurt made from full fat milk retained significantly higher percentage of serum within its structure, thus being characterised by decreased syneresis in comparison to the yoghurt made from skimmed milk. These results are in agreement with previous studies (Keogh and O'Kennedy, 1998) and may be explained by the presence of fat globules which may limit casein aggregation, preventing the shrinkage and rearrangement of the three-dimensional network into a more compact structure (Fox et al., 2000b).

Figure 4.13. Syneresis of yoghurt a) samples containing β-glucan; b) samples containing inulin, PHGG and SMP

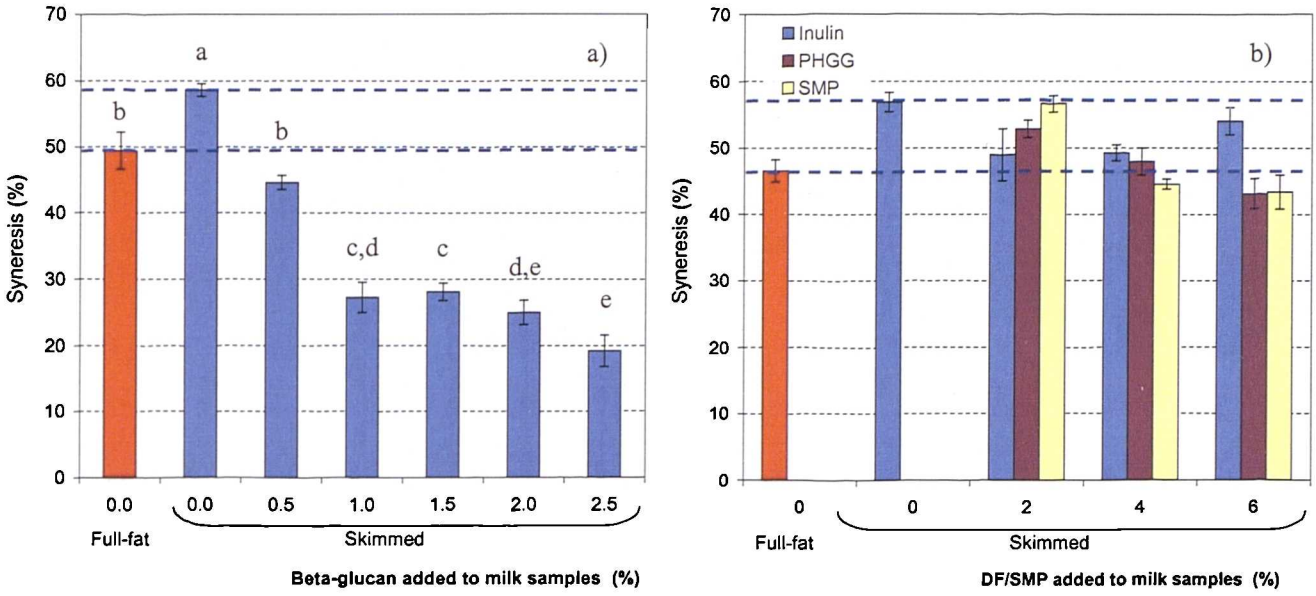


Table 4.9. ANOVA table summarising the rheological attributes and syneresis behaviour of yoghurt containing inulin and PHGG (the values represent means at a given treatment level)

Sample	Syneresis	Apparent viscosity (Pa*s)	Apparent viscosity (cP)	Storage modulus (Pa)	Tanδ
FF_co	*49.3±2.8*	*1.8±0.01*	*19967±3458*	*824±25.9*	*0.280±0.002*
SM_co	*58.6±0.95*	*1.2±0.07*	*16900±3605*	*557±51.9*	*0.280±0.003*
Effect of the type of DF					
Inulin	50.7	1.6	19811	783	0.285
PHGG	47.9	2.9	25856	1611	0.294
Significance	NS	***	**	***	***
SEM	1.7	0.2	1413	102	0.001
Effect of the level of DF addition					
2%	52.8	2.3	18933^{b}	850^{b}	0.284^{b}
4%	47.2	2.04	24683^{a}	1371^{a}	0.289^{b}
6%	46.8	2.42	26721^{a}	1370^{a}	0.297^{a}
Significance	NS	NS	*	*	***
SEM	1.7	0.2	1731	125	0.002

- within the same column, the values with the same letter are not significantly different;
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

Yoghurt samples containing β-glucan showed a significantly reduced syneresis in comparison to the SM_co yoghurt ($p<0.001$), and increasing levels of β-glucan in the formulations led to decreasing values for syneresis (Figure 4.13a). Yoghurt containing 0.5% β-glucan had a syneresis level comparable to the FF_co, while higher percentages of β-glucan addition significantly improved the ability of the yoghurt to retain larger amounts of serum within the structure. This low susceptibility to syneresis was previously reported for yoghurt-like fermented oat products and it was attributed to the ability of β-glucan to entrap water within the three dimensional network of the product (Martensson et al., 2001).

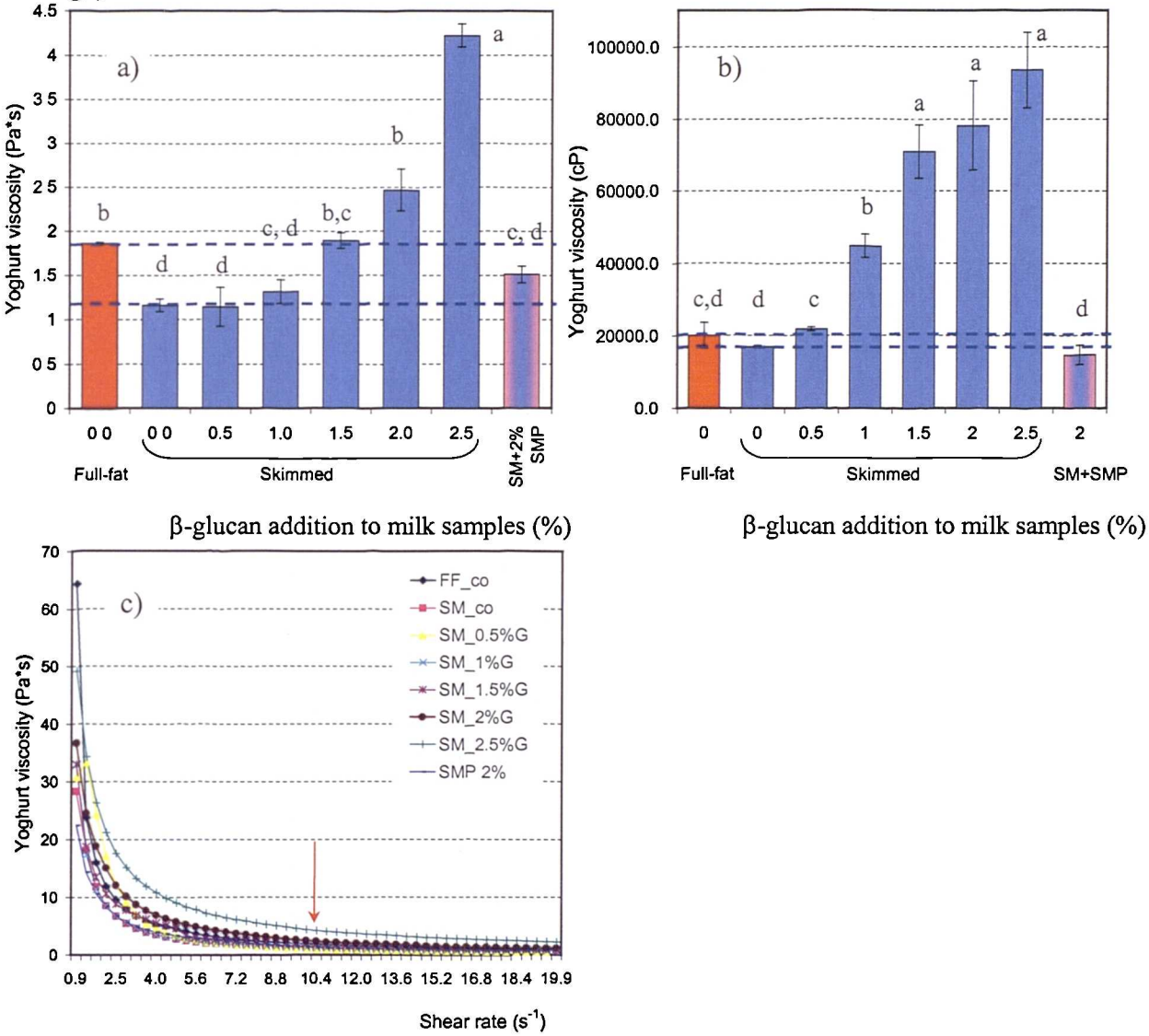
A similar trend could be observed for the yoghurts containing inulin or PHGG (Figure 4.13 b). Although their effect was not as strong as seen for β-glucan, the mean values presented in Figure 4.13b and Table 4.9 indicate that both inulin and PHGG reduced the syneresis of low fat yoghurt, bringing it at levels comparable to FF_co yoghurt especially at higher levels of addition. Although the mean values presented in Table 4.9 suggests that overall PHGG performed better than inulin and increasing levels of DF addition led to decreasing values for syneresis, statistical analysis found no significant difference between the type or level of DF used in the formulation ($p>0.05$).

4.3.2.2 Yoghurt rheological and textural characteristics

Rheological measurements covered both destructive and non-destructive tests and they were performed using both a controlled stress rheometer and a Brookfield rheometer and different geometries (cone and plate and coaxial cylinders respectively). Overall, the results support previous studies (de Lorenzi et al., 1995) indicating that yoghurt can be described in rheological terms as a viscoelastic material with a weak structure and demonstrating shear-thinning behaviour as presented in Figure 4.14c. Since the behaviour of all types of yoghurt was similar (similar shape for viscosity *vs.* shear rate curves), for

comparison purposes and statistical analysis, the apparent viscosity data will be presented as values at specific testing points: at a shear rate of $10s^{-1}$ for the tests performed on the controlled stress rheometer, and at 0.5rpm and a shear rate of $0.47s^{-1}$ for the tests performed on the Brookfield rheometer.

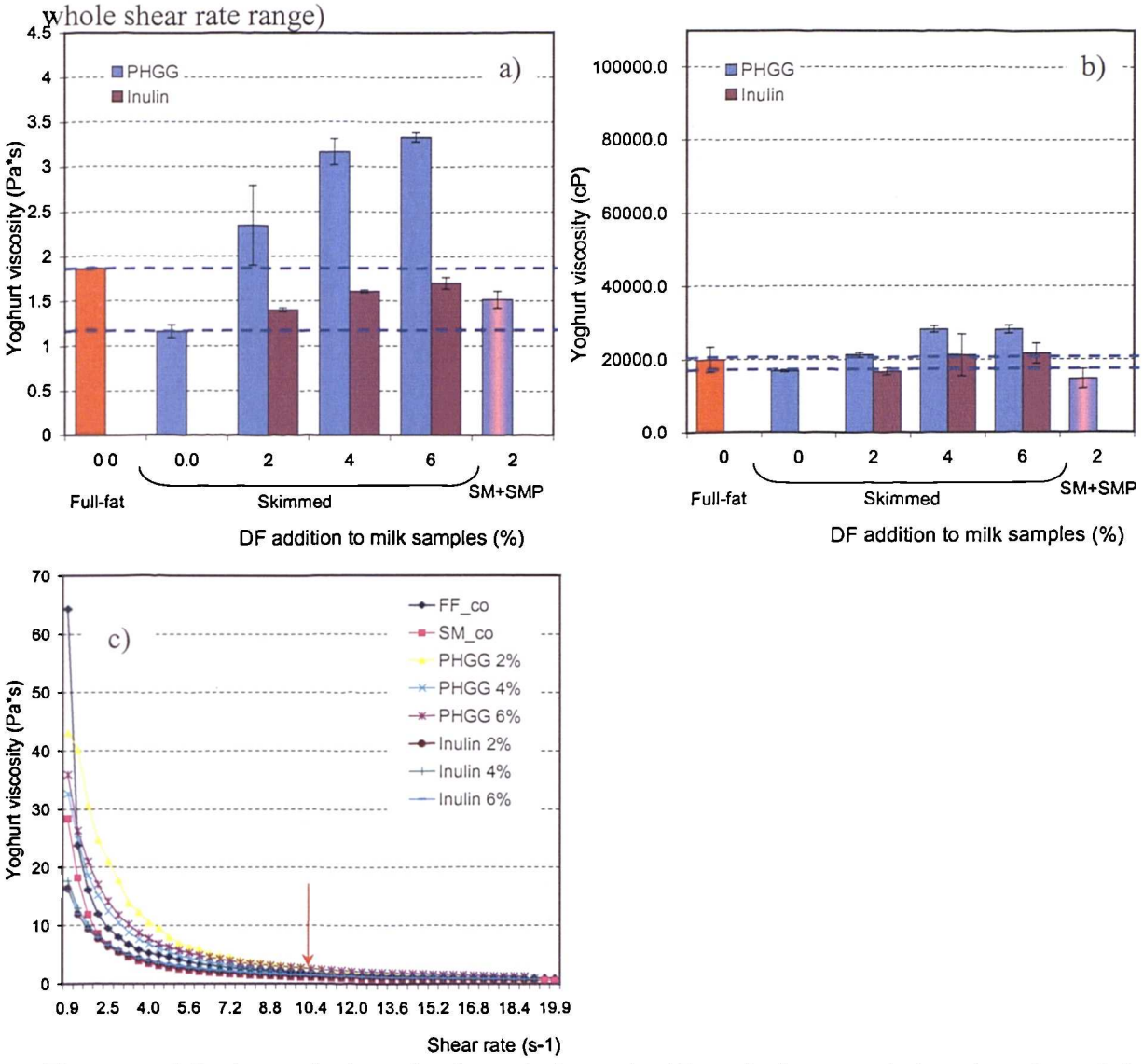
Figure 4.14. Apparent viscosity of yoghurt samples containing β-glucan as determined with a) a controlled stress rheometer - at a shear rate of $10s^{-1}$; b) Brookfield rheometer - at 0.5rpm and a shear rate of $0.47s^{-1}$; c) a controlled stress rheometer (the whole shear rate range)



The effect of β-glucan addition on the apparent viscosity of low fat yoghurt is illustrated in Figure 4.14. As expected, the results indicate that the viscosity of low fat yoghurt was significantly lower than that of its full fat counterpart and this is in agreement with

previous studies (Shaker et al., 2000), and also with the known reduced quality and thus acceptability attached to low fat yoghurts.

Figure 4.15. Apparent viscosity of yoghurt samples containing inulin and PHGG as determined with a) a controlled stress rheometer - at a shear rate of $10s^{-1}$; b) Brookfield rheometer - at 0.5rpm and a shear rate of $0.47s^{-1}$; c) a controlled stress rheometer (the whole shear rate range)



The use of β-glucan in low fat formulations significantly increased the viscosity of the product as suggested by Figure 4.14a and b, and statistical analysis. Thus, when added at levels of 1% (w/w) or higher, the viscosity of the product seemed significantly improved in comparison to SM_co and even to FF_co. One would think that this improvement in product viscosity was purely related to increased total solids present in the formulations,

since yoghurt manufacturers use this method to improve the texture and to reduce the syneresis of their products. To test this hypothesis, rheological measurements were also performed on low fat yoghurt containing 2% added skimmed milk powder (SMP). Interestingly, the viscosity of this product was similar to SM_co (Figure 4.14).

The effects of inulin and PHGG and various levels of addition on the apparent viscosity of low fat yoghurt are summarised in the ANOVA Table 4.9 and illustrated in Figure 4.15. Regardless of the instrument used for rheological measurements, statistical analysis suggests that yoghurts containing PHGG were significantly more viscous than those containing inulin ($p<0.001$, $p<0.01$). If the overall mean values for apparent viscosity of yoghurts containing inulin or PHGG are compared with the mean viscosity values for FF_co and SM_co, it can be observed that both inulin and PHGG appeared to improve the viscosity of the products in comparison to SM_co. However, while inulin brought the viscosity of the low fat product towards values closer to those of FF_co, PHGG significantly increased the viscosity of the products in comparison to both controls (FF and SM) (Figure 4.15 and Table 4.9). Moreover, the graphs presented in Figure 4.15 indicate an increase in viscosity with increasing levels of DF used; statistical analysis confirmed this trend on data obtained from the Brookfield rheometer (Table 4.9).

It is interesting to note the magnitude of the differences in yoghurt viscosity between the formulations containing β-glucan, and inulin or PHGG. The average apparent viscosity of yoghurt containing 2% β-glucan (2.46 Pa*s), as assessed using the controlled stress rheometer was well above the viscosities of yoghurts containing inulin which ranged from 1.4Pa*s (at 2% inulin addition) and 1.7Pa*s (for 6% inulin addition), but comparable to the viscosity of yoghurt containing 2% PHGG (2.3Pa*s). However, 2.5% β-glucan produced a yoghurt with a higher viscosity (4.2Pa*s) than that of the product containing

6% PHGG (3.3Pa*s)! These differences could be related to differences between the molecular weights, shapes of the molecules and spatial conformation the polysaccharides adopt in solution, all of these altering significantly the flow behaviour and thus the viscosity of the products.

The measurements of apparent viscosity of yoghurts using large deformation, destructive tests, provide information on product properties that may be related to the consistency of the gel during consumption. The results presented so far indicate that generally the incorporation of selective DF in low fat milk formulations increased the product viscosity in comparison to SM_co and often even in comparison to FF_co (depending on the type of DF and level of addition).

However, dynamic/oscillatory rheological tests can complement the large deformation tests by providing useful information on the product viscoelastic behaviour. While maintaining the structure not destroyed (tests performed within the linear region), these tests gave information on the elastic modulus (G'), viscous modulus (G") and the loss tangent (tanδ) and the results are presented in Figures 4.16 and 4.17 and summarised in Table 4.9. With regard to viscoelastic characteristics of FF-co *vs* SM_co, the results indicate that G' of FF_co was higher than of SM-co suggesting a stronger gel structure. These support previous research studies reporting similar tendencies: G' increases with an increasing fraction of fat (van Vliet (1988) cited by Lucey and Singh (1998)). They are also in agreement with the observations on the susceptibility to syneresis: higher G' observed for FF_co sample in comparison to SM_co is related to the strength and the number of bonds in the casein network, which on the other hand determine the susceptibility of strands to breakage.

Figure 4.16. Storage modulus (G') and tanδ (at 9.5Hz) of yoghurt samples containing DF as evaluated with the controlled stress rheometer a) G' for samples containing β-glucan; b) tanδ for samples containing β-glucan; c) G' for samples containing inulin and PHGG; d) tanδ for samples containing inulin and PHGG

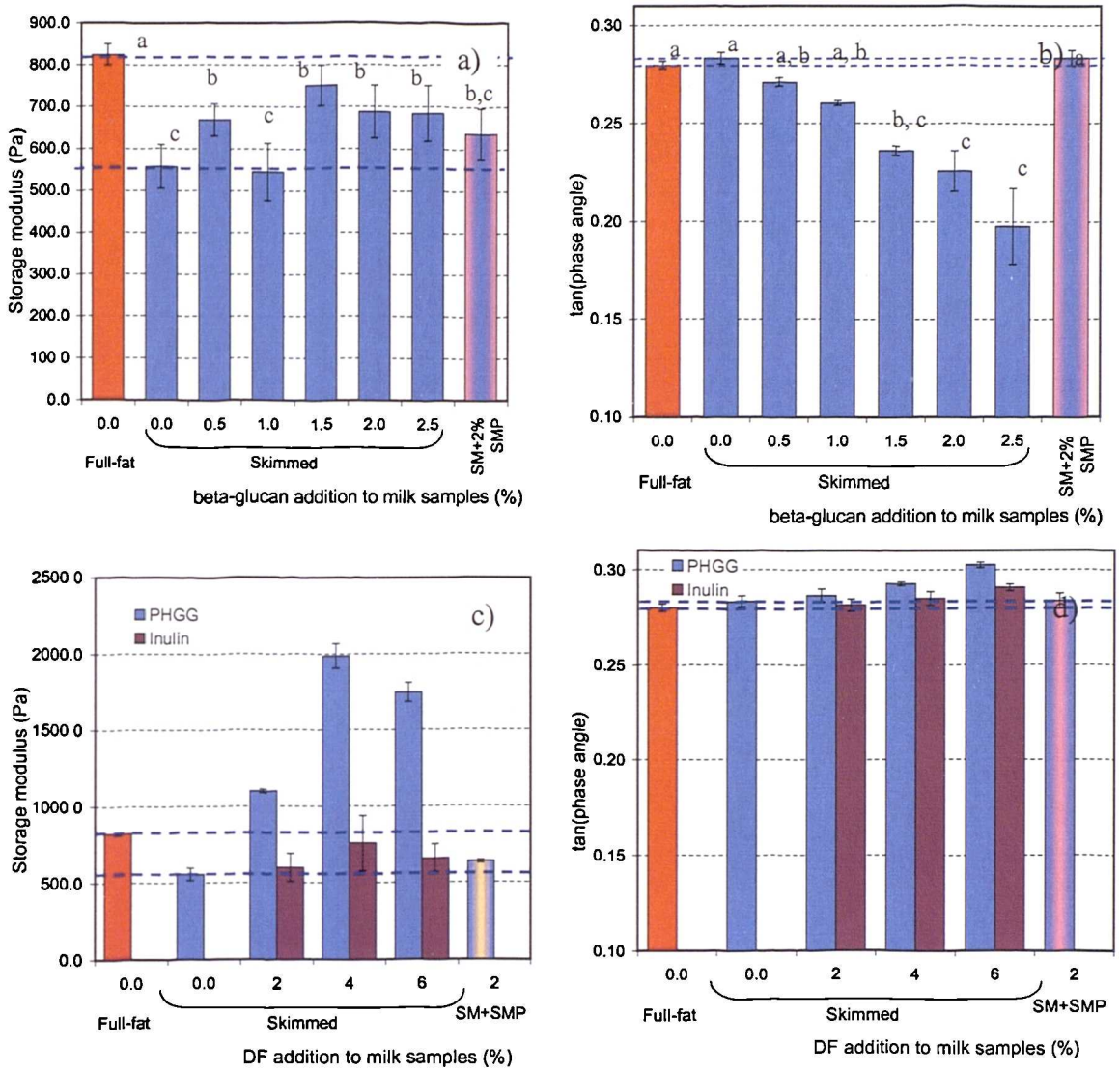


Figure 4.16a indicates that yoghurts containing β-glucan were characterised by increased G' values in comparison to SM-co (except for SM_1%G). This may be explained on one hand by the increased levels of total solids in samples containing β-glucan, and on the other hand by the thermodynamically incompatibility between casein and β-glucan. As extensively explained in paragraph 4.3.1.1, β-glucan may promote self association of casein resulting in increased gel strength (higher G') and at the same time casein may

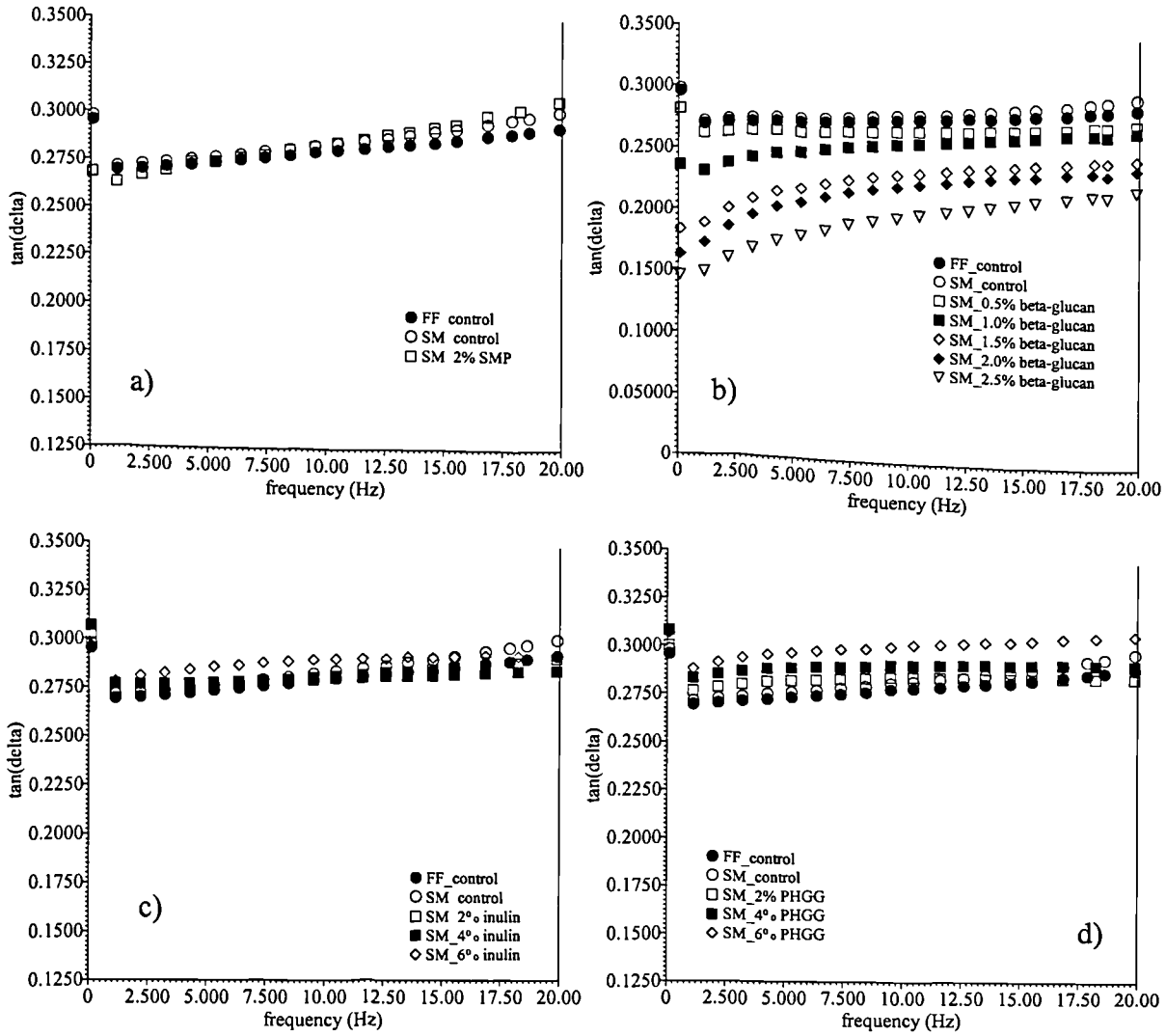
facilitate the association of β-glucan, which at high addition levels could result in the formation of a gel network alongside casein network.

The tanδ values of yoghurts containing above 1% β-glucan also support this idea as they are lower in comparison to both FF_co and SM_co (Figures 4.16b and 4.17b); it is important to observe that the higher the levels of β-glucan, the lower the tanδ values were. This indicates that the presence of β-glucan in yoghurt formulation modifies its viscoelastic behaviour, intensifying its gel-like characteristics. It is encouraging to note that a similar trend was observed during the experiment described in section 4.3.1.3. Thus, these findings on yoghurt viscoelastic characteristics support the results and explanation given for rheological characteristics of low fat curd (section 4.3.1.3).

The viscoelastic characteristics (G' and tanδ) of the yoghurts containing inulin and PHGG are summarised in Table 4.9 and presented in Figures 4.16b and 4.17c,d. Statistical analysis showed that yoghurts containing PHGG were characterised by significantly higher G' and tanδ values than those containing inulin ($p<0.001$, Table 4.9). Moreover, increasing levels of inulin/PHGG resulted in increasing values for both G' and tanδ ($p<0.001$, Table 4.9). The overall average G' and tanδ values of yoghurts enriched with PHGG were higher than those corresponding to both FF_co and SM-co. For yoghurts containing inulin the overall average tanδ and G' values indicated also a slight increase in comparison to those of SM_co. The rheological characteristics of yoghurts containing either PHGG or inulin suggests that the behaviour of these DF differs from the behaviour of β-glucan. The increased G' values for these yoghurts could be related to increased total solids and also to similar interactions casein-polysaccharides as explained for β-glucan. However both inulin and especially PHGG lead to an increase in tanδ of the low fat yoghurt samples (Figures

4.17c,d and Figure 4.16d), suggesting a shift in the viscoelastic characteristics of these yoghurts towards more viscous-like behaviour. Thus, at the levels used in the yoghurt formulations PHGG and inulin contributed essentially to the product viscosity (increased tanδ=G"/G' indicate that the viscous modulus G" is increasing at a higher rate than G').

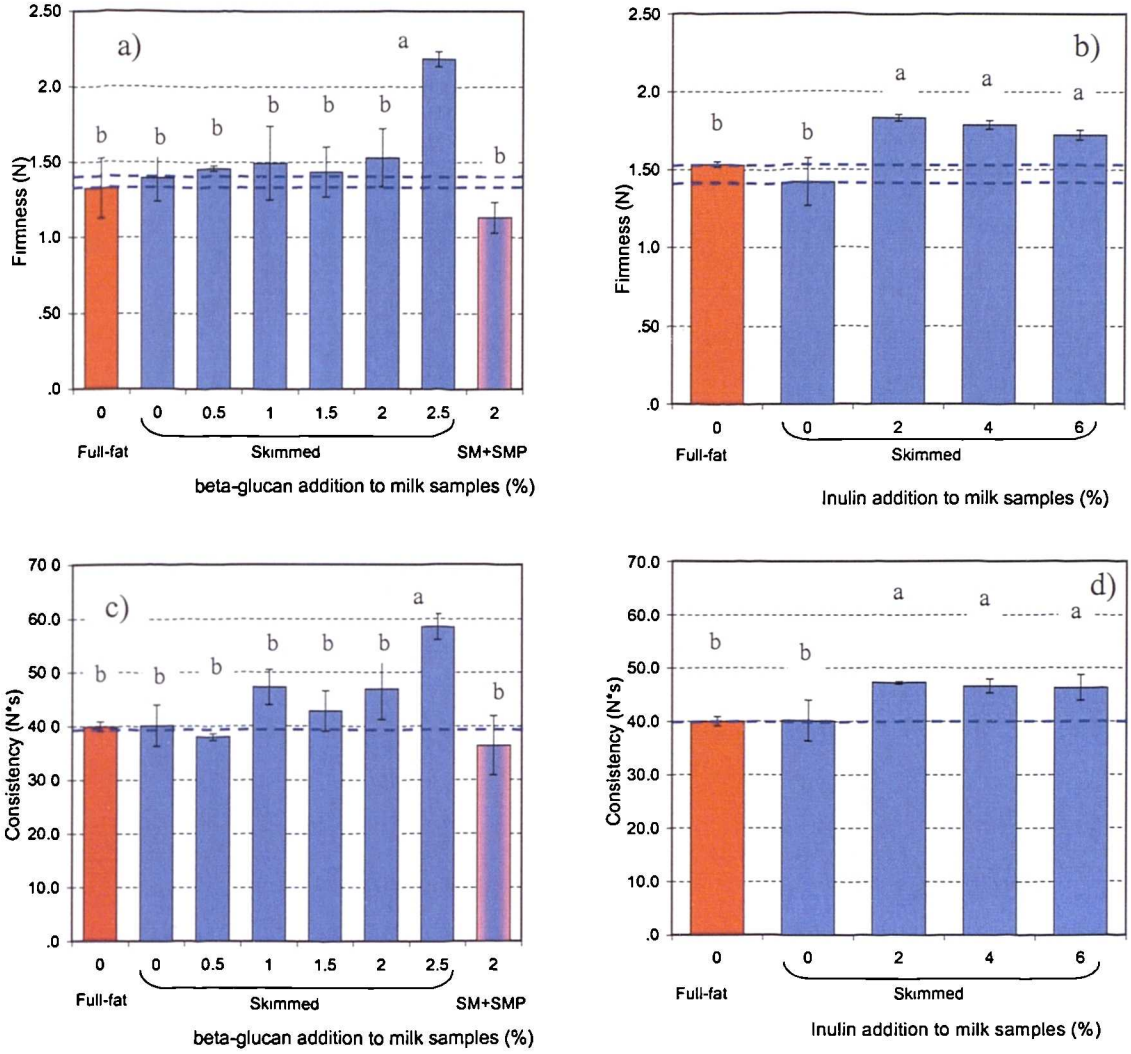
Figure 4.17. Tanδ of yoghurt samples containing DF as evaluated with the controlled stress rheometer a) control samples; b) samples containing β-glucan; c) samples containing inulin; d) samples containing PHGG



As previously mentioned, these effects are more obvious for the formulations containing PHGG than for inulin and may be related to differences in molecular weight, shape of the

molecules and conformation of these polysaccharides, with direct implications on viscosity development (as explained in section 1.3.4.2.3).

Figure 4.18. Textural attributes of yoghurt samples containing DF a) firmness for samples containing β-glucan; b) consistency for samples containing β-glucan; c) firmness for samples containing inulin; d) consistency for samples containing inulin



Textural attributes of DF enriched yoghurts (gel strength/firmness and consistency) were evaluated following a back-extrusion test performed on a Texture Analyser. The results for the samples containing β-glucan and inulin are presented in Figure 4.18 (texture analysis was not performed on samples containing PHGG due to insufficient PHGG material). FF-co and SM-co samples were not significantly different in terms of their

firmness or consistency and this supports previous research results (Schmidt and Bledsoe (1995) cited by Lucey and Singh (1998)).

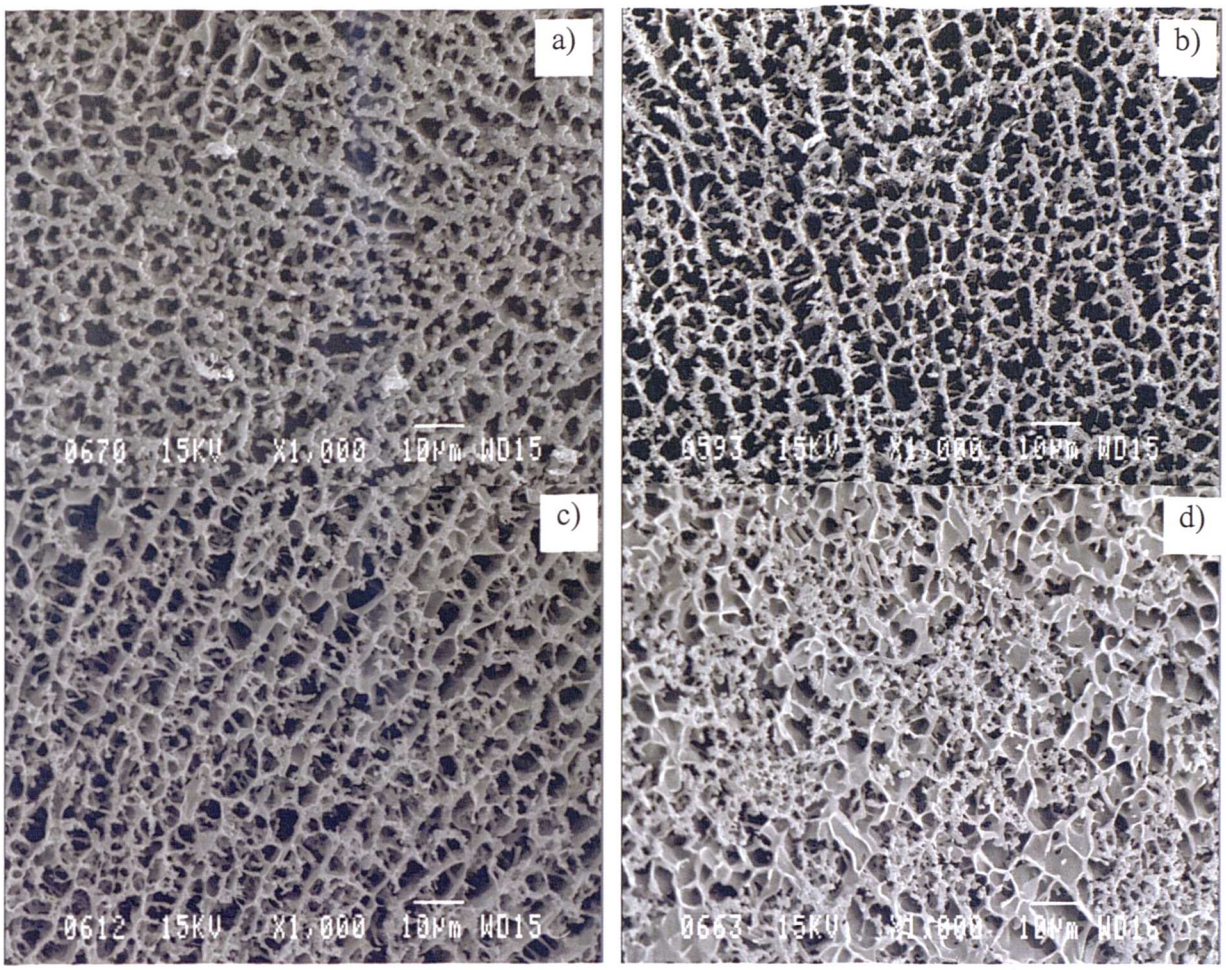
The incorporation of either β-glucan or inulin in yoghurt formulation resulted in a slight increase in product firmness and consistency in comparison to both FF-co and SM_co ($p<0.05$). For samples containing β-glucan, the highest firmness and consistency of the product was obtained for formulations containing 2.5% addition level. The increase in gel strength/firmness and consistency for the samples containing DF (β-glucan or inulin) could be again explained by increased levels of total solids and interactions between casein and polysaccharides which may contribute to increase in the strength of casein network. The texture results alongside the rheological results are in agreement with the trends observed for yoghurt syneresis; increased gel strength (G' and firmness) would make it less susceptible to rearrangements within the network, and consequently less susceptible to shrinkage and serum (whey) expulsion.

4.3.2.3 Yoghurt microstructure

Representative scanning electron micrographs of the yoghurt samples investigated are presented in Figures 4.19 and 4.20. Similar to what was reported previously by Kalab et al. (1983) the micrographs of control yoghurts (FF-co and SM_co) presented in Figures 4.19a,b indicate that the gels consist of a coarse network of casein particles linked together in clusters, chains and strands. This arrangement of casein micelles created relatively large free spaces inside the network which are known to entrap serum (whey). A clear difference can be observed between the structures of FF_co and SM-co yoghurts as illustrated by Figures 4.19a and b respectively. The full fat formulation was characterised by a denser, more 'branched' structure, with apparent thicker casein strands in comparison to low fat yoghurt, which had a more open structure, with larger pores (uniformly distributed) and

fine casein strands. The appearance of the SM-co micrograph (Figure 4.19b) suggests a weaker structure in comparison to FF_co (Figure 4.19a), more susceptible to breakage and internal rearrangements which are in agreement with lower G' and higher syneresis values obtained (paragraphs 4.3.2.2. and 4.3.2.1).

Figure 4.19. SEM micrographs (x 1000) for yoghurt containing β-glucan: a) FF_co; b) SS_co; c) SM_1%G; d) SM_2.5%G

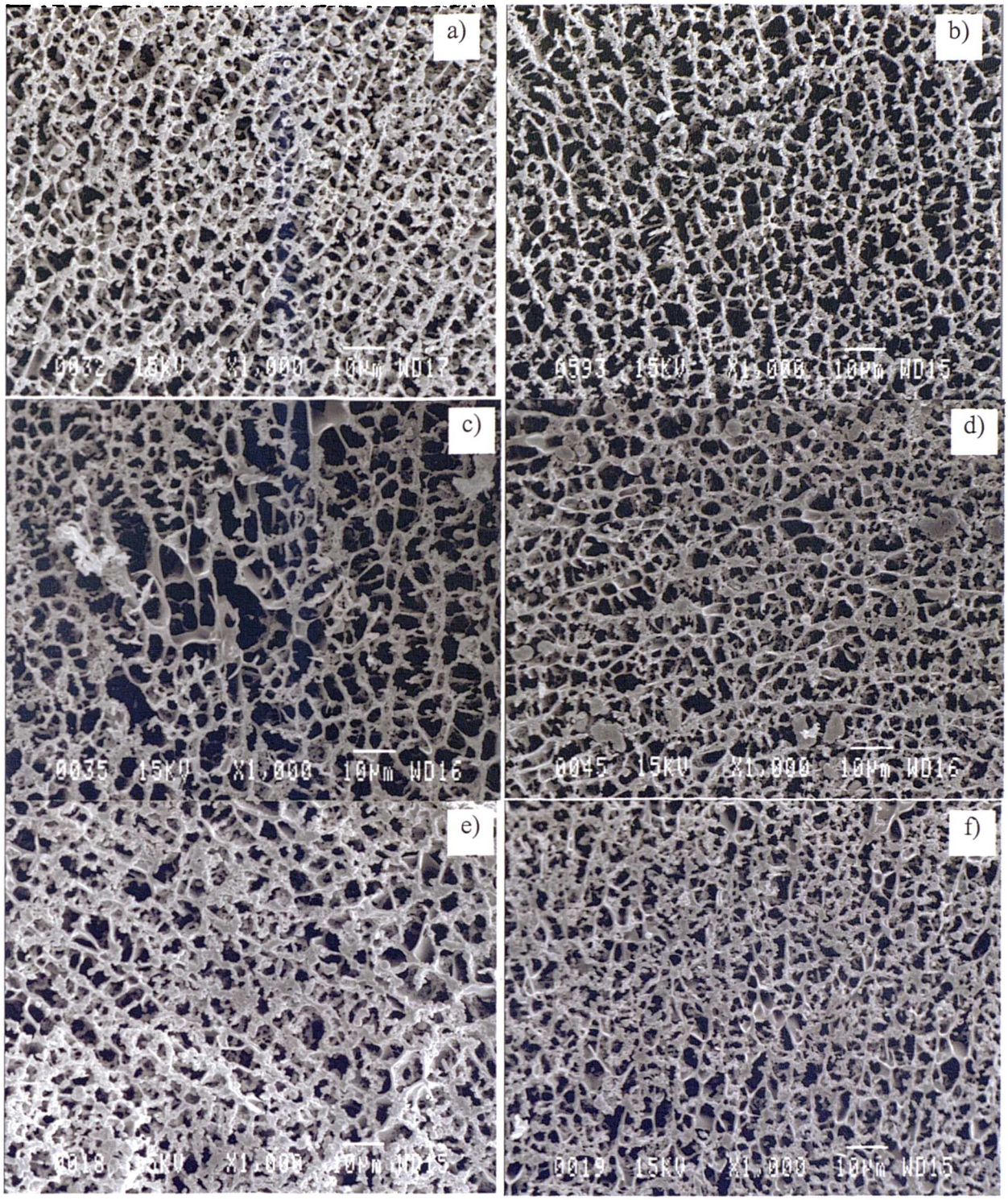


The incorporation of β-glucan in low fat formulations appeared to modify the structure of the final product and this is illustrated by the micrographs presenting yoghurt containing 1% and 2.5% β-glucan (Figures 4.19 c and d). At 1% β-glucan addition the structure already started to change (Figure 4.19c); although it was still more open than for FF_co, it can be noticed that

some smaller voids appeared in the casein network alongside the large ones, indicating that at least two levels of pore sizes were present. Equally interesting are the changes/increases in the thickness of casein strands; thus this structure leen FF_co and SM_co. The modifications in the yoghurt internal structure which started to be visible in samples containing 1% β-glucan are very obvious when higher levels of β-glucan were used. Although made from the same skimmed milk, the microstructure of the yoghurt containing 2.5% β-glucan (Figure 4.19 d) was found to be radically different from SM_co, but more similar to FF_co; it was characterised by a denser network, composed of thicker strands and creating free spaces/pores similar in size to the ones found in FF_co. Within this structure fused casein particles can be observed leading to the formation of a network which appeared to be slightly thicker and denser even than FF-co. This internal organisation explains the observed decreasing susceptibility to syneresis for samples containing increasing levels of β-glucan (Figure 4.13) and also the differences in the flow behaviour of the yoghurt as shown by the rheological and textural characteristics (increased viscosity and increased firmness - Figures 4.14, 4.16, and 4.18).

Similar changes in the microstructure of low fat yoghurt were obtained for formulations containing inulin or PHGG (Figures 4.20c-f). For samples containing inulin the differences in product structure (Figures 4.20c,d) were not as striking as seen for β-glucan. Nevertheless, the sample containing 6% inulin (Figure 4.20d) was comparable in terms of structure to FF_co having a stronger network and smaller pores than SM_co, and this supports the results obtained on syneresis and rheological attributes, which indicated improved behaviour in comparison to SM_co yoghurt (Figures 4.13, 4.15, and 4.16).

Figure 4.20. SEM micrographs (x 1000) for yoghurt containing inulin and PHGG: a) FF_co; b) SS_co; c) SM_2% inulin; d) SM_6% inulin; e) SM_2% PHGG; f) SM_6% PHGG.



Samples containing increasing levels of PHGG however, followed a similar pattern of structure modification as discussed for β-glucan; at 2% addition PHGG appeared to promote the formation of a gel structure characterised by a compaction of the casein network which promoted the appearance of two levels of pore sizes (Figure 4.20e). At 6% PHGG addition (Figure 4.20f), the structure of the yoghurt was similar to FF_co; nevertheless there appeared to be regions with higher density than the average. This could explain the higher apparent viscosity, G' and tanδ of samples containing 6% PHGG than of FF_co (Figures 4.15 and 4.16).

The changes of the internal organisation of low fat yoghurt in the presence of β-glucan, inulin or PHGG may be related to the existence of milk proteins - polysaccharides interactions which were detailed earlier in this chapter. The thermodynamic incompatibility between caseins and polysaccharides appeared to promote modifications in the microstructure of the products, with direct impact on product's viscoelastic properties and finally on their quality.

4.3.2.4 Sensory evaluation of yoghurts

From the range of yoghurts produced, eight types were selected for sensory evaluation on the basis of the results obtained from rheological and microstructural investigations. The samples subjected to sensory evaluation were: FF_co, SM_co, SM_0.5%G, SM-1.5%G, SM_2% inulin, SM_6% inulin, SM_2% PHGG, SM_6% PHGG, SM_2% SMP and SM_6% SMP. The results are summarised in Table 4.10 and also illustrated in Figure 4.21.

In terms of appearance attributes as perceived visually, statistical analysis indicated that the yoghurt samples were significantly different only in relation to whey separation ($p<0.001$, Table 4.10). Control yoghurts (FF_co and SM_co) and the formulation

containing 2% inulin received the highest scores for whey separation. All the other samples were given significantly lower scores indicating a significantly decreased tendency for syneresis. Similar trends were indicated by the whey separation scores as perceived by scooping; the highest scores were again assigned to control yoghurts, but also to yoghurts containing low levels of DF or SMP (Table 4.10). At high level of addition, all DFs used appeared to significantly reduce yoghurt susceptibility to syneresis in comparison to control products (Figures 4.21a,c,e, $p<0.001$, Table 4.10), the level of whey separation being comparable to products to which 6% SMP was added. These confirm the trends observed and discussed in paragraph 4.3.2.1 of this chapter.

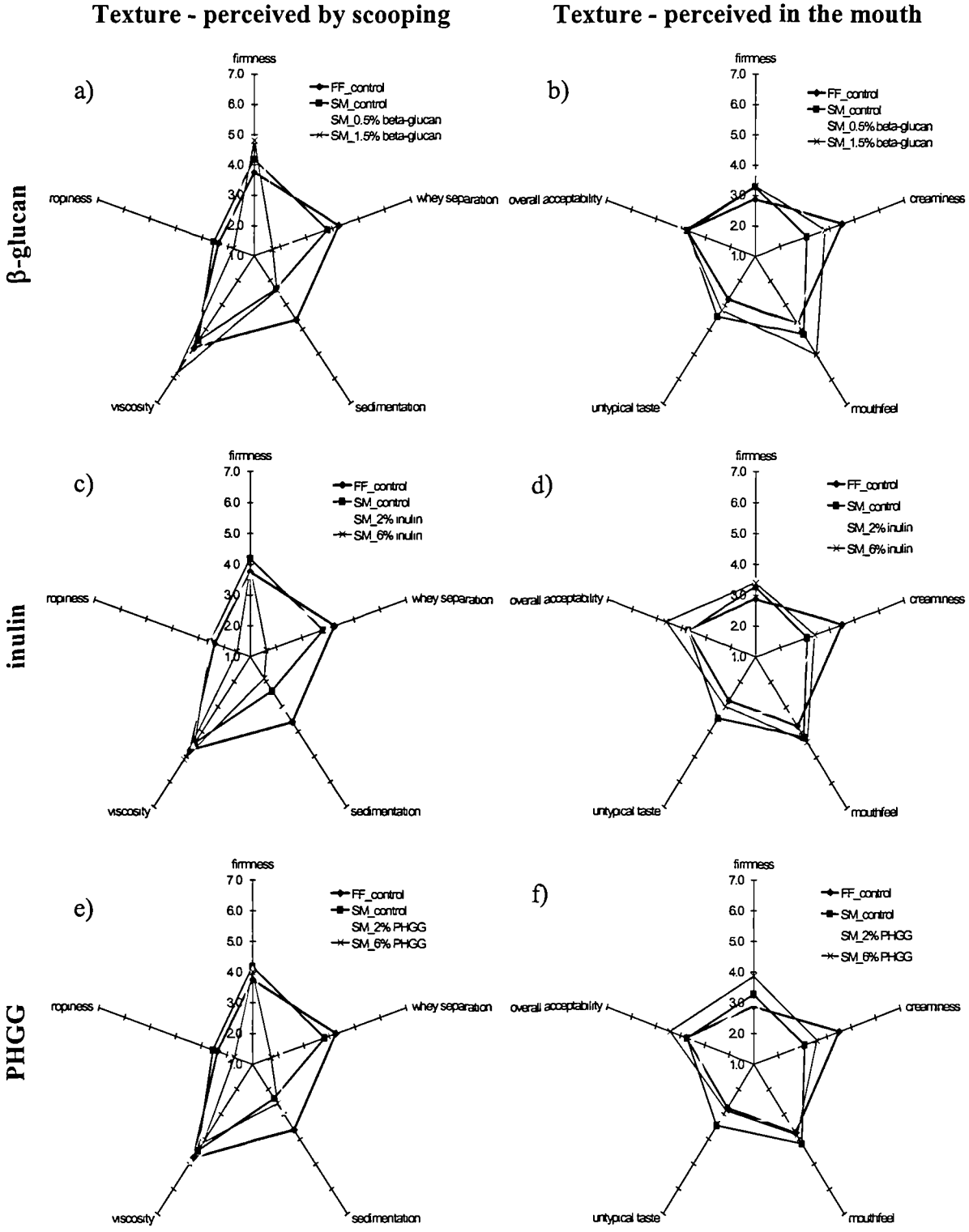
The sensory scores also indicate that the panellists found the samples tested as having similar firmness, except for the yoghurt containing 2% SMP, which received significantly lower scores ($p<0.001$, Table 4.10). This was also confirmed by the mean values for the firmness as assessed in the mouth which indicate no significant difference between the samples ($p>0.05$).

Table 4.10. Mean values for the sensory attributes of DF enriched yoghurt

	FF_co	SM_co	SM_0.5%G	SM_1.5%G	SM_2% inulin	SM_6% inulin	SM_2% PHGG	SM_6% PHGG	SM_2% SMP	SM_6% SMP	Significance
Appearance (as perceived by visual inspection)											
Whey separation	4.4^{a}	4.7^{a}	1.6^{b}	1.7^{b}	5.0^{a}	1.4^{b}	1.9^{b}	1.3^{b}	2.4^{b}	1.5^{b}	***
Colour	5.0	5.2	5.0	5.0	4.6	5.7	5.0	6.0	5/2	5.9	NS
Aspect of the surface	5.8	5.3	5.8	5.1	4.7	5.0	4.8	5.0	5.7	6.1	NS
Glossiness	5.7	5.4	5.2	5.2	4.6	5.0	4.9	4.6	5.2	5.5	NS
Texture/body (as perceived by scooping)											
Firmness	3.7^{a}	4.1^{a}	4.8^{a}	4.8^{a}	3.6^{a}	3.9^{a}	4.1^{a}	4.0^{a}	3.1^{b}	5.2^{a}	**
Whey separation	4.2^{a}	$3.8^{a,b}$	$2.4^{a,b,c}$	1.7^{c}	4.0^{a}	1.6^{c}	$2.1^{a,b,c}$	$1.7^{b,c}$	$2.8^{a,b,c}$	1.2^{c}	***
Sedimentation	3.6	2.7	2.5	2.4	3.1	1.9	2.9	2.6	2.2	1.5	NS
Viscosity	4.7	4.4	5.1	5.8	4.5	5.1	4.9	4.1	4.2	6.4	NS
Ropiness	2.4	2.5	2.2	1.8	2.5	1.5	1.9	1.7	2.2	1.6	NS
Texture/body (as perceived in the mouth)											
Firmness	2.9	3.3	3.7	3.3	3.4	3.4	3	3.8	2.5	3.7	NS
Creaminess	$4.5^{a,b}$	2.9^{b}	$3.7^{a,b}$	$3.8^{a,b}$	$3.4^{a,b}$	$3.4^{a,b}$	2.7^{b}	$3.6^{a,b}$	2.8^{b}	5.1^{a}	*
Mouthfeel	$4.7^{a,b}$	$3.8^{a,b}$	$3.7^{a,b}$	$5.0^{a,b}$	3.3^{b}	$4.4^{a,b}$	$3.4^{a,b}$	$3.8^{a,b}$	$4.8^{a,b}$	5.5^{a}	*
Untypical taste/flavour	2.7	3.4	3.1	3.2	3.2	3.0	3.1	2.8	3.0	2.1	NS
Overall acceptability	3.7	3.7	4.1	3.8	3.7	4.6	3.5	4.3	3.6	4.9	NS

- within the same column, the values with the same letter are not significantly different;
*** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; NS - not significant

Figure 4.21. Sensory attributes of yoghurt as affected by DF addition: a-b) β-glucan; c-d) inulin; e-f) PHGG



Interestingly, the panellists found no significant differences in the viscosity of the samples tested and this is somehow surprising. Bearing in mind that the results from instrumental

rheological tests showed significant differences (paragraph 4.3.2.2 of this chapter), it appears that the sensory analysis performed was not sensitive enough to highlight the differences previously observed in the viscosity of the products as assessed instrumentally. In terms of creaminess the lowest scores (related to watery texture of the products) were given by the panellists to low fat control yoghurt and also to low fat yoghurts containing 2% PHGG or 2% SMP (Table 4.10 and Figure 4.21). Incorporation of β-glucan into formulations or inulin or PHGG at high levels (6%), significantly improved the perceived creaminess of the product ($p<0.05$). Equally important, in these formulations the mouthfeel of the products was also improved in comparison to low fat formulations, the resulting texture being perceived as smoother ($p<0.05$, Table 4.10). It is interesting to note that sensory attributes such as colour, glossiness, aspect of the surface, untypical taste/flavour were not affected by the incorporation of DFs into the formulation. However, most importantly the overall acceptability of the products was good, the panellists showing no particular preference for a type of product ($p>0.05$). Nevertheless, the mean values for overall acceptability indicate that there was a tendency that yoghurt samples containing 6% PHGG or 6% inulin were preferred to the controls.

The results from these experiments suggest that the DFs selected for this study could be used successfully in low fat yoghurt formulations. Interactions between DF and milk protein were seen to promote changes in product structure, which were directly related to changes in rheological properties, and in several cases led to improved sensory attributes of the final products.

4.4 Conclusions

The present study showed that selected DFs (β-glucan, inulin and PHGG) have the potential to be used in milk formulations with direct application in obtaining low fat milk products with characteristics similar to their full fat counterparts. This was demonstrated in two types of products (cheese and yoghurt). SEM[1] investigations indicated that the microstructure of the products studied (rennet and acid induced skimmed milk gels) changed drastically in the presence of DF. This was in agreement with the changes observed on their viscoelastic behaviour, and the origin of the changes are thought to be primarily related to the existence of segregative casein-polysaccharide interactions that promote demixing phenomena.

The present study also demonstrated that various different microstructures may be obtained depending on the type of DF, its characteristics, and level used. This enables the production of low fat dairy products, with non-compromised quality characteristics, and which also bring the added health benefit of DF.

Chapter 5. Concluding comments

During the past few decades major global health transitions have occurred, changes that have profoundly altered life expectancy and our ways of living, but also led to an epidemic of non-communicable diseases. This epidemic is now accelerating world wide at a worrying rate, resulting in a discrepancy between health care needs and resources.

Almost all Western countries are experiencing an obesity epidemic, with the problem becoming increasingly common in young adults and children. Along with the increase in obesity, the incidence of type 2 diabetes, CVD and hypertension have increased dramatically causing not only a depreciation of the overall quality of life of individuals, but also leading to large numbers of premature deaths. The growing concerns on health issues amongst consumers, governments, health authorities, and the scientific community have led to formulations of dietary guidelines for prevention and management of these epidemics. A recent joint FAO/WHO report (WHO, 2003) made dietary recommendations for the prevention of diet related chronic diseases, the general belief being that small changes in risk factors can have a significant positive impact in the long term. Among other recommendations it was suggested to decrease the intake in free sugars below 10% of the daily energy, to increase the total DF intake (minimum intake 25g /day), and to restrict the intake of saturated fatty acids to less than 10% of daily energy intake. The same report listed the intake of low GI foods as a possible factor in reducing the risk of weight gain,

obesity and developing type 2 diabetes. However, the dietary habits of the consumers do not reflect these recommendations and part of the reason is related to a relative shortage of such products on the market and also to their inferior palatability (e.g. low fat yoghurt) (Wilkinson et al., 2000).

In an attempt to address these issues, this study investigated the behaviour of a range of DFs in cereal and dairy food formulations with the aim to screen for DF ingredients that may lead not only to nutritionally valuable, but also to good quality, palatable functional foods. A range of insoluble and/or soluble DF were used in cereal and milk formulations, having as objectives:

- to assess the effects of various DFs on the microstructure of the resulting DF enriched products;
- to widen the knowledge surrounding the effects of DF enrichment on the textural, rheological attributes and overall quality and acceptability parameters of cereal and dairy based products;
- to establish a relationship between the type of DF, level of addition, microstructural organisation of the food product and its quality;
- for the cereal products to investigate the effects of DFs on starch digestibility, and consequently on the GI of the products;
- for the dairy products to assess the potential use of DFs in low fat formulations.

For the investigations on cereal products a range of soluble (guar gum, inulin, β-glucan, xanthan gum, LBG) and insoluble DFs (pea fibre and bamboo fibre) were chosen to be used in pasta and bread formulations at various levels (2.5, 5.0, 7.5 and 10% from the

flour). Chapters 2 and 3 describe the results of these experiments. The behaviour of DF enriched pasta/bread products during cooking/storage and *in vitro* digestion was discussed in relation with the changes observed in the structure of the products.

Generally, both the type and level of DF addition were shown to have a significant effect on the quality attributes of these products.

The results on pasta indicate that various DFs had different effects on the cooking qualities of DF enriched products. Thus, inulin, guar gum, bamboo fibre and LBG appeared to not affect the cooking qualities of the products since the cooking losses obtained were comparable to the control. In contrast, pea fibre and β-glucan led to increased cooking losses in comparison to the control, indicating a deterioration of the product structure with starch exposure to swelling and rupture, while xanthan gum significantly decreased the losses resulted during cooking.

Overall, DF enriched pastas showed reasonably good textural characteristics, in many cases similar to the control. Xanthan gum and LBG appeared to consolidate the structure strength of pasta (indicated by higher firmness and elasticity values in comparison to the control), and these observations support and expand the research of Edwards et al (1995). It is important to underline that the effects associated with xanthan gum and LBG may represent an important selling point showing that DF enriched products could still have an *al dente* texture, and moreover in these cases slight overcooking is expected to not dramatically alter the quality of the final product. The use of all the other types of DFs led to slight decreases in product firmness and elasticity under the conditions of the experiment. Similar trends have been noticed previously (Knuckles et al., 1997, Marconi et al., 2000, Edwards et al., 1995) but for different levels of DF and other type of DF preparations. Nevertheless, the quality of the products in terms of textural attributes could

be improved by optimising the recipe (added gluten) or optimising the cooking time.

Equally interesting are the results related to DF enriched bread. On average increasing levels of DF caused gradual depreciation of bread quality parameters (volume, specific volume, height) in comparison to the control, and these agree with previously reported effects (Laurikainen et al., 1998). The most positive and promising results on bread quality characteristics were associated with the use of bamboo fibre, LBG, pea fibre and guar gum, which generated results comparable to the control. However, other DFs (β-glucan, inulin and xanthan gum) significantly depreciated the final bread quality, and this was not entirely unexpected considering the work of Wang et al. (2002a), Keagy et al. (2001), and Knuckles et al. (1997).

The evaluation of the textural attributes of DF enriched breads during storage generated attractive results, too. Except for inulin and β-glucan, all the other DFs produced breads with firmness and springiness values comparable (pea fibre) or improved (guar gum, xanthan gum, LBG, bamboo fibre) in comparison to the control (decreased firmness and increased springiness). These effects were either due to the production of initially softer crumbs (guar gum, LBG) or to reduced rates of staling (bamboo fibre, xanthan gum and pea fibre) in comparison to the controls. These findings extend and complement previous research on bread staling (Wang et al., 2002, Guarda et al., 2004, Barcenas et al., 2004) reporting such effects of certain DF but when used at significantly lower levels. Sensory analysis carried out on breads containing the highest levels of LBG and bamboo fibre (10% from the flour) confirmed the results obtained from the quality evaluation and texture analysis. In the light of general belief that DF addition in bread formulation is associated with poor quality bread, the present observations become even more exciting and

encouraging since they reveal that there is a real potential in developing good quality DF enriched bread.

The current research served to expand on previous knowledge in the area linking the structure of the food products with their physical properties, and certain nutritional aspects (starch digestibility). SEM investigations provided a valuable insight into the internal structure of both bread and pasta products as affected by DFs used. It is important to note that the observations made in the case of pasta were also seen in bread products, although the initial internal structure of those two food products is notably different. One of the conclusions drawn was that generally all DFs especially at high levels of addition seemed to lead to reduced degree of starch swelling in comparison to the controls, regardless of the cereal product investigated. Furthermore, the structure of the products enriched with insoluble DFs or inulin was generally found to be more comparable to their controls in the sense that the starch granules although less swollen, appeared to be distinct within the protein-DF matrix. In comparison, soluble DFs (except for inulin) significantly altered the structure of products. In these cases the starch granules appeared to be coated in a mucilaginous looking layer similar to what has been found previously by Brennan et al. (1996), and they seemed to retain their shape, to be less swollen and distinct from one another, but at the same time they became part of the matrix that forms the product structure. These structural differences persisted from the initial production of the products (pasta/bread) and were still evident after they have been digested for 300 min. As such, the manipulation of food structure was seen to play an effective role in controlling the physical and potentially nutritional quality of a cereal product.

Previous research studies have indicated that certain DFs alter postprandial glycaemia in both healthy and diabetic subjects (Gatti et al., 1984, Potter et al. 1981, Bourdon et al.,

1999). This effect on blunting glycaemic increase, together with delaying gastric emptying, and enhanced satiety have been associated with the consumption of soluble DF. This study expands this area through the meaningful findings related to the rate of starch digestion following the *in vitro* experiments conducted. In agreement to other studies carried out *in vivo* and *in vitro* (Wolever et al., 1986; Casiraghi et al., 1992), this work indicates that pasta leads to significantly lower glucose response than an equivalent amount of carbohydrate in form of bread. More importantly, as a step forward this work proved that certain DFs when used in pasta or bread formulations could lead to even slower rates of sugars released than from the controls. Increasing levels of DF resulted in significantly reduced product digestibility. The lowest rates for starch digestion and consequently the lowest HIs and predicted GIs were obtained when soluble DFs (except for inulin) were incorporated in the formulations. Nevertheless, the use of bamboo fibre, an insoluble DF seemed to have a similar effect on the rate of starch digestion as the soluble DF. The SEM micrographs of digested products were in agreement with the parameters calculated following the *in vitro* digestion, and provided a visualisation of what was concluded for starch digestion. Ranking of the DFs used according to their effect on products digestibility was possible and it is interesting and encouraging noting that the order found for DFs in relation to their effect in bread was similar to that found in pasta. Thus these results confirm that the effect of DFs on starch digestibility is real and it manifests independent of the product internal structure (in porous as well as in compact structure). The reduced rate of starch digestibility in products containing certain DFs is proposed to be the result of a combination of denominator factors: reduced starch swelling and rate of amylose leaching out of the granules; formation of a layer coating starch granules, which may act as a barrier between starch and α-amylase; potential inhibition of α-amylase by DF.

A pilot *in vivo* study confirmed the results previously found during the *in vitro* digestion of

bread, indicating a significant decrease of the predicted GIs of bread containing LBG or bamboo fibre (at 10% addition) in comparison to the WWB, while no significant difference was found when the two types of breads were compared to each other. These GI values (determined either *in vitro* or *in vivo*) can prove extremely useful if it takes into account the importance of low GI cereal foods beyond the immediate low glycaemic response (i.e. insulinaemic effects and second meal effects), which recommend them as having a preventive effect for the risk of various chronic diseases. Also it can be of considerable interest for the food producers that recently became interested in low GI foods under the pressure of health authorities increasingly concerned about the nation's health in relation to the dietary habits.

Although these results can not claim to have found the ultimate answer on the mechanisms involved in decreased starch digestibility when in combination with certain DFs, 'new insights' were provided to widen the knowledge on this subject. In particular, these sets of experiments showed that the use of DFs offers a possible and suitable way to design high fibre, low GI functional cereal foods and to improve their nutritional quality as far as GI is concerned. Thus the physiological benefits of high DF intake can be complemented by the metabolic merits of a low GI diet. In addition it was demonstrated that certain DFs do not diminish the quality, textural properties and sensory acceptability of the final products. Since on the market there are not many carbohydrate rich foods eliciting a low postprandial glycaemic response, the knowledge that certain DF are effective in lowering blood glucose concentration offers a significant potential in the development of new functional food products designed to diminish the risk of developing chronic diseases and that can also be of considerable therapeutic use (e.g. the management of diabetes).

The studies on dairy products focused on the effects of three types of soluble DFs: β-glucan, inulin, and PHGG on products (fresh curd and yoghurt) microstructure and rheological properties, in relation to the level of DF addition and also to the percentage of fat in milk. The goal was to assess the behaviour of these DFs in milk formulations for potential development of functional dairy products (enriched with DF) with low fat content.

Several technological benefits became obvious for fresh curd and yoghurt produced from formulations containing DFs. Coagulation time and optimum cutting time of low fat milks containing β-glucan or PHGG decreased in comparison to the low fat controls, in certain instances reaching values comparable to those obtained for the full fat milk. In these cases an increase in curd yield and a decrease in the amount of proteins lost in the whey were also observed, especially in relation with increasing levels of DF used. These effects could not be reproduced by the formulations containing inulin. In the low fat yoghurts the presence of DF improved their stability against mechanical damage, as it was shown by reduced syneresis, and again the best results were associated to the use of β-glucan and PHGG. For yoghurt, these results were also confirmed by sensory analysis carried out on a selection of samples.

Rheological tests performed indicated that in both fresh cheese and yoghurt, the DFs used altered the viscoelastic behaviour of the final products. Thus, in the fresh cheese made from either semi-skimmed or skimmed milk, increasing levels of DFs led on average to decreasing G' and tanδ values in comparison to the controls, indicating the development of weaker and at the same time more elastic structures. The results indicate that several low fat curds showed rheological behaviour closer, and possibly similar to that of their full fat counterparts. For instance, this was achieved when β-glucan was added at concentrations

of 1.0% to 1% fat milk or 1.0, 1.5% to 0.1% fat milk, or when 4% PHGG was added to 1% fat milk. These results were also confirmed by the large deformation rheological measurements (texture tests) performed on the samples. However, these trends were more obvious for β-glucan or PHGG containing curds as opposed to curds made from formulations containing inulin.

Similarly, changes in the flow behaviour of low fat yoghurts (increased apparent viscosity) were obtained for the formulations containing DF. The effects were evident especially in relation to the use of β-glucan or PHGG, which is in agreement to what was observed for DF enriched curds. Oscillatory rheological tests gave a fuller understanding of product viscoelastic characteristics as affected by the type and level of DF, and helped explain the susceptibility of the low fat yoghurts to syneresis. Thus on average all DF increased G' values in comparison to SM-co. This trend was explained by the increased levels of total solids in samples containing DF, and on the other hand by thermodynamical incompatibility between casein and polysaccharides. Complementary information was acquired by observing tanδ values which indicated clearly that each type of DF used behave differently in the milk system. In formulations containing β-glucan the resulting tanδ were lower in comparison to both controls (full fat and skimmed milk), showing that the presence of β-glucan intensified yoghurt's gel-like characteristics. It is important to note that this supports the similar trend observed for rheological characteristics of low fat curd. In contrast, both inulin and especially PHGG produced an increase in tanδ of the low fat yoghurt samples, implying a shift towards more viscous-like behaviour. The effects were once again more obvious for the formulations containing PHGG than for inulin and this was explained by the differences in the molecular weight, shape of the molecules and their conformation.

An important part of the work focused on gathering information about the structure of the curd and yoghurt products, particularly important to better understand and control the texture and stability of the final dairy foods.

As presented in Chapter 4, dramatic changes of curds structures were induced by the presence of the DF studied. Increasing levels of DF in low fat formulations resulted in increasing pore sizes and in the formation of a more open network in comparison to skimmed milk control curd, which in several cases (1% fat milk and 1% β–glucan, and 0.1% fat milk and 2% β-glucan) resembled the structural configuration of full fat curd. Similarly, the weak, very opened structure of the skimmed milk yoghurt was seriously altered by the DF, becoming a denser network, composed by thicker strands and creating pores comparable in size to the ones found in full fat control yoghurt. This was obvious, especially for the formulations containing β-glucan or PHGG, and for high levels of addition.

The internal organisation/conformation of the low fat milk products was in agreement with changes observed in their flow behaviour as identified by the results on rheological and textural parameters. The origin of these changes is believed to be related primarily to the existence of segregative casein-polysaccharide interactions that were detailed in Chapter 4. The structural investigation work is particularly important not only for understanding the rheological behaviour of the products, but also because the information in this area is lacking. Very few studies have been published that deal with the microstructure of milk-polysaccharides gels (Sanchez et al., 2000; Kalab et al., 1975, Hood and Allen, 1977). SEM investigations used for these experiments proved to be an efficient method to detect changes in casein networks such as those induced by the presence of polysaccharides.

The results presented in Chapter 4 demonstrate that the DFs selected (β-glucan, inulin and PHGG) can be used in milk formulations. Interactions between DF and milk protein were seen to promote changes in product structure, which were directly related to changes in rheological and textural characteristics, and in several cases led to improved sensory attributes of the final products. The present study shows that very original microstructures can be obtained depending on the nature of the type and level of DF used. This could open new opportunities in tailoring milk protein based gels, and more specifically low fat dairy products with non-compromised quality characteristics and which also bring the health benefits of DF (which can contribute towards reaching the daily recommended level). The knowledge of the mechanisms occurring in casein-polysaccharide mixed systems is of great importance in order to develop specific properties in dairy products.

Summary of the main conclusions:

- DF can be successfully used to design good quality functional cereal or dairy products, with acceptable and in some cases improved sensory attributes. However, it was shown that there is no generic solution/rule, which can be applied in order to lead to good quality food products. A careful selection of suitable DF and levels needs to be carried out in relation to the type of product to be made in order to ensure that such products meet consumer expectation with regards to their sensory characteristics.
- DF enriched cereal products were shown to bring additional health benefits, through their lowered GI in comparison to the control products (without DF), as demonstrated by *in vitro* and *in vivo* studies. It was proved that the effects of DF on starch digestibility are evident regardless of the product internal structure (they are effective in porous as well as in compact structures). Ranking of the DF used according to their effect on the GI of cereal foods was achieved.

- In the dairy products interactions between DF and milk protein were found to promote substantial changes in product structure, with direct influence on their rheological properties; in several cases improved sensory attributes of the final products were obtained.
- Various different microstructures in milk products can be obtained depending on the type of DF, its characteristics, level used and also on the fat content of milk. This enables the production of low fat dairy products, with non-compromised quality characteristics and which also bring the added health benefits of DF.

Future work

The effect of DF on starch digestibility and consequently on the GI of starchy foods, is definitely an area that warrants further investigation, especially now with the ongoing consumer awareness on low GI foods coupled with a shortage of such products on the market.

This study showed that a reduction in the GI of starch foods could be achieved not only in relation to the use of soluble DF, but also for certain insoluble DF. It would be therefore interesting to determine the exact mechanisms generating these effects, and also the minimum concentration needed to achieve significant difference. For example, are these effects due to the restricted starch swelling, changes of the product microstructure or include also inhibition of α-amylase?

Thus further *in vitro* digestion studies on model starch systems are needed to be carried out to investigate the effect of the type of DF (LBG, xanthan gum, β-glucan, bamboo fibre, guar gum), ratios between starch and DF and different moisture contents. It would be also

interesting to compare these effects in relation to different products structure and different cooking procedures (e.g. cooking as opposed to extrusion-cooking).

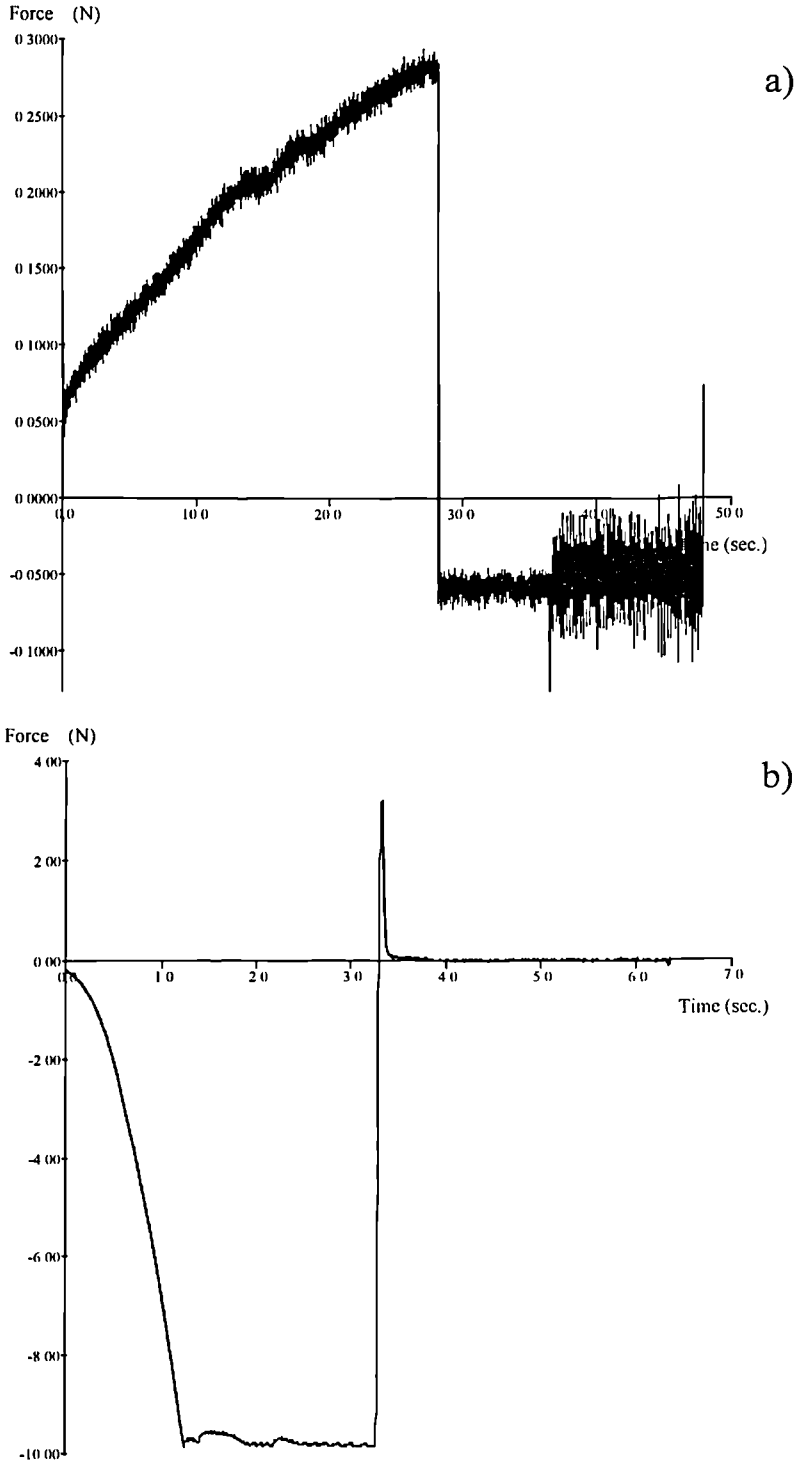
However, the ultimate aim must not be forgotten, and this is to generate knowledge which can be used by the food producers to design good quality DF enriched cereal products with low GI and acceptable sensory properties. Thus, understanding the interaction between DF and food components and their behaviour during various processing stages is essential. Phase diagrams of starch and various DFs mixtures could prove to be useful. In addition investigations on model systems involving selective DF in combination with starch and gluten could be considered to focus on the effect of DF on rheological characteristics, water distribution between individual components, food microstructure, starch retrogradation and storage characteristics.

It was also demonstrated that in milk products certain DFs promote modifications in the microstructure of the products which enabled the generation of low fat milk products with characteristics similar to their full fat counterparts. However, a deeper understanding of the interactions between milk proteins and various DF in relation to various processing stages is needed. Thus phase diagrams of casein-PHGG and casein β-glucan mixtures are needed. Moreover it would be interesting to monitor the changes in structure and rheological properties of the mixtures during various stages of processing in relation to both levels of DF and fat present in the milk formulations.

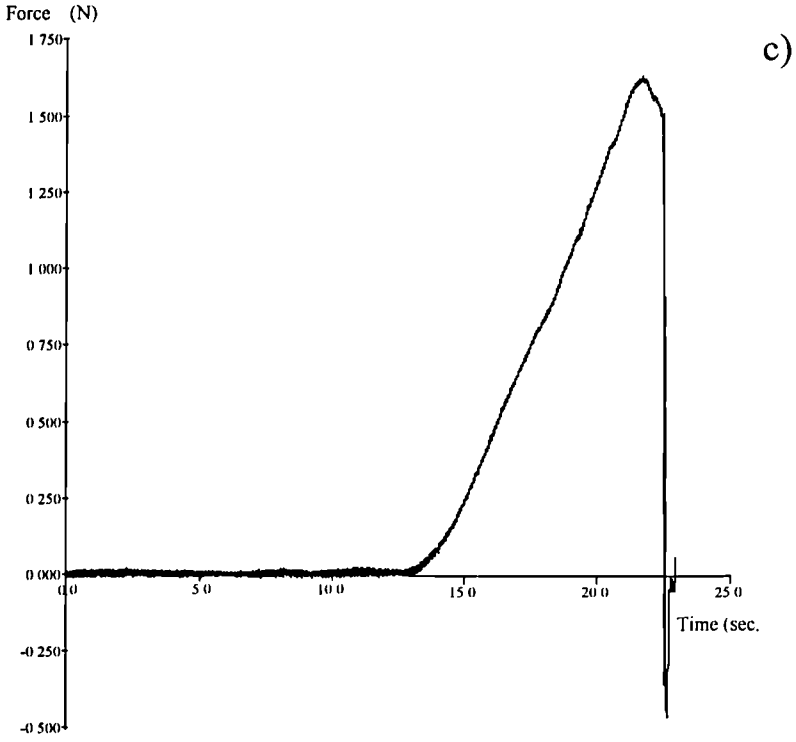
Hopefully all of this information will be of use for food manufacturers and it will potentially lead to a wider range of DF enriched products becoming part of our daily diet.

Appendices

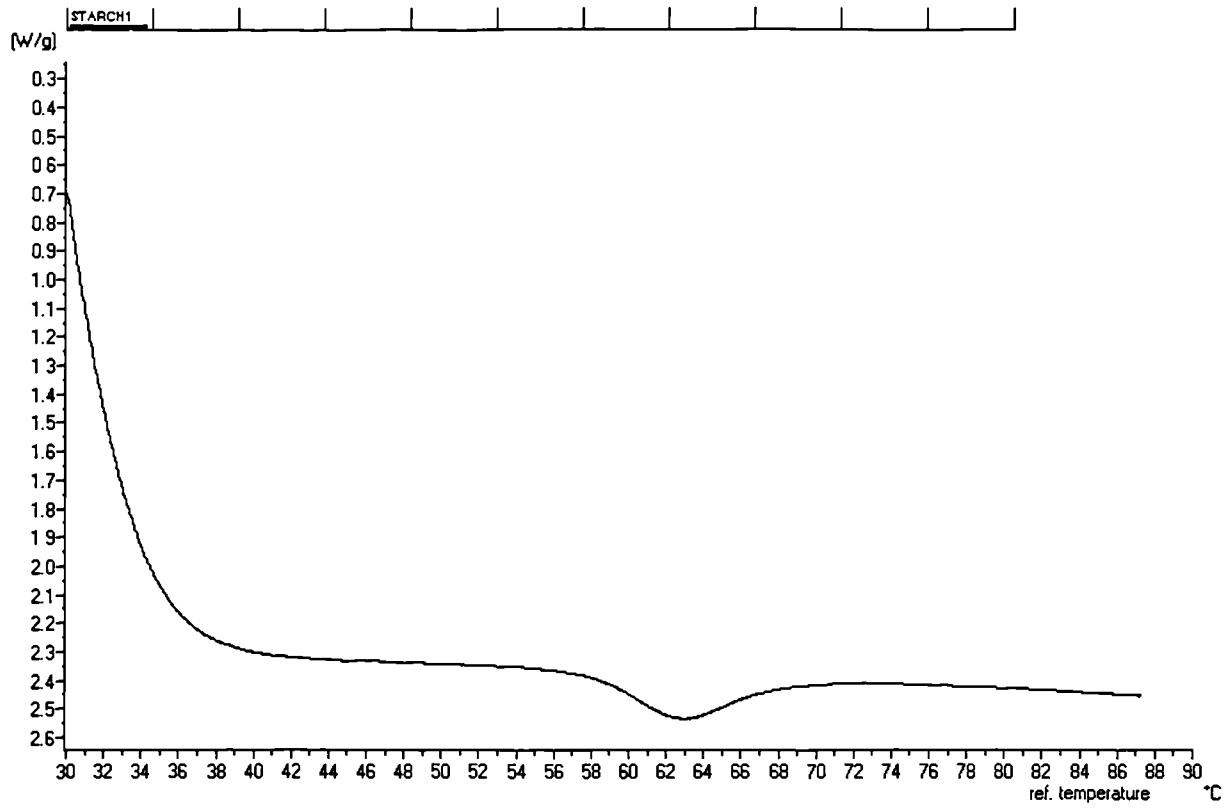
Appendix 2.1. Texture Analyser - typical tests for assessment of pasta textural characteristics: a) tensile test; b) adhesive test; c) firmness test



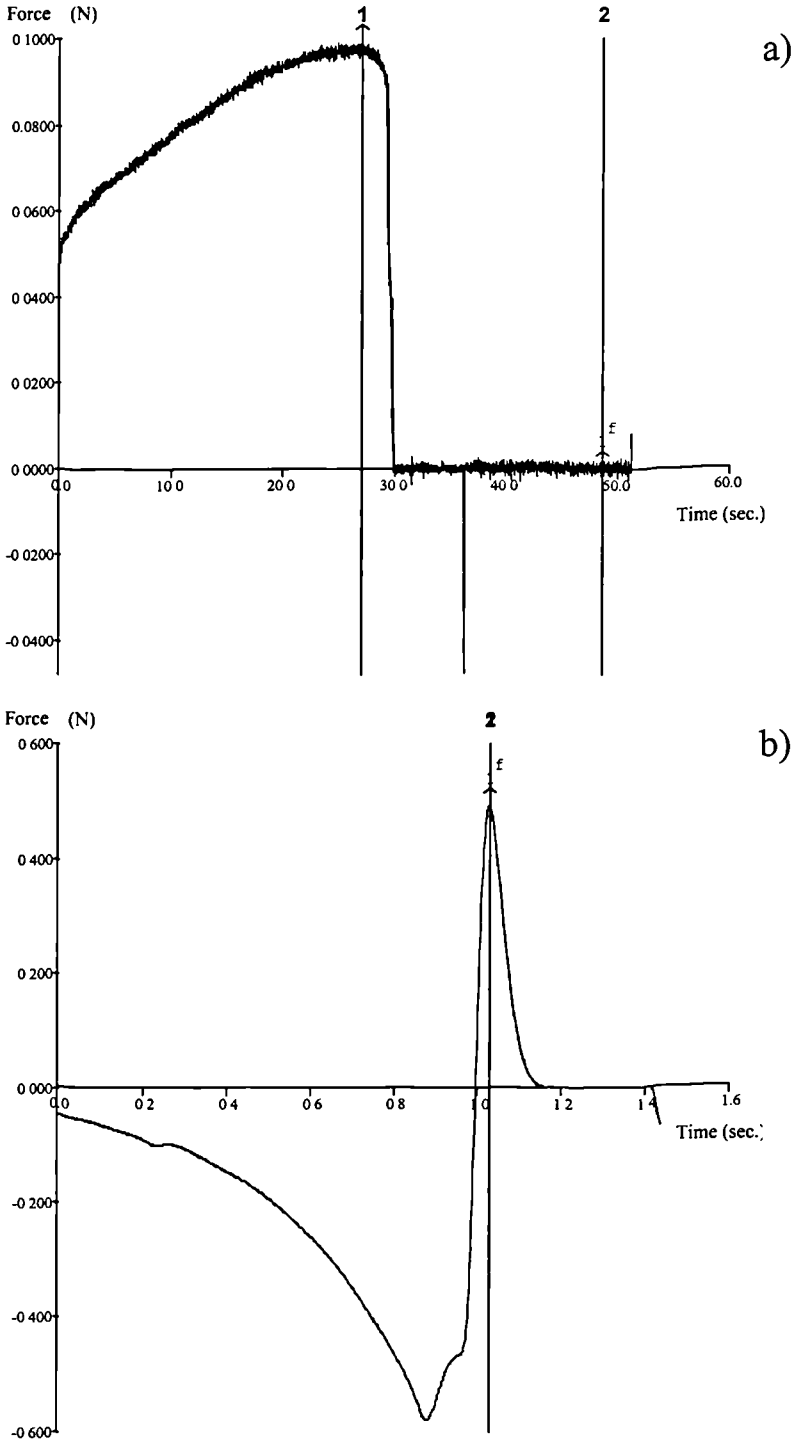
Appendix 2.1. (continued)



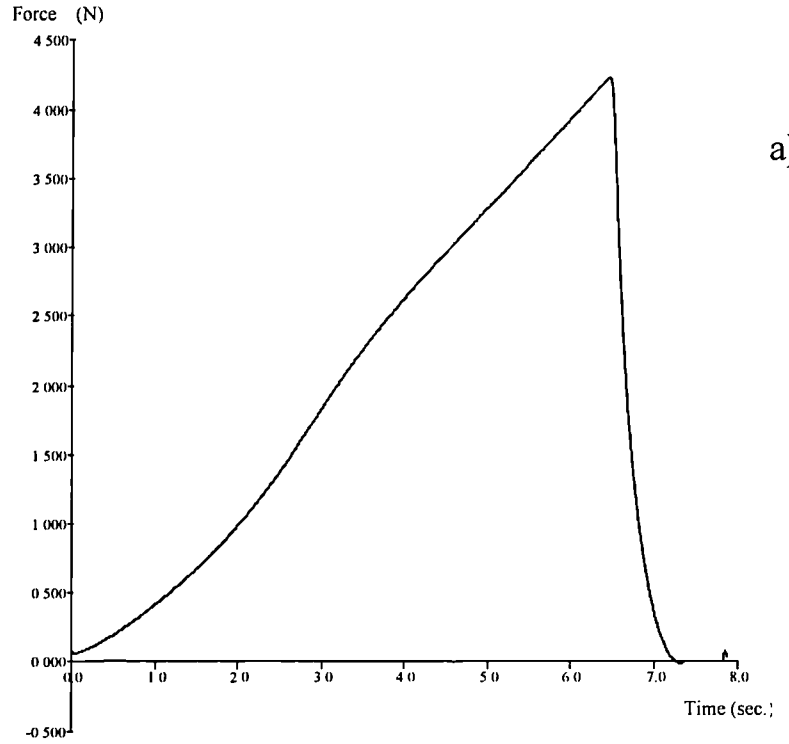
Appendix 2.2. Differential Scanning Calorimetry (DSC) test - typical curve for starch gelatinisation



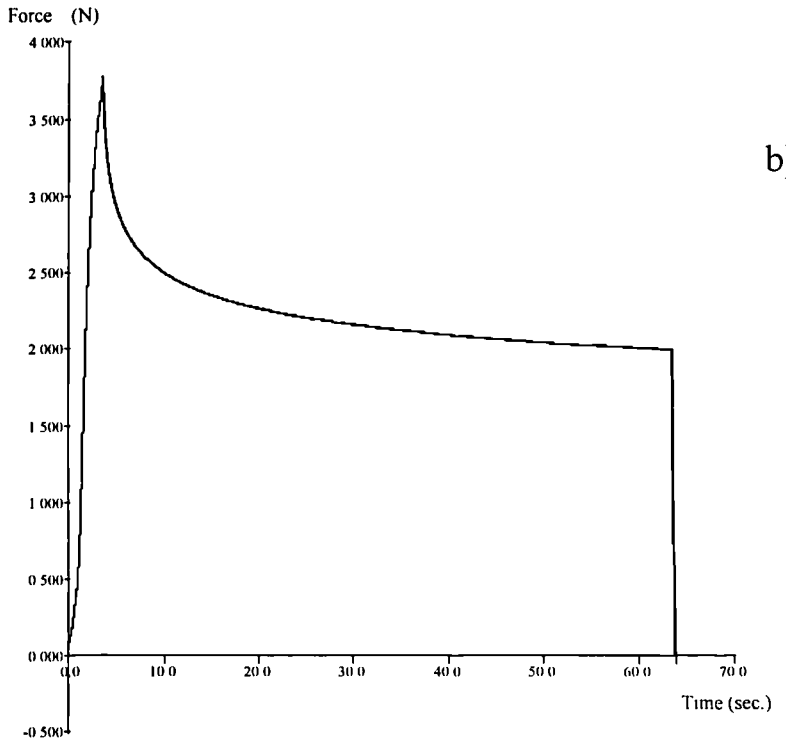
Appendix 3.1. Texture Analyser - typical tests for assessment of dough textural characteristics: a) tensile test; b) adhesive test.



Appendix 3.2. Texture Analyser - typical tests for assessment of bread textural characteristics: a) crumb firmness; b) crumb springiness.



a)



b)

Appendix 3.3. Assessment of satiety/hunger

Date: **Subject code:**
Sample code:

After each blood sample is taken, please tick the appropriate box, which corresponds to your feeling of hunger.
-10 represents extreme hunger
+10 represents extreme satiety

Time 0 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 15 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 30 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 45 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 60 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 90 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Time 120 min

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10

Thank you for your time! ☺☺☺☺☺☺

Appendix 3.4. Bread sensory evaluation form

Panelist name: .....

Sample code:

Date:

Bread sensory evaluation

Please tick the box at the point which best describe the sensory characteristic of the sample

Crust colour

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

dislike extremely like extremely

Crumb colour

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

dislike extremely like extremely

Crumb texture

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

dislike extremely like extremely

Flavour

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

dislike extremely like extremely

Taste

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

dislike extremely like extremely

Overall acceptability

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9

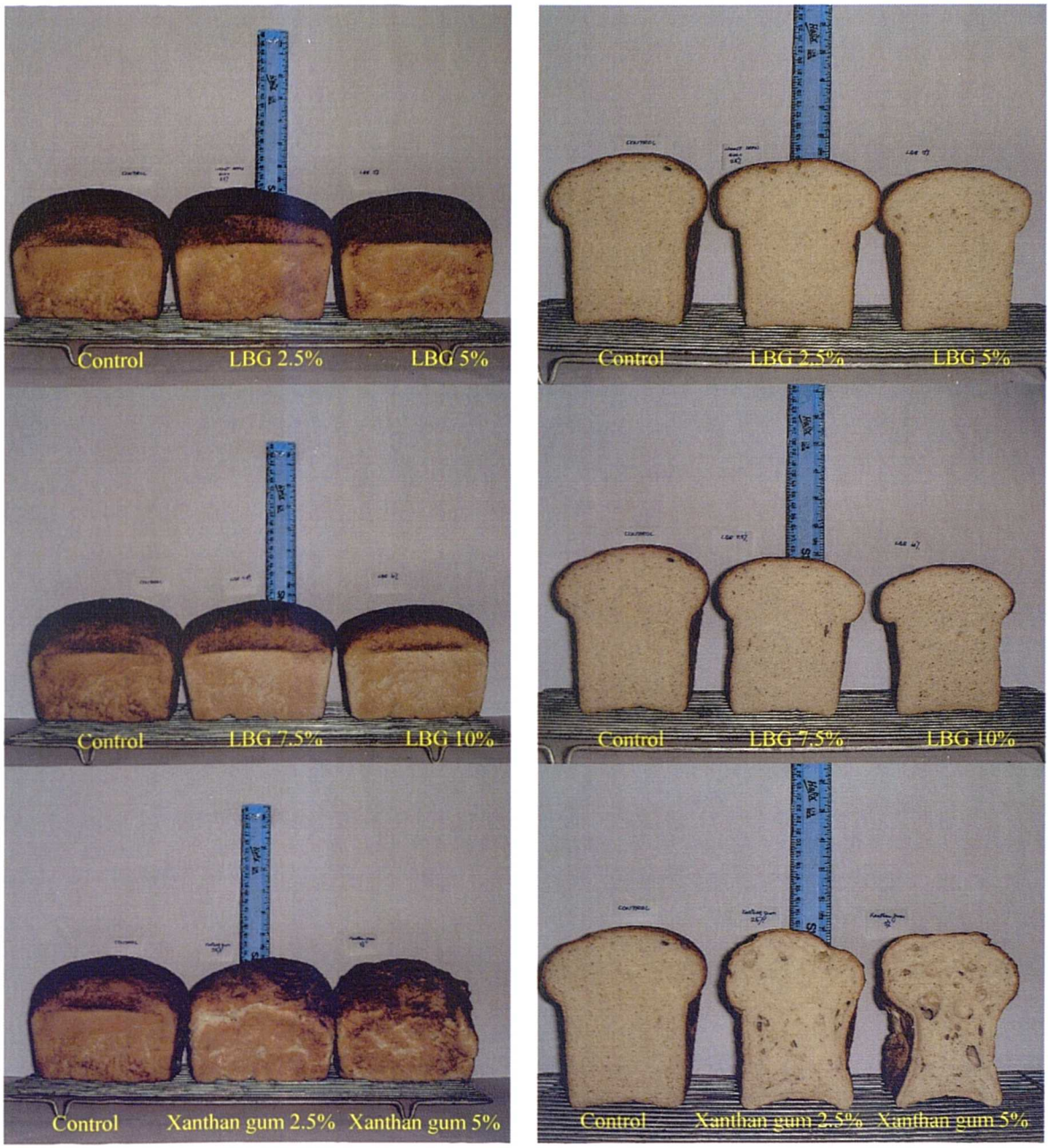
dislike extremely like extremely

Thank you for your time! ☺☺☺☺☺☺

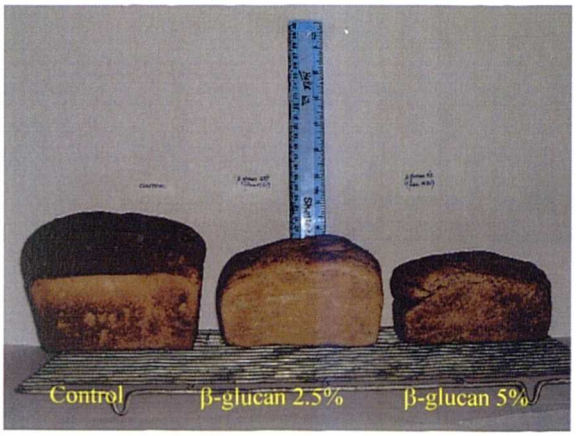
Appendix 3.5. Digital images of DF enriched bread

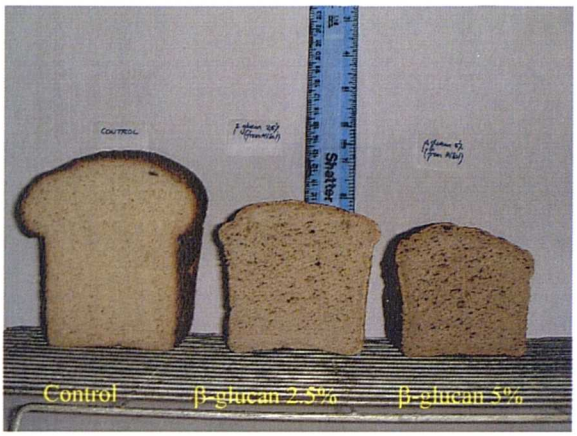
Appendix 3.5. (continued)

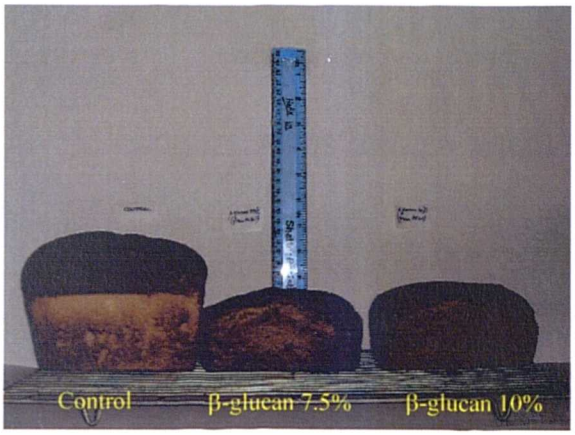
Appendix 3.5. (continued)

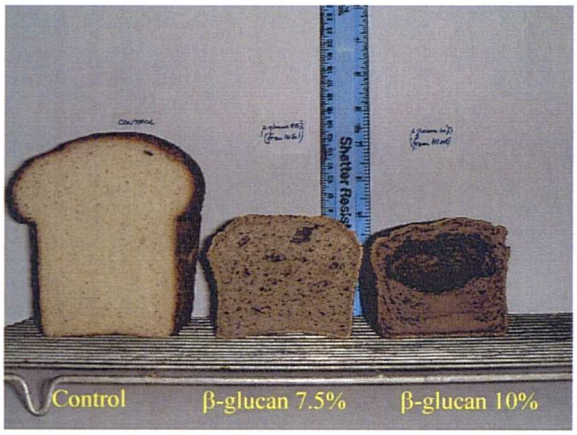


Appendix 3.5. (continued)

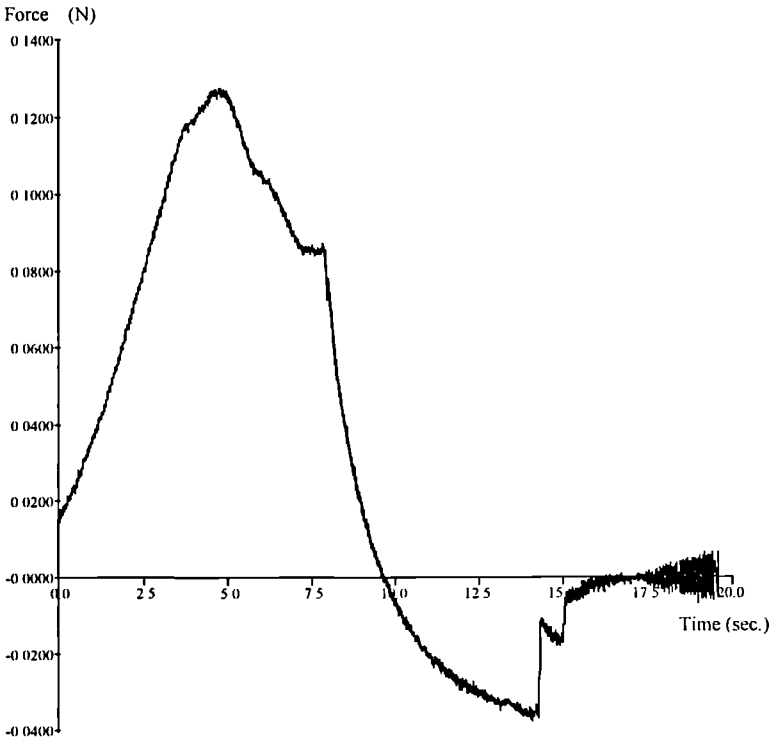




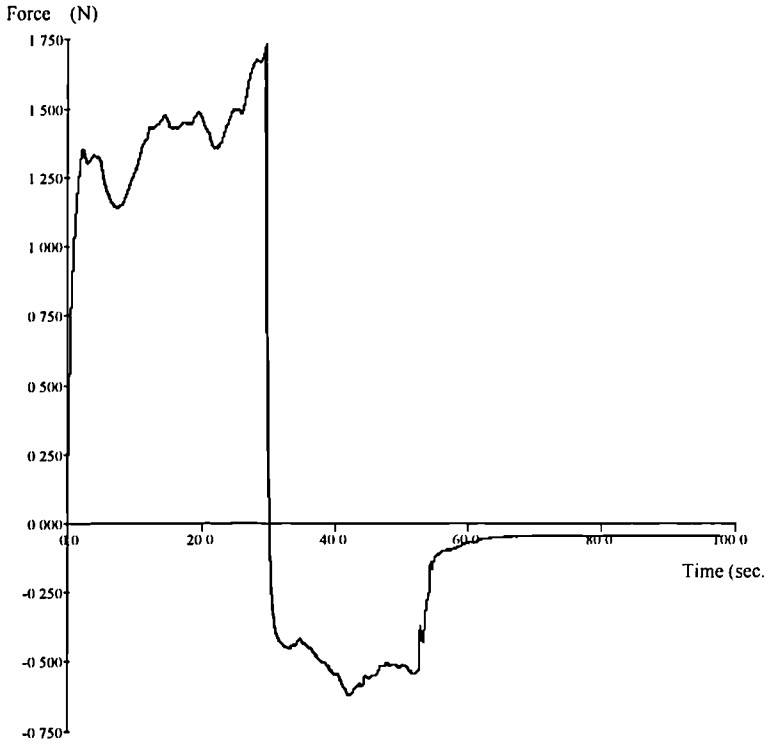




Appendix 4.1. Texture Analyser - typical test for assessment of curd firmness



Appendix 4.2. Texture Analyser - typical test for assessment of yoghurt firmness and consistency



Appendix 4.3. Sensory evaluation form for yoghurt samples

Sample code:

Panelist name: .....

Score sheet for sensory evaluation of yoghurt Date: / /

Please tick the box at the point which best describe the sensory characteristic of the sample.

Appearance – perceived by visual inspection of yoghurt surface

whey separation	□	□	□	□	□	□	□	
	none	1	2	3	4	5	6	7 extreme
colour	□	□	□	□	□	□	□	
	untypical							typical
aspect of the surface	□	□	□	□	□	□	□	
	very rough							very smooth
glossiness	□	□	□	□	□	□	□	
	very dull							very shiny

Texture/body –as perceived by scooping

firmness	□	□	□	□	□	□	□	
	very soft							very firm
whey separation (appearance)	□	□	□	□	□	□	□	
	none							extreme
sedimentation (appearance)	□	□	□	□	□	□	□	
	none							extreme
viscosity	□	□	□	□	□	□	□	
	very thin							very thick
ropiness	□	□	□	□	□	□	□	
	none							extreme

Texture/body – as perceived in the mouth

firmness	□	□	□	□	□	□	□	
	very soft							very firm
creaminess	□	□	□	□	□	□	□	
	watery							very creamy
mouthfeel	□	□	□	□	□	□	□	
	very coarse							very smooth
Untypical taste/flavour	□	□	□	□	□	□	□	
	none							very strong
Overall acceptability	□	□	□	□	□	□	□	
	dislike extremely							like extremely

Thank you for your time! ☺☺☺☺☺☺☺☺☺

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