SOURDOUGH BREAD ENRICHED WITH SOLUBLE FIBRES: DEVELOPMENT, CHARACTERISATION AND NUTRITIONAL ASPECTS OF A FUNCTIONAL FOOD PRODUCT

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Abstract

The application of sourdough in breadmaking has multiple technological and nutritional benefits. However, the use of soluble fibres in sourdough technology is a currently understudied area. Given that the UK fibre intakes (at average of 14g/d for adults) fall short of government recommendations, the aim of this PhD was to develop soluble fibre-enriched sourdough bread with a low glycaemic index (GI). The PhD comprised three key phases: 1) product development, 2) physico-chemical and sensory characterisation, and 3) GI analysis of the fibre-enriched sourdough breads.

After undertaking a product development trial, the physico-chemical properties of five sourdoughs and the resultant breads were assessed using pH measurements, Chen-Hoseney dough stickiness rig and rapid viscosity analysis (RVA). Bread volume, texture analysis, C-Cell image analysis, fibre and resistant starch determination were also conducted. Consumer acceptability of the developed breads was assessed using an untrained sensory panel ($n = 100$). The content of lactic acid and ethanol was studied using high-performance liquid chromatography (HPLC) analysis. The glycaemic and satietogenic properties of sourdough bread enriched with soluble fibre (XG/GA/Pec), control sourdough bread and white wheat bread (WWB) were tested in a cross-over study using 11 healthy participants (mean age 35 $\pm$ 10 years, BMI 23.7 $\pm$ 2.86 kg/m^2), a standard seven-point protocol and Satiety Labelled Intensity Magnitude (SLIM) scale.

The results of this study showed a negative correlation between the value of pH and the dough stickiness for control sourdough bread ($R^2 = 0.618$) and for control sourdough bread with added wheat bran ($R^2 = 0.532$). The RVA results showed that the addition of 10% of soluble significantly influenced the gelatinisation and properties of starch in flour pastes. The research showed that the addition of 10% soluble and insoluble fibre (wheat bran) to sourdough bread significantly ($p < 0.05$) increased content of total dietary fibre from 3% to ~12% (dry matter basis). The developed fibre-enriched sourdough breads did not differ significantly from non-enriched version in their scores in the consumer acceptability test ($p > 0.05$).

The values of GI obtained in the intervention study were 66 for control sourdough bread ($p = 0.03$) and 59 for XG/GA/Pec ($p = 0.006$) when compared to glucose reference food. The subjects reported greater satiety after consumption of bread with

XG/GA/Pec than after consuming white wheat bread ($p = 0.036$). These results show that sourdough bread enriched with soluble fibres played a role in reducing glycaemia and in increasing the perception of satiety following ingestion.

This PhD makes contribution to knowledge by applying high amounts of soluble fibres, which are routinely used in much lower concentrations in food industry. The research described within this thesis provides a formula and processing conditions for the production of a functional food product. This study adds to the existing knowledge of food science and human nutrition. Within this thesis it is demonstrated that sourdough and soluble fibre may act simultaneously on the gastrointestinal tract and jointly exert effects on postprandial blood glucose concentration and satiety. By demonstrating prolonged satiety of bread characterised by lower GI, this PhD makes a contribution to the debate on the satietogenic properties of dietary carbohydrates. Further research is now needed to explore the hormonal and metabolic effects after ingestion of soluble fibre-enriched sourdough bread. Future studies of the fermentability of these breads by colonic microflora could also provide insight into their prebiotic properties.

List of contents

List of tables

List of figures

List of abbreviations

AA – acetic acid

AUC – area under the curve

CFU – colony forming unit

CHO – carbohydrate

DF – dietary fibre

EPS – exopolysaccharide

GA – gum arabic

GI – glycaemic index

GR – glycaemic response

HePS – hetero-polysaccharide

HoPS – homo-polysaccharide

iAUC – incremental area under the curve

IDF – insoluble dietary fibre

LA – lactic acid

LAB – lactic acid bacteria

NSP – non-starch polysaccharide

Pec – pectin

RDI – recommended daily intake

RS – resistant starch

SDF – soluble dietary fibre

SLIM – satiety labelled intensity magnitude

TDF – total dietary fibre

TS – total starch

VAS – visual analogue scale

XG – xanthan gum

Chapter 1   Introduction

1.1. Background

Sourdough fermentation is one of the oldest biotechnological processes (Hammes et al., 2005). The use of cereal fermentation was common in Ancient Egypt where it was applied in production of leavened bread and beer (Hansen and Schieberle, 2005). The extent of the Roman Empire's reach contributed to the spread of sourdough technology to most European countries. During the migration of Europeans to America, sourdough starter was transported across the Atlantic Ocean. In sourdough cultivated in San Francisco, lactic acid bacterium named *Lactobacillus sanfranciscensis* was first described more than 40 years ago (Kline and Sugihara, 1971). Sourdough is presently applied in breadmaking in many regions of the world, with Mediterranean countries using it routinely in the production of wheat bread (Hansen and Schieberle, 2005). Rye flour, not as abundant in gluten as wheat flour, has diminished breadmaking properties in comparison to wheat flour. Therefore in rye-harvesting countries of Central and North Europe, sourdough acidification of rye flour-water mixtures allowing for higher solubility of pentosans is a technological necessity (Hansen and Schieberle, 2005).

Sourdough fermentation provides technological improvement resulting in bread richer in flavour (Rehman et al., 2006) and characterised by firmer texture. Through delayed staling and its natural resistance to microbial spoilage, sourdough fermentation allows food manufacturers to reduce the amount of improvers and preservatives used in bread production, adding to the natural appeal of this type of food commodity (Katina, 2005). Sourdough has been successfully applied in increasing the palatability of whole-grain, fibre-rich and gluten-free breads (Poutanen et al., 2009).

The functionality of sourdough can extend beyond its technological benefits. Research into the properties of sourdough demonstrated that *in vitro* decomposition of phytic acid (up to 100% phytate hydrolysis) taking place during fermentation (Lopez et al., 2001) shows the potential to increase the fraction of soluble minerals. Certain bacteria associated with sourdough fermentation can produce folic acid (Kariluoto et al., 2006), although this potential is still to be explored. Bacterial

fermentation may result in proteolysis of the gliadin fraction of gluten. This mechanism was suggested to be responsible for improving the tolerability of bread to coeliac patients (Di Cagno et al., 2002). Some lactic acid bacteria may also produce exopolysaccharides (EPS). EPS may act as a soluble fibre influencing the microflora of human gut. In an *in vitro* study, it was demonstrated that EPS has bifidogenic potential (Dal Bello, 2001), which might be worth exploring further in animal and human studies. Sourdough breads are characterised by improved glycaemic properties due to the content of organic acids, and their ability to slow gastric emptying (Liljeberg et al., 1995). This property of sourdough will be explored in depth in chapter 2 of this thesis.

According to a recent UK report on bread and bakery products, in 2011 96.7% of main shoppers bought bread. In addition, sliced packaged was the most often bought type of bread, while the market for speciality breads (including sourdough bread) observed a slight decline in comparison to previous years (Mintel, 2012).

Bread's potential to be a functional food is reflected by high levels of consumption. Despite the fact that the concept of functional foods or food ingredients is quite widespread, no uniform or universally accepted definition of functional food is in operation (Diplock et al., 1999, Schieber, 2012). Doyon and Labrecque (2008) reviewed more than 100 existing definitions to elucidate a few re-emerging key concepts:

1. functional food should offer a health benefit, *e.g.* to maintain health;
2. functional food may be produced by either fortification with a functional ingredient or removal of undesired or unhealthy ingredients (*e.g.* salt);
3. the functionality should be enhanced beyond the basic function of food as a vehicle for nutrients;
4. functional food should be a food commodity routinely consumed as a part of a daily diet.

It has been stressed that functional foods must remain foods (not a supplement, tablet or capsule), and must exert their effects in quantities that are normally consumed (Diplock et al., 1999, Roberfroid, 2000). Doyon and Labrecque (2008) mentioned the issue of proven health benefit is a part of only two of over a hundred definitions they reviewed. In keeping with the view of Doyon and Labrecque is the guidance

recently issued by European Food Safety Authority (EFSA) for food manufacturers wanting to make a health claim (EFSA, 2012). As a result of EFSA ruling, many manufacturers of "functional" food products had to remove their products from the market or repackage them (Mintel, 2011). This suggests that food manufacturers are keen to explore the market for functional foods but tighter regulation of this market is required in order to safeguard consumers from misleading health claims. Therefore, the need for a universally accepted definition of functional foods has been highlighted.

The present trends in the functional food science address the imbalance between energy expenditure and energy intake and the resulting obesity, insulin resistance, diabetes, hypercholesterolaemia and increased risk of cardio-vascular disease (Saris et al., 1998). Sourdough and soluble fibre have a range of health benefits (Trowell, 1972, Anderson et al., 1994, Salmerón et al., 1997a, Salmerón et al., 1997b, Di Cagno et al., 2002, Katina et al., 2005, Kariluoto et al., 2006, Poutanen et al., 2009, Slavin et al., 2009, Brennan et al., 2012, Calasso et al., 2012) which can, doubtlessly, address some of the current health issues.

Indigestible non-starch polysaccharides (NSP) – dietary fibre (DF) is an important food component. Its importance in human nutrition has been a subject of more than 50 years of research in many scientific disciplines. Adequate fibre intake is beneficial in prevention and management of constipation and of importance in the management of other large-intestinal diseases such as irritable bowel syndrome (IBS) (Slavin et al., 2009). Moreover, fibre consumption has been linked to body weight reduction and maintenance (Slavin, 2005). Dietary fibre may contribute to reduced risk of type 2 diabetes (Salmerón et al., 1997a, Salmerón et al., 1997b) and reduced blood cholesterol concentrations (Anderson et al., 1994). Recently it was shown that viscosity of the fibre may determine its hypocholesterolaemic effect (Vuksan et al., 2011). In addition, certain dietary fibres were shown to induce the apoptosis of colonic adenocarcinoma cells (Olano-Martin et al., 2003a) and to increase survival rate of animals suffering from melanoma (Takeuchi et al., 2009).

Fibre consumption in the UK falls short of 18g/day recommended by the government and remains at a level of 14g/day (Bates et al., 2011, Nelson et al., 2007). The fortification of food with missing nutrients is one way to address the

inadequacies in nutrient intake. Bread is a common staple food in many countries around the world. By increasing fibre content of bread it may be possible to address the inadequate fibre intake of, at least, a part of a population.

Certain types of fibre were shown to exert a reducing influence on postprandial glycaemia (Granfeldt et al., 1995, Brennan et al., 2012). The consumption of sourdough breads may reduce glycaemic response in healthy subjects (De Angelis et al., 2007, De Angelis et al., 2009). However, the application of soluble fibre in sourdough bread making is currently understudied. The possibility for simultaneous action of sourdough and soluble fibre on postprandial glycaemia presented in this chapter led to development of the current PhD project and formulation the following aims.

1.2. Aims

1. To develop and characterise a range of sourdough breads enriched with soluble fibres.
2. To elucidate the influence of sourdough and soluble fibre on dough, the structure of resultant breads and to analyse the consumer acceptability of sourdough breads enriched with soluble fibres.
3. To assess the nutritional aspects of sourdough bread and sourdough bread enriched with soluble fibre.
4. To develop a model describing the influence of soluble fibre upon the glycaemic and satietogenic properties of sourdough breads.

1.3. Structure of the thesis

This PhD thesis focuses on application of sourdough and dietary fibres in the production of a functional food product, i.e. a product with enhanced nutritional properties. Therefore, in chapter 2, the literature consisting of peer-reviewed journal articles is critically appraised with the focus placed on sourdough and soluble fibre as functional food ingredients. Chapter 2 also reviews the health benefits of low-glycaemic index (GI) foods as well as the methodological issues of GI measurement. Moreover, literature concerning satiety and satiety measurement is also reviewed in chapter 2.

Chapter 3 reviews the breadth of methods used, and the analysis of results obtained during the product development stage of this PhD study. Chapter 4 details the methodology and results of consumer acceptability of the breads obtained in the course of the first stage of this study. Chapter 5 presents the methodology applied in measurement of glycaemic index and satiety response, followed by analysis of data obtained in the third stage of this PhD study. A critical discussion of the findings of the study against a broad body of literature follows in chapter 6. The discussion of the results provides a conceptual basis to develop a model describing the mode of action of the sourdough breads enriched with soluble fibres, and it is also presented in chapter 6.

Chapter 2   Literature review – sourdough and dietary fibre

2.1. Introduction

Within this chapter a critique of existing research is presented. Sourdough and soluble fibres are functional ingredients of breads developed as one of the aims of this project. Therefore, specific focus is placed on sourdough and dietary fibre technology, and their nutritional properties. As a result of the review of the secondary research published in the scientific journals and presented in chapter 2 of this thesis, it is argued that the breads proposed as a subject of this PhD project fit within the current trends and definitions of functional foods. This chapter serves as an introduction to the idea of functional food and demonstrates up-to-date research achievements in the field of sourdough technology. The nutritional aspects of sourdough breads are presented in the following sections of this chapter in order to demonstrate its role as a functional ingredient, and an independent functional food. Because the lines of research involving sourdough and dietary fibre have been joined together within this thesis, the state of current knowledge of dietary fibre technology and its nutritional aspects is also presented. The soluble fibres chosen as subject of this project are introduced and their functional aspects are duly discussed. In addition to nutritional aspects of the sourdough breads enriched with soluble fibre forming the scope of this PhD study, glycaemic response and satiety will also be introduced and critically discussed in chapter 2.

2.2. Literature overview

The scope of existing literature concerning sourdough and sourdough breads is broad. In order to make the literature review succinct, two main themes were identified within published research, technological and nutritional.

Sourdough has been at the focus of researchers' attention for at least four decades. Description of *Lactobacillus sanfranciscensis* and its association with *Saccharomyces exiguus* (Sugihara et al., 1971, Kline and Sugihara, 1971) in San Francisco sourdough bread was a prelude to the research into the technological and nutritional properties of this breadmaking technology. Sugihara et al. (1971) and Kline and Sugihara (1971) revealed the symbiotic association between two types of microorganisms, lactic acid bacteria and yeasts and its influence on the technological

properties of San Francisco sourdough. Research into sourdough has developed in the 1990s and it is continuing to be a focus for food technologists and nutritionists.

Studies were performed into technology of sourdough products (Wehrle et al., 1997, Schober et al., 2003, Arendt et al., 2007, Gocmen et al., 2007). Sourdough fermentation was found to affect the dough properties negatively (Gocmen et al., 2007) and leads to bread with diminished properties (Armero and Collar, 1998). Arendt et al. (2007) reported that lactic acid fermentation improves properties of the dough. Studies into shelf-life of sourdough breads showed that fermentation can be helpful in prolonging the shelf life of bread through delaying staling (Martinez-Anaya, 1996, Katina et al., 2006a) and inhibition of microbial spoilage (Corsetti et al., 1998b).

Nutritional aspects of sourdough breads form another theme in published research.. Sourdough has been the focus of research into bioavailability of minerals (Leenhardt et al., 2005, Sanz-Penella, 2012) liberated from complexes with phytic acid by the phytase activity of microorganisms in low pH (Lopez et al., 2001, Chaoui et al., 2003). The potential of sourdough microbiotae to synthesise folate was a focus of several studies (Kariluoto et al., 2004, Kariluoto et al., 2006). Proteolytic potential of sourdough fermentation has been explored as a tool in production of celiac-friendly breads (Di Cagno et al., 2002, Di Cagno et al., 2004). These aspects of sourdough technology will be explored in more detail in later sections of this chapter.

The glycaemic properties of sourdough breads are also of interest to researchers. High-GI diets have been linked to the prevalence of type II diabetes (Salmerón et al., 1997a, Salmerón et al., 1997b, Brand-Miller, 2003) coronary heart mortality, certain types of cancer and elevated blood levels of triglycerides and LDL cholesterol (Brand-Miller, 2003). The need to develop low-GI bread has recently been stressed by researchers (De Angelis et al., 2009). Previously, sourdough was successfully used in development of low GI wheat bread (De Anglis et al., 2007, De Angelis et al., 2009) and was demonstrated to produce improved glycaemic response even in subjects with impaired glucose tolerance (Maioli et al., 2008). Numerous studies explored the metabolic aspects of consumption of sourdough bread (Liljeberg et al., 1995, Liljeberg and Björck, 1996, Liljeberg and Björck, 1998, Östman et al., 2002).

The addition of fibres to bread was the subject of research in the past (Frati Munari et al., 1998, Lu et al., 2000, Atkinson et al., 2008). It was demonstrated that fibre has a detrimental effect on bread structure (Pomeranz et al., 1977; Rossell et al., 2001). However, it is disputed on whether fibre addition has an influence on postprandial glycaemia, and whether the presence of 'whole grains' contributes towards lower glycaemic response (Björck et al., 2000). The effect of fibre addition upon postprandial glycaemia seems to be variable and the methodological details are sometimes unavailable (Atkinson et al., 2008). Only a limited number of studies have been performed so far to assess the usefulness of sourdough fermentation in conjunction with soluble dietary fibre in developing functional breads. For instance, De Angelis et al. (2007) and De Angelis (2009) applied rye and oat fibre in production of low-GI sourdough breads.

Previous research showed that viscous soluble fibre may reduce postprandial glycaemia (Brennan et al., 2012). This thesis offers an insight into technology and nutritional aspects of sourdough breads enriched with soluble fibres, gum arabic, xanthan gum and pectin. The soluble fibres selected for this study have been studied extensively, with regards to their health benefits (Abd-Allah et al., 2002; Olano-Martin et al., 2002; Takeuchi et al., 2009; Calame et al., 2011). Despite the fact that soluble fibres used in this PhD study were shown to produce prolonged satiety (Di Lorenzo et al., 1988, Calame et al., 2011), the findings of studies concerning the satietogenic properties of carbohydrate remain inconclusive (Anderson and Woodend, 2003).

So far, diverse foci of the major research themes in the field of study have been introduced. Technological studies seem not to agree on whether the application of lactic acid fermentation in breadmaking imparts positive or negative effects on properties of doughs and breads. Furthermore, the application of soluble dietary fibre in sourdough breads seems to be understudied. Little is known so far about the satietogenic properties of sourdough breads and previous studies do not offer a unanimous conclusion to the link between glycaemic properties and satiety. Research presented in this thesis addressed the technological and nutritional aspects of sourdough breads enriched with soluble fibres. The focus was placed on the

properties of sourdoughs enriched with XG, GA, Pec and bran, and glycaemic and satietogenic effects of consumption of resultant breads.

2.3. Functional foods

According to Mintel (2011), in 2010 the functional food market in the UK was valued at £785 million; it is forecasted that in 2016 this value will be in excess of £1 billion. Even the recent EFSA's clampdown on the misleading health claims seems to have had a relatively minor impact on the growth of this sector of food industry (Mintel, 2011). According to Mintel (2011), women are the main consumers of functional foods. After breakfast cereals, yoghurt pots, fruit juices, and, jointly, frozen fish and dairy spreads, bread is the fifth most purchased functional food commodity. According to the recent National Diet and Nutrition Survey (NDNS) and Low-Income Diet and Nutrition Survey (LIDNS), white bread was the most commonly consumed type of this commodity (Nelson et al., 2007, Bates et al., 2011). Functional food health claims are approached by consumers with scepticism (Mintel, 2011). Therefore, the health claims attached to foods should be supported by strong and transparent evidence of their potential health benefits.

Several socio-economic factors define the composition of modern diets (Falguera et al., 2012). The three leading trends in today's food market are: functional foods, organic foods and sustainably sourced foods. Consumer demand leads the market, and it seems that the concern for health and fitness is the leading driver of the current food market (Mintel, 2011, Falguera et al., 2012). Further in this chapter, it will be demonstrated that both sourdough and soluble dietary fibre can contribute towards the production of a functional food product.

2.4. Sourdough

Sourdough is an ancient biotechnological process, which has been used in production of leavened bakery products for thousands of years (Sadeghi, 2008). Sourdough is a fermented mixture of flour and water, characterised by sour taste. The properties of sourdough are a result of its acidity.

Sourdough is the oldest leavening method and various civilisations around the world have been used sourdough as a breadmaking technique. However, relatively new (19th century) baker's yeast-leavened bread constitutes the most popular bakery product. De Vuyst and Neysens (2005) state that in addition to the staple character of bread, sourdough bread also defines cultural and geographical identity of certain countries or regions. An example of such product is world-famous San Francisco sourdough bread. Tradition of making various breads and cakes based on the application of sour starters is usually very old, *e.g.* Milanese Christmas cake, Panettone (De Vuyst and Neysens, 2005).

Sourdough can be traced back to the times of ancient Egypt where also beer was produced with the use of cereal fermentation (Poutanen et al., 2009). Due to its properties, sourdough technology in our times is regarded as a natural alternative to bread additives (Katina, 2005, Sadeghi, 2008). The current market trends show that despite falling consumption of bread, in general the demand for artisan and healthier breads (including sourdough) is on the increase (Mintel, 2009).

It was not only the technological application in bakeries that turned the attention of researchers back towards this ancient technology. Sourdough is renowned for its nutritional benefits and it has been the subject of comprehensive research and reviews (De Vuyst and Neysens, 2005, Katina, 2005, Poutanen et al., 2009). These technological and nutritional benefits of sourdough breadmaking technology have been related to the sourdough microbial composition (Paramithiotis et al., 2007).

Because of its microbial composition, sourdough can be considered as a complex ecosystem (Gobbetti, 1998) and the mutualistic interactions between the sourdough lactic acid bacteria and yeasts, forming the basis of this ecosystem, have been studied by a number of researchers. Spontaneous sourdough develops in mixtures of cereal meals and water (Hammes et al., 2005). Lactic acid bacteria (LAB) are responsible for souring of the mixture, and yeast produce the leavening gas – carbon dioxide (Vrancken et al., 2010). The presence of the microorganisms in spontaneous sourdoughs is dependent upon their presence in flour. Cereal flour normally contains 2×10^4 - 6×10^6 CFU/g (Stolz, 1999, De Vuyst and Neysens, 2005). These microorganisms gain their metabolic activity in the presence of water.

2.4.1. Use of selected starter cultures

The industrial application of sourdough enabled the development of selected starter cultures. There are two groups of commercially available LAB starter cultures: 1) defined (where single or multiple strains are present in a known number) and 2) undefined (the number of starter strains is unknown) (Giraffa, 2004). The use of carefully selected strains of microorganisms can help to obtain a natural and healthy product with desired characteristics (Leroy and De Vuyst, 2004). Starter strains selection relies upon numerous factors: their competitiveness; antagonism against spoilage and infectious microorganisms; the rate of production of metabolites; influence on organoleptic properties; contribution to decomposition of anti-nutritional factors; probiotic action (Holzapfel, 1997, 2002). Starters composed of specific single or mixed strains of LAB with yeasts are now commercially available and enable the production of sourdough in a one-stage process (Robert et al., 2006).

2.4.2. Types of sourdough fermentation

The sourdough process can be divided into three types (I, II and III) depending on the technology used in the production of sourdough (De Vuyst and Neysens, 2005, Siragusa et al., 2009). Each type of sourdough fermentation is characterized by different fermenting microflora, which in turn is dependent upon several exogenous (temperature, redox potential) and endogenous (flour and dough microbial and chemical composition) factors (Arendt et al., 2007). Fermentation parameters such as dough yield, the addition of salt, the quality and quantity of the starter used, as well as the number of propagation steps, fermentation time and oxygen availability all determine the selection of dominating microorganisms in sourdough (De Vuyst and Neysens, 2005, Arendt et al., 2007, Corsetti and Settanni, 2007). The three types of sourdough fermentation are presented in Table 1 jointly with the microorganisms most typical for each type of sourdough.

2.4.2.1. *Type I sourdoughs*

Type I sourdoughs are obtained traditionally, through spontaneous fermentation of flour-water pastes, and are characterized by daily refreshments (continuous propagation) (Corsetti and Settanni, 2007, Gaggiano et al., 2007). In contrast with

types II and III sourdoughs, this type does not require the addition of baker's yeast, mainly because highly adapted species of yeast (such as *Candida humilis*) develop in the type I sourdough ecosystem (Hammes et al., 2005). The predominant species of LAB is *L. sanfranciscensis* (De Vuyst and Neysens, 2005). Type I sourdough has the widest application (Siragusa et al., 2009). It can be further divided into subtypes:

a) Type Ia sourdoughs are pure culture sourdoughs. The microbial composition is stable and the microflora is highly adapted to the sourdough ecosystem and resistant to contamination with other organisms. This type of sourdoughs is derived from natural fermentation. San Francisco sourdough is an example of type Ia sourdough.

b) Type Ib sourdoughs are mixed culture sourdoughs obtained from wheat or rye flours, or mixtures of the two in a multiple fermentation process. The microbial composition of such sourdough is determined by the fermentation conditions which select the dominating and accompanying strains of microorganisms.

c) Type Ic sourdoughs are fermented in higher temperatures in tropical regions and are associated with highly specialised yeast, *Issatchenkia orientalis* (De Vuyst and Neysens, 2005). Sudanese sourdough bread (kisra) prepared from sorghum flour and fermented in temperatures ranging 35-40 °C is an example of type Ic sourdough.

2.4.2.2. *Type II sourdoughs*

In type II sourdoughs, the fermentation time is long (2-5 days), the temperature of the process is higher (> 30 °C) and the water content is increased compared with type I sourdough (De Vuyst et al., 2009). This type of sourdough is used mainly as a dough acidifier as it contains quite high concentration of acids: pH < 3.5 after 24h fermentation (De Vuyst and Neysens, 2005). Type II sourdoughs can be stored for up to one week, thus can be made in large quantities (silo) (De Vuyst and Neysens, 2005). Their fluid or semi-fluid consistency enables pumping; they also can be dried (Decock and Cappelle, 2005, De Vuyst and Neysens, 2005). The acid-resistant, thermophilic species of LAB present in type II sourdoughs are similar to lactobacilli found commonly in humans' and animals' digestive systems (Tieking et al., 2003).

2.4.2.3.   *Type III sourdoughs*

Type III sourdoughs are dried preparations containing lactic acid bacteria resistant to drying (Decock and Cappelle, 2005, Corsetti and Settanni, 2007). *Lactobacillus brevis*, *Pediococcus pentosaceus* and *L. plantarum* strains have such properties (De Vuyst and Neysens, 2005). Type III sourdoughs are obtained by the use of defined starter cultures, sourdough fermentation and the subsequent evaporation of water (Gaggiano et al., 2007). Sourdoughs of this type are a good way of obtaining the 'authentic bread taste' in modern industrial bread making and are often used in industrial bakeries due to the consistent quality of the resultant bread (Decock and Cappelle, 2005).

2.4.3. Sourdough microorganisms

The sourdough ecosystem usually consists of one or more species of LAB and one or more species of yeast. The sour taste of sourdoughs originates from lactic acid and, occasionally, acetic acid which are the products of the metabolism of LAB. Sourdough yeasts produce carbon dioxide (leavening gas) and alcohols. The ratio LAB:yeast in sourdoughs is approximately 100:1 (Sadeghi, 2008). Heterofermentative LAB can also produce CO_2 as a result of their metabolism (Corsetti and Settanni, 2007).

The LAB and yeast present in sourdough may be of natural origin (contamination of raw ingredients) or be introduced from a starter culture. More than 50 species of bacteria and 25 species of yeasts have been identified to date in sourdoughs of various type and origin (Ottogalli, 1996, Arendt et al., 2007). The species of bacteria most often isolated from spontaneous sourdough fermentations are *Lactobacillus sanfranciscensis*, *L. brevis* and *L. plantarum*, and, among the yeasts *Saccharomyces cerevisiae* is often present, either occurring naturally or as an added species (Gobbetti, 1998, Paramithiotis et al., 2007).

Heterofermentative (able to use more than one source of energy) LAB are the dominant type of microorganism in the sourdough ecosystem. The majority of sourdough LAB belong to genus *Lactobacillus* but also species of *Leuconostoc,*

Weisella and *Pediococcus* genera do occur (De Vuyst and Neysens, 2005). Amongst the yeast, species belonging to *Saccharomyces* and *Candida* dominate.

Table 1. Classification of sourdoughs and the corresponding characteristic microflora

Type Ia	Type Ib	Type Ic	Type II	Type III
Obligate heterofermentative *L. sanfranciscensis*	Obligate heterofermentative *Lactobacillus* spp. (related to *L. brevis*) *L. brevis* *L. buchneri* *L. fermentum* *L. fructivorans* *L. pontis* *L. reuteri* *L. sanfranciscensis* *Weisella cibaria*	Obligate heterofermentative *Lactobacillus* spp. (related to *L. pontis*) *L. fermentum* *L. reuteri*	Obligate heterofermentative *L. brevis* *L. fermentum* *L. frumenti* *L. pontis* *L. panis* *L. reuteri* *L. sanfranciscensis* *W. confusa*	Obligate heterofermentative *L. brevis*
	Facultative heterofermentative *L. alimentarius* *L. cassei* *L. paralimenatrius* *L. plantarum*			Facultative heterofermentative *L. plantarum* *Pediococcus pentosaceus*
	Obligate homofermentative *L. acidophilus* *L. delbrueckii* *L. farciminis* *L. mindensis*	Obligate homofermentative *L. amylovorus*	Obligate homofermentative *L. acidophilus* *L. delbrueckii* *L. amylovorus* *L. farciminis* *L. johnsonii*	
Yeasts *Candida humilis* *Saccharomyces exiguous*	Yeasts *C. humilis*	Yeasts *Issatchenkia orientalis*	Yeasts No yeasts *S. cerevisiae* might be added	

Source: De Vuyst and Neysens (2005)

Of more than 20 species of yeast found in sourdoughs, the following are the most commonly present: *Saccharomyces cerevisiae*, *S. exiguus* (*Torulopsis holmii*, *Candida holmii*, *S. minor*), *C. humilis* (*C. milleri*) and *Issatchenkia orientalis* (*C. krusei*) (Pulvirenti et al., 2004). These species very often form associations with LAB (De Vuyst and Neysens, 2005). Various yeast strains are responsible for the production of volatiles in sourdough, thus influencing the flavour of bread (Damiani et al., 1996). Ethanol and carbon dioxide are the major metabolites of yeast (Paramithiotis et al., 2006).

2.4.4. Sourdough microorganisms' interactions

The flavour and texture of sourdough rye and wheat breads originates from the carbohydrate catabolism by LAB (Spicher and Nierle, 1988, Gobbetti et al., 1994b). Flour composition (the availability of fermentable carbohydrates) and the presence of other microorganisms (yeasts) have an impact on the LAB metabolism (Gobbetti et al., 1994b).

As with other fermented foods, the stability of interactions between sourdough LAB and sourdough yeast can be achieved if the microorganisms involved are not competitive for the main source of carbon (Paramithiotis et al., 2007). In case of sourdough, the main sources of energy are the mono- and disaccharides (maltose) present in the flour. The non-competitive mode of their metabolism by sourdough microorganisms has been identified as key to the mutual existence of both types of microbes (Paramithiotis et al., 2007). It was shown that the yeast metabolic activity towards the fermentable carbohydrates of flour has a strong influence on LAB fermentation during the sourdough process (Peppler and Reed, 1987, Gobbetti et al., 1994b). Sourdough yeasts and LAB only partially compete for sources of nitrogen. It was demonstrated that yeasts produce and excrete the essential amino-acids which facilitate the growth of LAB in co-cultures (Gobbetti et al., 1994a).

2.4.4.1.   L. sanfranciscensis *and* S. exiguus*: an example of perfect symbiosis*

One of the bacterial species most widely isolated from sourdough of various kinds and multiple origins, is *Lactobacillus sanfranciscensis* (Gobbetti and Corsetti, 1997). The species' name reflects the origin of the first isolate of this bacterium from the San Francisco sourdough by Kline and Sugihara (1971). The reason for the widespread occurrence of this microorganism is its great adaptability to the environmental conditions, which is due to the fact that *L. sanfranciscensis* is able to use various electron acceptors depending upon their availability (Gobbetti and Corsetti, 1997). Preferentially, *L. sanfranciscensis* uses maltose (rather than glucose) as a source of energy, by the means of hydrolysing it to glucose, which, in turn, is excreted outside the bacterial cell in order to prevent its excessive intracellular concentration (Paramithiotis et al., 2007).

The excretion of glucose has a significant impact on *L. sanfranciscensis* and the surrounding environment. Firstly, it can be used by the bacteria as a source of energy after the exhaustion of maltose. Secondly, it can be used by mutually existing maltose-negative yeasts, such as *S. exiguus*, thus ensuring the continuity of LAB-yeast association (Sugihara et al., 1971, Gobbetti and Corsetti, 1997, Paramithiotis et al., 2007). Moreover, the study of simple co-cultures of sourdough microorganisms showed that the non-competitive use of carbohydrates by the two types of organisms enhances LAB contribution to flavour development through the increased production of bacterial metabolites (Gobbetti et al., 1994b).

The strict mutual association between *L. sanfranciscensis* and *S. exiguus* has been shown to be present in sourdoughs from San Francisco (Kline and Sugihara, 1971, Sugihara et al., 1971) and in sourdoughs used for production of Panettone.

2.5. Technological considerations of the use of sourdough bread making technology

The behaviour of dough results from the properties of proteins contained in the flour. The hydration of these proteins (gluten) during the mixing of dough yields a continuous network which exhibits a unique, viscoelastic behaviour (Gocmen et al., 2007). Viscoelastic properties allow for dough to retain the gas produced during the fermentation but also to expand (leaven) (Gocmen et al., 2007).

The rheological properties of dough and its behaviour arise from the viscosity and elasticity of gluten. Acetic acid produced during the heterofermentative fermentation of flour carbohydrates accounts for shorter and harder gluten chains, whilst more elastic gluten structures are due to the presence of lactic acid (Lorenz, 1983, Corsetti and Settanni, 2007).

The pH of a sourdough started with LAB or mixed cultures of LAB and yeast was reported to be as low as $3.72 - 4.35$ (after 8 hour fermentation) which relates to quite an acidic environment (Damiani et al., 1996). However, Wehrle and Arendt (1998) reported the pH of controlled sourdough to be 4.6 after 10 hours fermentation and cessation of metabolic activity of sourdough microorganisms at pH values lower than 4.

Flour properties (such as water absorption) and dough mixing properties which impact dough handling and baking characteristics and influence the baked product (Catterall, 1998, Arendt et al., 2007), can be studied with the use of farinograph (Belitz et al., 2004). Rheological properties of doughs as affected by addition of salt, lactic acid and acetic acid were studied (Tanaka et al., 1967, Maher Galal et al., 1978, Wehrle et al., 1997). The increase of farinograph water absorption, decrease of mixing time and weakening of the dough were reported as a result of organic acid addition (Tanaka et al., 1967, Maher Galal et al., 1978, Wehrle et al., 1997, Arendt et al., 2007). Maher Galal et al. (1978) explained dough weakening by the net positive charge present in acidic environment and its influence on the solubility of proteins, which leads to unfolding of gluten molecules and their electrostatic repulsion preventing the creation of bonds (Maher Galal et al., 1978, Arendt et al., 2007). In other words, development of elastic gluten network may be difficult in acidic conditions, and could result in reduced extensibility and increased resistance to extension (Tanaka et al., 1967, Arendt et al., 2007) This, however, remains in disagreement with the findings of Gocmen et al. (2007) who reported the loss of resistance to extension of doughs fermented with the use of sourdough technology. The disagreement in the findings may, however, be a result of methodological differences of the studies (strain of LAB used, quality of flour, formula used to prepare sourdough).

Wehrle et al. (1997) reported that acidification of dough improves the elasticity of dough, which was confirmed by Schober et al. (2003) in a study of the effect of salt and acid addition upon gluten characteristics. In addition to these findings, Thiele et al. (2002) suggested an increase of proteolysis of unfolded chains of gluten, which also could have an effect on the dough softness and elasticity (Clarke et al., 2004, Arendt et al., 2007). Technological benefits associated with the use of sourdough in breadmaking have been noted. However, improved dough handling properties and bread volume are disputed. Dough acidification is a necessity in rye breadmaking. In rye flour, which is less rich in gluten than wheat flour, an acidic environment promotes the solubility of rye flour pentosans and dough water absorption (Martínez-Anaya and Devesa, 2000, De Vuyst and Neysens, 2005). The increase of solubility of pentosans decreases their negative influence on gluten network; additionally, low pH prevents undesirable influence of α-amylase on rye breadcrumb (Cauvain, 1998).

Thus, the acidification of rye-flour dough has an apparent advantage: strengthening and softening the dough. In wheat doughs, however, the proteolytic activity of sourdough microorganisms negatively affects the formation of a gluten network (Kawamura and Yonezawa, 1982), which results in a weakening of dough structure and poorer dough handling properties (Gocmen et al., 2007).

Gocmen et al. (2007) demonstrated that the effects of sourdough on the properties of both doughs and resultant breads depend on the technological parameters (temperature and time of fermentation) used in breadmaking. Lower fermentation temperature and long fermentation time resulted in poor handling properties and unsatisfactory appearance of the resultant breads. In addition, the level of use of sourdough in the breadmaking seems to be of importance. The application of 20% of sourdough prior to incubation was shown to produce doughs with better handling properties and bread of better acceptability in comparison to 40% addition of sourdough (Gocmen et al., 2007). Therefore, the technological parameters have to be adjusted to enable production of sourdough bread with optimised characteristics.

2.5.1. Physico-chemical properties of sourdough breads

Regarding the current research, the relationship between the physico-chemical properties of bread and its sensory properties is crucial. The relationship between the texture of bread and its sensory perception was proposed by Brady and Mayer (1985). The related textural and sensory properties are summarised in Table 2.

Table 2. The textural description of bread properties and related sensory description

Textural description	Sensory description
Hardness	Force required to compress between molar teeth
Cohesiveness	Force necessary to break apart; degree of compression by molar teeth before breaking
Elasticity	The degree of quickness of recovery after compressing force of molar teeth is removed
Chewiness	Amount of time required to chew until ready to swallow.

Source: Brady and Mayer (1985)

In opposition to the properties of dough, which depend mainly on the properties of gluten, the properties of a baked product depend mainly on starch as this is the main

compound present in bread (Arendt et al., 2007). The process of baking sees the transformation of the foamy structure of dough into the spongy texture of bread crumb which results from protein denaturation as well as starch swelling and gelatinization (Belitz et al., 2004).

The texture of bread is affected by the rheological properties of dough and baked bread properties such as volume and crumb structure. Additionally, water content plays a role. The acidification of dough through the utilization of sourdough technology was linked to improved softness of sourdough bread crumb (Katina et al., 2006a). Katina et al. (2006a) explained this increased crumb softness as resulting from higher loaf volume. Therefore, the improvement of technological parameters of bread offered by sourdough may contribute to improved sensory profile of sourdough breads.

2.5.1.1. *Staling*

Staling is the wide spectrum of changes occurring during bread storage. It involves crust softening, crumb hardening, an increase in crumbliness, a change in taste (loss of the freshly-baked bread aroma) and mouthfeel of bread. Staling was attributed to structural changes in starch (retrogradation of gel back into crystalline form) (D'Appolonia and Morad, 1981) that take place directly after baking (for amylose) and over period of days (5 days for amylopectin) (Arendt et al., 2007).

Other factors that need to be considered with regards to the rate of bread staling are the temperature of bread storage, moisture content (linked with water activity) of the bread and the migration of water within the bread crumb (Corsetti et al., 2000). Arendt et al. (2007) list the methods for quantifying the rate of bread staling. It is noted, however, that due to its sensitivity, the perception of bread staling by a consumer cannot be compared with results obtained by the use of any of the instrumental methods (Sidhu et al., 1996, Arendt et al., 2007). The use of sourdough technology and the acidification of the dough it offers, enhances starch hydrolysis by sourdough microorganisms and increases proteolysis to prolong the period of bread staling (Corsetti et al., 1998a, Corsetti et al., 2000).

Armero and Collar (1998) report that sourdough generally produces breads, the crumb of which is characterized by increased firmness in comparison to straight (baker's yeast-leavened) bread. However, Corsetti et al. (1998a) put forward a suggestion that the use of specific strains of lactic acid bacteria may delay the staling. The formation of EPS by LAB, and dextrins through starch hydrolysis, was suggested to be one of the mechanisms of prolonged staling of sourdough bread (Martinez-Anaya, 1996, Katina et al., 2006a). The delay in staling is dependent upon the acidification rate offered by the bacterial strain used, and the strains giving the lowest pH and highest total titratable acidity (TTA) were shown to produce higher volume, softer texture and the lowest staling rate. This finding was in keeping with an earlier study (Barber et al., 1992).

In another study (Katina et al., 2006b), the combination of sourdough fermentation of wheat bran with enzymes (α-amylase, xylanase, and lipase) addition was reported to be the most effective method for improving the crumb softness and delaying staling (measured as crumb firmness). The antistaling effect of the technology used was explained by the degradation of starch by α-amylase. The softer crumb of breads obtained from wheat bran subject to sourdough fermentation was in line with findings of Corsetti et al. (2000).

2.5.1.2. *Antimould activity of sourdough lactic acid bacteria*

Bread is a perishable food commodity and the most common spoilage occurring in bread is caused by moulds (Corsetti et al., 1998b). The LAB present in sourdough fermentation are responsible for prolonging the shelf-life of bread. LAB were also shown to postpone the growth of mould and reduce the production of aflatoxin in commercial silage (Hassan and Bullerman, 1995).

The antimould activity attributed to sourdough LAB was named an important factor during the selection of an appropriate starter culture in sourdough breadmaking (Corsetti et al., 1998b). The extended shelf-life of sourdough bakery products was attributed to the mixture of lactic and acetic acids (Röcken, 1996). However, in addition to lactic and acetic acid, it is the mixture of synergistically acting organic acids that exhibits antimould activity, with caproic acid shown to be the most potent antifungal produced by a strain *L. sanfranciscensis* CB1 (Corsetti et al., 1998b). In

addition, LAB have been shown to inhibit the growth of bacteria responsible for rope spoilage of bread (*Bacillus spp.*) (Messens and De Vuyst, 2002, Sadeghi, 2008). Several authors stressed the fact that natural food preservation offered by the use of sourdough technology adds to the natural, additive-free appearance of the sourdough breads (Messens and De Vuyst, 2002, Gobbetti et al., 2005).

2.5.2. Sensory properties of sourdough breads

According to Corsetti & Settanni (2007), the flavour and aroma of bread are the main determinants of bread quality taken into account by bread buyers. Amongst other useful properties of LAB, their influence upon the flavour of sourdough bread has been a subject of research and reviews. Hansen and Schieberle (2005) name several factors influencing the flavour of sourdough bread: flavour compounds originating in flour; flavour compounds generated by LAB and yeast and influenced by their interactions; aroma precursors generated as a result of enzymatic activity of flour and microorganisms; then, finally, production and losses of flavour and aroma compounds during the thermal processing of dough.

Two groups of flavouring compounds are produced during sourdough fermentation: non-volatile (acids) and volatile (Rehman et al., 2006). With regards to the non-volatile flavour compounds, homofermentative LAB metabolise hexoses mainly to lactic acid, while the heterofermentative LAB produce acetic acid, ethanol and CO_2 as well as lactic acid (Damiani et al., 1996, Hansen and Schieberle, 2005). The production of lactic acid in sourdough depends mainly on flour type, and the production of acetic acid is determined by the strain of LAB used (Hansen and Hansen, 1994).

The fermentation quotient (FQ), which is the ratio of lactic and acetic acid produced during the sourdough fermentation, may account for the quality of bread (Corsetti and Settanni, 2007). The acidification of dough by LAB results in bread with an improved crumb structure and the sensory properties usual for the sourdough bread (Spicher et al., 1981, Gobbetti et al., 1994a).

Within the volatile compounds responsible for the flavour of sourdough bread, aldehydes, furans, aliphatic hydrocarbons, aromatic hydrocarbons, alcohols,

sulphuric compounds, terpenes, pyrroles and esters were identified (Gobbetti et al., 1995b, Rehman et al., 2006, Bianchi et al., 2008). The production of volatile compounds in sourdough is related to the activity of LAB and yeast. The content of volatiles depends upon several factors like temperature and time of fermentation, dough yield and water content (Gobbetti et al., 1995b, Hansen and Schieberle, 2005). The type of flour used is not without a consequence to the bread flavour because different ash content causes variances in the taste of bread (Katina et al., 2006a). Also the type of micro-organisms used for fermentation has an effect on sourdough bread flavour (Katina et al., 2006a). Gobbetti et al. (1995b) showed that prolonged bacterial proteolysis and dough acidification by LAB synergistically enable optimum yeast metabolism. They also reported the positive influence of the addition of fructose to sourdough on the volatile profile of the resulting bread, and the positive effect of the addition of fructose and citrate to sourdough on the content of iso-alcohols and methylaldehydes.

2.6. Sourdough as functional food

Figure 1 summarises the effect that the application of sourdough has upon the nutritional properties of bread.
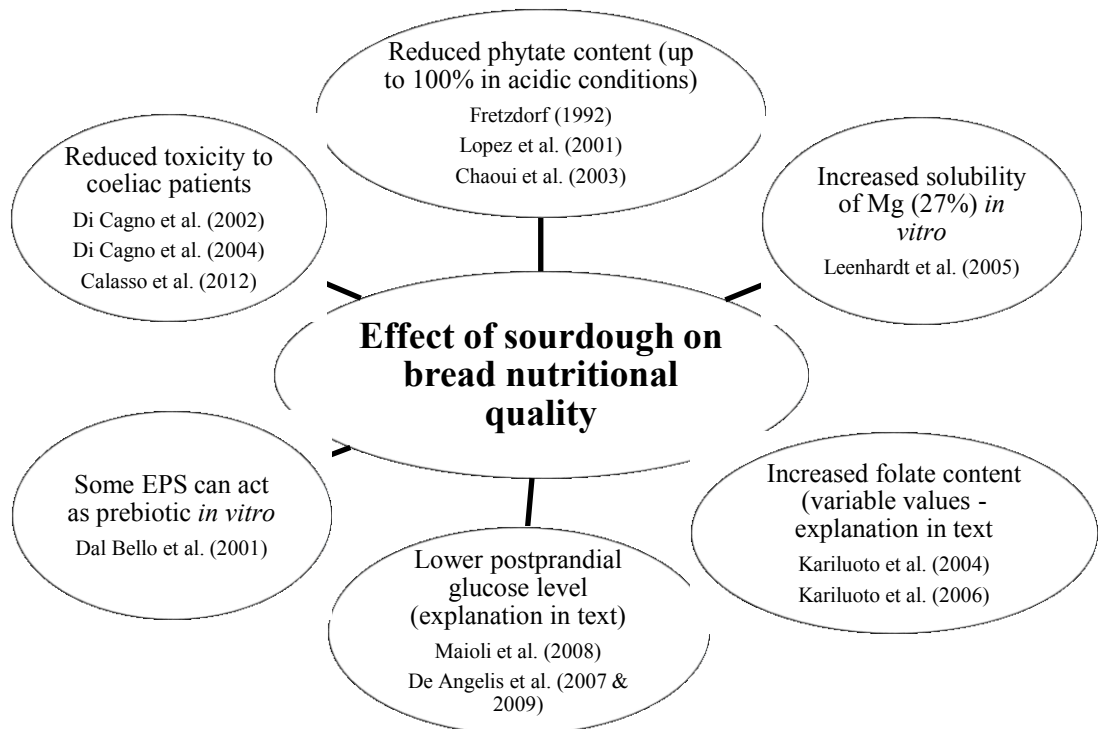
Figure 1. Effect of sourdough on nutritional quality of bread (adapted from Arendt et al., 2007)

It can be seen that there is more than one possible mechanism by which sourdough can improve the nutritional value of white wheat bread. The findings emerging from the review of the literature concerning the health benefits of sourdough are presented in the following subsections.

2.6.1. Mineral bioavailability of sourdough breads

Flour, in particular wholemeal, was reported to contain relevant amounts of minerals such as potassium, magnesium, zinc and iron (Leenhardt et al., 2005). Nonetheless, cereal flour contains phytate (phytic acid, PA, *myo*-inositol hexakisphosphate) which chelates multivalent cations, making them unavailable for absorption. Phytases are a group of cereal, bacterial and yeast enzymes catalysing the hydrolysis of phytic acid to smaller particles (IP_1 – IP_4), which have less ability to chelate the multivalent metal ions (Leenhardt et al., 2005). It was shown that the activity of phytase results in the decrease of PA status in sourdough and increases the solubility of magnesium (Leenhardt et al., 2005). The study by Leenhardt et al. (2005) focused on the increase of solubility of magnesium *in vitro*, and demonstrated that, in sourdough, after four hour fermentation around 27% of total Mg was solubilised (compared with 7% in dough which was not leavened in any way, 10% in yeast-leavened dough and 20% in dough acidified with lactic acid).

A more recent study by Sanz-Penella et al. (2012) investigated the *in vitro* bioaccesibility and bioavailability (using Caco-2 cell lines) of iron from wholemeal breads obtained through fermentation with phytase-active *Bifidobacterium infantis* and *B. pseudocatenulatum.* Sanz Penella et al. (2012) showed that only a small fraction of iron was made available to Caco-2 cells when dough was fermented by phytase-active bifidobacteria. Significantly, more iron was taken up by Caco-2 cells exposed to digests of sourdough with added fungal phytase. No significant change in the concentration of ferritin in Caco-2 cell lines was shown. However, there was an increase in iron availability for absorption (Sanz-Penella et al., 2012).

Both of the studies (Leenhardt et al., 2005, Sanz-Penella et al., 2012) demonstrated that sourdough fermentation and its associated increase in phytase activity are able to liberate minerals from complexes with phytic acid. The findings of the research point towards the possibility of increasing the mineral bioavailability of cereal products.

Nevertheless, the effect that sourdough has on mineral bioavailability remains to be investigated further. It appears that phytase activity increases during sourdough fermentation, and thus the *in vitro* solubility ('bioaccesibility') of minerals is enhanced. However, this effect seems to be small and might prove insufficient to improve the bioavailability of minerals from bread. Further on that note, research into bioavailability of minerals from sourdough-fermented breads has been limited to *in vitro* studies. Future research in this area could bring a deeper understanding of the conditions that need to be fulfilled to sufficiently improve bioavailability of minerals from sourdough breads.

2.6.2. Folate content of sourdough breads

Folic acid (pteroyloglutamic acid), referred to as folate in its naturally occurring forms, is one of the water-soluble vitamins belonging to the vitamin B group. It is known that *in utero* deficiency of folic acid leads to neural tube defects and, in adult humans, to megaloblastic anaemia (Kariluoto et al., 2006). Folic acid-containing co-enzymes participate in *de novo* synthesis of DNA within the cell, and thus in cell replication; folic acid can also play a role in the prevention of cardiovascular disease (FSA, 2010b).

Cereal products, especially rye, are naturally rich sources of folic acid in diet (Kariluoto et al., 2006). In the year 2000, it was recommended by the Department of Health that the foods should be fortified with folic acid to the quantity of 0.24mg/100g. However, despite the long term debates and consultations, the UK has no legislation regarding the fortification of flour with this vitamin (FSA, 2010b).

It was shown that sourdough fermentation of flour can increase the folate content in bread due to the presence of a specific microflora (Kariluoto et al., 2004). It was suggested that this increase in folate level might partially counteract the loss of 25% of folate that occurs during the baking of bread (Kariluoto et al., 2004). However, in the same study, it was argued that the competition for nutrients by sourdough microorganisms can lead to the inhibition of growth of folate-synthesising yeasts.

Indeed, in a subsequent study by Kariluoto et al. (2006) it was found that some of the microorganisms associated with sourdough fermentation actually contribute to the

depletion of total folate content. In a sterile mixture of rye flour and water, the content of folates was 5.2 ± 0.6 μg/100g, higher than that of sterile rye flour and water inoculated by strains of *L.sanfranciscensis*, *L.acidophilus*, *L. brevis* and *L. plantarum* (2.9 ± 1.0 – 4.2 ± 0.6 μg/100g). However, folic acid content was elevated in presence of LAB such as *L. bulgaricus* (8.1 ± 3.1 μg/100g) and *Streptococcus. thermophilus* (10.4 ± 2.3 μg/100g). Moreover, four strains of yeast often occurring in sourdoughs (*C. millerii*, *S. cerevisiae* ALKO 743 and TS 146, and *Torulaspora. delbrueckii*) produced significantly higher concentrations of folate than LAB (15.3 ± 4.2 – 22.8 ± 4.8 μg/100g). The higher end of this range was not significantly different from the folate content of unsterilised rye flour and water subject to spontaneous fermentation (26.6 ± 10.4 μg/100g). Two species of bacteria responsible for the increased folate content of this spontaneous ferment were identified as *Enterobacter cowanii* and *Pentoea agglomerans* (Kariluoto et al., 2006). Therefore, sourdough's contribution towards increased folate content of bread seems uncertain and offers further possibility of research.

2.6.3. Glycaemic index of sourdough breads

Glycaemic index (GI) is the difference in glycaemic response of a food product in relation to the response generated by pure glucose (100%). It is normally measured as the area under the curve of glucose concentration in blood following ingestion. There are three classes of products: low GI (<55%), medium GI (55%-70%), and high GI (>70%) foods (De Angelis et al., 2009). Low-GI and rich in fibre food has been linked with lower prevalence of heart disease, diabetes, obesity, colorectal cancer and breast cancer.

Sourdough bread was shown to produce better postprandial glucose response in comparison to bread leavened with baker's yeast in subjects with impaired glucose tolerance (Maioli et al., 2008). In another study, it was demonstrated that sourdough technology has an application in the production of low-glycaemic index white wheat bread (De Angelis et al., 2009). This glycaemia-lowering effect was attributed to lower digestibility of starch (De Angelis et al., 2009).

The values of GIs for various sourdough breads and breads enriched with soluble fibres are summarised in Table 3. There is no agreement as to the value of GI of

sourdough bread. Unpublished observation by Atkinson et al. (2008) assigned a GI value of 79 to bread containing 2% of vinegar and 2.5% sourdough. In the study by De Angelis et al. (2007), sourdough bread with oat fibre had GI of 53.7. De Angelis et al. (2009), through the application of rye and oat fibre and souring the dough with LAB, obtained bread with a GI of 41.1. Due to the use of 6% and 12% of arabinoxylan-containing fibre, Lu et al. (2000) obtained breads with a GI of 56 and 41 respectively. The addition of 2% of pectin resulted in bread with GI of 85 but 2% addition of guar gum reduced this value to 66 (Atkinson et al., 2008). No methodological details were available to the above results published in International Tables of GI and GL by Atkinson et al. (2008). Perhaps if the details of manufacture of breads with 2% guar gum and breads with 2% pectin were known, it would be possible to explore the difference in influence of these two fibres on postprandial glycaemia. A possible explanation of the influence of pectin and guar gum might be the difference of stickiness of both gums and their water binding capacity and their effect on starch gelatinisation during baking. Hence, although the other data have been approved in peer reviewed journals, the results of Atkinson et al. (2008) have to be interpreted with caution.

Table 3. Studies concerning glycaemic response of sourdough breads and breads containing soluble fibre

Bread	No of subjects	GI[‡]	Reference
white bread (served with 15g of Psyllium mucilage)	n = 10	65	Frati Munari et al. (1998)
white bread (6% arabinoxylan-rich fibre)	n = 14	56	Lu et al. (2000)
white bread (12% arabinoxylan-rich fibre)	n = 14	41	Lu et al. (2000)
white bread (2% pectin)	n = 10	85*	Atkinson et al. (2008)
white bread (2% guar gum)	n = 10	66*	Atkinson et al. (2008)
white bread (2% vinegar + 2.5% sourdough)	n = 10	79*	Atkinson et al. (2008)
white bread (served with 18g of vinegar)	n = 12	63	Östman et al. (2005)
white bread (served with 23g of vinegar)	n = 12	73	Östman et al. (2005)
white bread (served with 28g of vinegar)	n = 12	54	Östman et al. (2005)
white bread	n = 15	72	De Angelis et al. (2007)
sourdough bread with oat fibre and fermented to $\Delta pH = 1.5$	n = 15	53.7	De Angelis et al. (2007)
sourdough bread (70% wheat flour, 30% corn flour)	n = 16	not given	Maioli et al. (2008)
sourdough bread (5.59% rye fibre, 2.25% oat fibre)	n = 20	41	De Angelis et al. (2009)

[‡]with glucose as reference (GI = 100); *unpublished data, source: Atkinson et al. (2008).

2.6.3.1. *Mechanisms of sourdough breads' reduced GI*

As shown in the section 2.6.3, sourdough's potential to reduce the glycaemic index of foods has been established. Researchers also investigated the influence of the

sourdough upon the postprandial glycaemia to elucidate the mechanism behind this phenomenon. Liljeberg et al. (1995) observed that sourdough fermentation reduced postprandial glycaemia and Liljeberg and Björck (1996) suggested reduced gastric emptying as the potential mechanism for this action. The products of bacterial metabolism during sourdough fermentation are mainly lactic acid, with some amounts of acetic acid (depending on the strain of LAB). It was shown that organic acids may influence postprandial glycaemic response. Later, Liljeberg and Björck (1998) studied the glycaemic effects of white wheat bread with and without acetic acid. Acetic acid reduced the rate of gastric emptying and, therefore, slowed the release of glucose from starch, which resulted in a GI of 64 (Liljeberg and Björck, 1998). Additionally, the postprandial insulinaemia was attenuated. Also propionic acid was shown to reduce postprandial glycaemia in a dose-dependent manner (Liljeberg and Björck, 1996).

Lactic acid seems to have a different influence on postprandial glycaemia. LA did not influence gastric emptying (Liljeberg and Björck, 1996). The explanation for this fact was provided in an earlier research (Hunt and Knox, 1969), where it was determined that acids with lower molecular weights were more effective in slowing gastric emptying than acids with larger molecular weights. Moreover, it was demonstrated that the addition of lactic acid elicited a lower (by 17-28%) glycaemic response to wholegrain barley bread in comparison to bread with no organic acid added (Liljeberg et al., 1995). Therefore, another mechanism has to be responsible for lowering the postprandial glycaemia using lactic acid. The findings of the mechanistic research by Östman et al. (2002) showed that lactic acid reduced the glycaemic response to food only if it was present together with gluten in the food product (barley gruel), before heat treatment. The addition of lactic acid after thermal processing of barley gruels did not have an effect upon glycaemic response (Östman et al., 2002). Considering that gluten and starch interact in dough, which was proposed as explanation for reduced GI of gluten-containing breads (Jenkins et al., 1987a), Östman et al. (2002) proposed that lactic acid strengthens these interactions, therefore reducing the digestibility of the starch.

2.6.4. Production of exopolysaccharide by sourdough LAB

The improvement of the texture and keeping properties of baked products is often achieved by the use of polysaccharides (Arendt et al., 2007). Exopolysaccharides (EPS) are a miscellaneous group of microbial polymers excreted by bacteria into the environment (Tieking and Gänzle, 2005). LAB have the ability to synthesise exopolysaccharides either as homopolysaccharides (HoPS) or heteropolysaccharides (HePS) (De Vuyst et al., 2001, Van Hijum et al., 2006).

The EPS important to sourdough processes are HoPS produced from sucrose by extracellular glucosyltransferases (the product being glucans - reuteran, dextran, mutan) or fructosyltransferases (fructans - levan, inulin) (Gänzle et al., 2007). Some strains of *L. reuteri* LB121 were reported to produce glucan and levan (Van Geel-Schutten et al., 1998). Tieking and Gänzle (2005) reported that amongst the species of LAB found in type II sourdoughs (*L. reuteri*, *L. panis*, *L. acidophilus*, *L. frumenti*), there are a number of strains producing EPS. Some strains of *L. sanfranciscensis* are able to produce levan-type EPS which was shown to display bifidogenic (prebiotic) properties in an *in vitro* study (Dal Bello, 2001). The application of microbial polysaccharides in human nutrition should be further investigated to explore whether the potential shown in *in vitro* study (Dal Bello, 2001) could translate into benefits *in vivo*.

2.6.5. Other nutritional values

Sourdough breads have the potential to increase the tolerance of patients suffering from coeliac sprue to wheat. The mechanism of coeliac disease is not yet fully understood but rich in proline peptides (gliadins), which constitute a fraction of gluten proteins, are involved (Di Cagno et al., 2002, Di Cagno et al., 2004).

Some LAB have the potential to hydrolyse the prolamins and in this way might enable the production of bread that could be tolerated by coeliac patients (Di Cagno et al., 2002). Sourdough fermentation was successfully applied to the production of coeliac-"friendly" bread from mixture of wheat and and other flours of lower than wheat gluten content (oat, buckwheat and millet) (Di Cagno et al., 2004). More recently, it was also demonstrated that gluten-free sourdough can reduce the release

of nitric oxide (NO) and interferon-γ (INF-γ) from the duodenal cells and, therefore, promote the recovery of coeliac patients from the inflammatory phase of the illness (Calasso et al., 2012).

2.7. Dietary fibre

2.7.1. Dietary fibre: definitions, chemistry and physiological function

During the last two centuries the average consumption of dietary fibre (DF) decreased whilst the consumption of meat protein, refined cereal products and sugar increased (Kendall et al., 2010). The fibre content of wheat flour was reduced after the introduction of the modern roller mills at the end of the 19th century (Trowell, 1972). However, during the last few decades it was brought to the attention of researchers and medics that the appropriate dietary fibre intake reduces the risk of cardiovascular disease, diabetes and certain forms of cancer.

Several definitions of dietary fibre have emerged but no universally accepted definition exists. In the 1970s, dietary fibre was defined as the remains of plant cells resistant to hydrolysis by human enzymes (Trowell, 1972). The definition of dietary fibre established by the Dietary Fibre Definition Committee (AACC, 2001) states that

> "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."

Chemically, AACC definition distinguished four groups of compounds encompassed by the dietary fibre definition (Table 4).

Table 4. Constituents of dietary fibre

Non-starch polysaccharides and resistant oligosaccharides
- Cellulose
- Hemicellulose
  - Arabinoxylans
  - Arabinogalactans
- Polyfructoses
  - Inulin
  - Oligofructans
- Galactooligosaccharides
- Gums
- Mucilages
- Pectins

Analogous carbohydrates
- Indigestible dextrins
  - Resistant maltodextrins (from corn and other sources)
  - Resistant potato dextrins
- Synthesised carbohydrate compounds
  - Polydextrose
  - Methyl cellulose
  - Hydroxypropylmethyl cellulose
- Indigestible ("resistant") starches:
  - Physically inaccessible starch
  - Ungelatinised starch
  - Retrograded starch
  - Chemically modified starch

Lignin

Substances associated with the non-starch polysaccharide and lignin complex in plants
- Waxes
- Phytate
- Cutine
- Saponins
- Suberin
- Tannins

Source: AACC (2001)

In addition to the compounds listed in Table 4, Trowell et al (1976) and Elleuch et al. (2011) mention also resistant proteins, which pass through the intestine without digestion and/or absorption.

According to the definition created by the US Food and Drug Administration (FDA), the fibre in diet consists of three elements:

1) dietary fibre – sum of indigestible carbohydrates and lignin of plant origin that are found intact in food;

2) functional fibre – isolated non-digestible carbohydrates, the addition of which to food has beneficial effects; and

3) total fibre – the sum of dietary fibre and functional fibre (Trumbo et al., 2002, Slavin, 2005).

The definition agreed in 2008 by Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) states that dietary fibre includes carbohydrate polymers consisting of 10 or more monomers and not subject to hydrolysis in human small intestine. CCNFSDU distinguishes three categories of dietary fibre:

1) edible carbohydrate polymers occurring naturally in food;
2) carbohydrate polymers obtained from raw material proven to have a benefit to health; and
3) synthetic carbohydrate polymers proven to have a benefit to health (Cummings et al., 2009).

A limitation of CCNFSDU definition of dietary fibre is the omission of shorter than 10 monomers saccharides (*e.g.* lactulose). According to AACC (2001), physiological effects of dietary fibre are necessary for its definition. Insufficient intake of dietary fibre is a cause of constipation, increased coronary heart disease (CHD) risk and the instability of glucose and insulin levels in blood. The same report states that the primary characteristic of dietary fibre is the fermentation in the large intestine. Nevertheless, all of the definitions presented above agree that dietary fibre is non-digestible carbohydrate beneficial to human health.

Table 5 summarises the physiological functions of dietary carbohydrates. The digestibility of a carbohydrate in the small intestine determines the availability of monomers for absorption and, subsequently, constitutes a factor to influence the GI of carbohydrate-containing food product (Asp, 1996). Carbohydrates indigestible in the small intestine pass freely to the large intestine where they are subject (to various extent) to fermentation by colonic flora. Fermentability is largely dependent upon the solubility and viscosity of the carbohydrate (Asp, 1996).

Table 5. Physiological properties of food carbohydrates

Digestible	Non-digestible	
• Rate of digestion (GI)	• Viscosity	
• Proportion of absorbed monomers (fructose/glucose ratio)	• Structural features	
	• Water binding capacity	
	• Fermentability	
	Fermentable	Non-fermentable
	▪ Rate and site of fermentation	▪ Water binding capacity
	▪ Products of fermentation	

Source: Asp (1996)

Although Table 5 shows the physiological properties of digestible and non-digestible CHO, it does not consider the effect of dietary fibre ingestion on the serum concentration of cholesterol. In a comparative study by Anderson et al. (1994), it was shown that sources of viscous soluble fibres (such as psyllium husk, guar gum and pectin) are more potent in their ability to reduce serum and liver cholesterol levels than the sources of mainly insoluble fibres (brans). Several mechanisms of hypocholesterolaemic action of soluble fibres were proposed. Psyllium husk and oat fibre were shown to modify the excretion and metabolism of bile acids. Modification of intestinal transport of lipids was suggested. Also, SCFAs resulting from the large-intestinal fermentation of fibres could be implicated in cholesterol-lowering effects of dietary fibres (Anderson et al., 1994). The findings of Anderson et al. (1994) were recently supported by another study, which showed that the viscosity of the dietary fibres determined their ability to reduce the blood cholesterol (Vuksan et al., 2011).

The average recommended daily intake for DF in the USA is 25g/day for an adult (Slavin, 2005). This amount of fibre, equal to approximately 5 teaspoons of dry substance, is hardly achievable in the daily diet in highly-developed countries. According to the report by Bingham (1990) the average DF (as non-starch polysaccharides) consumption in the UK was 12.4g /day. Slavin (2005) cites 16.5-17.9 g/day for men and 12.1-13.8 g/day for women, the difference in consumption relating to the caloric intake. Recent UK nutrition surveys showed an average consumption of 14 g/day of NSP (Bates et al., 2011, Nelson et al., 2007). Despite the apparent rise in DF intake, this level of consumption still falls below the 18g/day recommended by British Nutrition Foundation (Buttriss and Stokes, 2008).

The main source of DF in bakery products are cereal brans, especially wheat bran (Katina et al., 2006b). However, resistant starch (RS) created during thermal processing of dough also might play an important role in increasing the fraction of resistant carbohydrates in breads. The properties of RS include hypocholesterolaemic effect, improvement of postprandial glycaemic response, control of diabetes, prevention of colonic cancer, and lower caloric load (8 kJ/g compared with 15 kJ/g for fully digestible starch) (Fuentes-Zaragoza et al., 2010).

2.8. Soluble fibre, fermentability and prebiotic effect

Kendall et al. (2010) stressed the importance of the inclusion of soluble, insoluble, particulate and viscous soluble fibre in definitions of dietary fibre. The health benefits of dietary fibre are mainly the effects of viscous fibre. The effects of viscous fibres recounted by Kendall et al. (2010) are: slower transit of food through the gastro-intestinal tract resulting in slower digestion and absorption (resulting in reduced glycaemia), enhanced fermentation in the colon, and binding of bile acids which may result in lowering of serum cholesterol. There were a number of acute studies in which no pronounced metabolic effect of particulate fibre was found, yet, in cohort studies, the cereal fibre was consistently linked to a reduced risk of coronary heart disease and diabetes (Salmerón et al., 1997a, Salmerón et al., 1997b, Liu et al., 2000). As shown in section 2.7.1, the viscosity of fibre was found to be a prerequisite of cholesterol-lowering action (Anderson et al., 1994, Vuksan et al., 2011).

According to definitions, what distinguishes dietary fibre from other dietary carbohydrates is its resistance to enzymatic digestion in human small intestine (Trowell, 1972, AACC, 2001, Trumbo et al., 2002, Cummings et al., 2009). Therefore, DF does not provide energy to human cells in the same way that proteins, available carbohydrates and lipids do. Instead, DF is used as a source of energy by bacteria inhabiting the large intestine yielding ca. 1-2 kcal/g (Slavin et al., 2009). It was shown that the fermentation characteristics of a dietary fibre depends on the chemical composition of this fibre, its degree of polymerisation and the arrangement of sugars in the fibre molecule (Jonathan et al., 2012).

In general, it is believed that insoluble fibre is not fermentable and soluble fibre is fermentable (Slavin et al., 2009). Tungland and Meyer (2002) summarised the fermentation properties of various dietary fibres; non-soluble fibres, such as cellulose, hemicellulose, lignin, plant waxes, chitin, chitosan and resistant starches were presented as fibres characterised by limited fermentability. Conversely, soluble fibres (including β-glucans, pectins, gums, inulin and oligosaccharides) are well fermented by the colonic microflora (Tungland and Meyer, 2002). With the

exception of inulin and gum arabic, soluble fibres produce viscous solutions in the human gut.

From the fermentability of soluble fibres stems their prebiotic effect. The idea of prebiotic as an indigestible food component which conveys health benefits to the host through positive influence on certain colonic bacteria was first introduced by Gibson and Roberfroid (1995). Since that time, the concept has been developed further and prebiotic dietary substances have been widely researched. Non-digestible carbohydrate (CHO) such as RS, resistant dextrins, NSP (including pectins and gums), oligosaccharides and sugar alcohols can be fermented by colonic microflora (Roberfroid et al., 2010). The main genera of bacteria inhabiting the colon are *Bacteroides, Bifidobacterium, Ruminococcus, Eubacterium, Lactobacillus* and *Clostridium* (Roberfroid et al., 2010).

The main products of bacterial fermentation of fibre in the colon are short-chain fatty acids (SFCAs): acetic, propionic and butyric acids; and gases: methane, hydrogen and carbon dioxide and hydrogen sulfide (Cummings et al., 1987, Roberfroid et al., 2010). SCFAs are produced from many groups of compounds. However, they are largely derived from indigestible starches and dietary fibres (Scheppach et al., 1995) such as cellulose, pectins, xylans, arabinogalactans, gums and mucilages (Macfarlane and Macfarlane, 2003). The molar ratio of acetate:propionate:butyrate produced in the large intestine is 60:25:10 mmol/l on average.

Propionate and acetate are quickly taken up by the cells of epithelium and then released by the basolateral cells to portal circulation, and thus are able to exert their physiological effects far from colon. Butyrate is a source of energy of colonocytes, modifies certain cellular activities, and was shown to inhibit the growth of colon cancer cell lines (Scheppach et al., 1995). Proteins reaching the large intestine are also subject to fermentation but, since their products of fermentation are toxic and linked to colonic cancer and inflammatory disease, it is believed to be beneficial to increase the fermentation of CHO in the gut (Roberfroid et al., 2010).

Roberfroid et al. (2010) reported the health implications of prebiotic substances. It was found that prebiotics in children may help to establish an increased number of bifidobacteria. Prebiotics may alleviate the symptoms of gastro-intestinal diseases

(traveller's diarrhoea, irritable bowel syndrome, inflammatory bowel disease) and exhibit an anticarcinogenic activity as well as increase the absorption of certain minerals.

2.9. Soluble fibres used in the primary research
2.9.1. Xanthan gum

Xanthan gum (XG) is the most important microbial polysaccharide (Katzbauer, 1998). It was discovered in the 1950s (García-Ochoa et al., 2000). Plant pathogen bacterium *Xanthomonas campestris* produces xanthan as an extracellular substance. XG was cleared by Food and Drug Administration (FDA) in 1969 for use as a thickener in food industry in the USA. In 1980 it was added to the list of approved thickeners and stabilisers by the European Commission, and in 1988 it was declared that the acceptable daily intake should be unspecified (Katzbauer, 1998). Xanthan gum figures on the list of additives approved by the EU as E 415 (FSA, 2010a).

XG is the most commercially accepted microbial polysaccharide (Rottava et al., 2009). It has been used widely as a thickener, stabilizer, emulsifier and, synergistically with other gums, as a gelling agent in food industry. XG has a number of applications in the bakery industry. It was shown to be of benefit in gluten-free and protein-rich products technologies (Sidhu and Bawa, 2002).

2.9.1.1. *Structure of xanthan gum*

XG is an anionic polysaccharide (Pelletier et al., 2001). In the primary structure of xanthan, the backbone and the side chains can be distinguished. The structure of the main chain of the molecule is identical to the structure of cellulose and consists of 1,4-β-D-glucose (Figure 2). The side chains consist of a trisaccharide: β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose (Katzbauer, 1998, García-Ochoa et al., 2000, Pelletier et al., 2001). The anionic character of the molecule comes from the pyruvate groups that are attached to the terminal mannose molecule. Additionally, the first mannose group can be acetylated (Pelletier et al., 2001).

XG shows complicated secondary structure in which stiff molecules of xanthan interact with one another to form double or triple helices (Milas and Rinaudo, 1979, García-Ochoa et al., 2000). Depending on the associations between the chains, the

molecular weight for XG ranges between $2x10^6$ and $20x10^6$ Da (García-Ochoa et al., 2000).
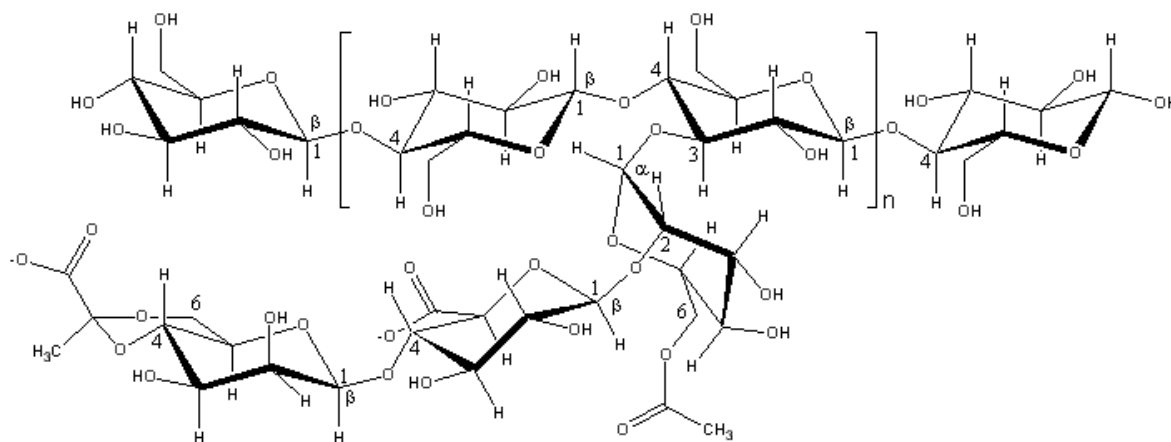
Figure 2. The structure of xanthan gum (from Chaplin, 2010)

2.9.1.2. *Physical properties of xanthan gum*

XG dissolves easily in cold and hot water but requires strong agitation when introduced into water to prevent the formation of lumps (Katzbauer, 1998). The temperature of dissolution affects the viscosity of the obtained solution by controlling the conformation of the large molecules of xanthan (Horton et al., 1985, Katzbauer, 1998).

In water, XG molecules undergo conformational changes from ordered to disordered conformation and vice-versa. In the ordered state the double stranded structure was reported, in which the trisaccharide side-chains are associated with the backbone. The ordered state can be induced by the reduction of temperature or the addition of small amount of salt (Pelletier et al., 2001).

2.9.1.3. *Health effects of XG*

The anti-carcinogenic properties of XG were a subject of study by Takeuchi et al. (2009). Oral administration of xanthan gum to mice inoculated with melanoma cells, retarded the growth of the tumour and prolonged the survival of mice. In the same study it was suggested that oral administration of xanthan gum might be beneficial for prevention of neoplasms. Nevertheless, there is a lack of similar studies performed on human cell lines.

2.9.2. Gum arabic

This section focuses on the body of research on the properties and the uses of gum arabic (GA) obtained from the exudates of trees from the *Acacia* species (Fam. *Leguminosae*) and renowned for its emulsifying and interfacial properties (Dror et al., 2006).

GA's origin is not uniform as various species of *Acacia* trees produce the exudates. Main species of importance in the production of GA are *A. senegal* and *A. seyal* (Islam et al., 1997). The definition of GA substance given by Joint Expert Committee for Food Additives (FAO/WHO) states that GA refers to the dried exudates of *A. senegal* and *A. seyal* (FAO, 1999, Verbeken et al., 2003). The gum produced by other *Acacia* species is sometimes referred to as gum acacia (Verbeken et al., 2003).

As GA is the oldest gum known to humans, the commercial importance of gum arabic has been great and its application has several thousand years of tradition (Verbeken et al., 2003, Islam et al., 1997). Ancient Egyptians used GA in paints for creating hieroglyphs and applied it as an adhesive medium for flaxen wrappings for the embalmment of corpses (Islam et al., 1997, Verbeken et al., 2003, Belitz et al., 2004). Presently, gum arabic is listed as an EU approved food additive as E 414 (FSA, 2010a).

Acacia trees occur in sub-Saharan regions of Africa. Sudan is the world's largest producer of GA (Belitz et al., 2004) and other producers include Nigeria, Chad, Mali and Senegal (Islam et al., 1997). Globally, the biggest importer of gum arabic is Europe. Within this continent, the United Kingdom and France are the key markets (Verbeken et al., 2003).

In commercial production of GA, natural exudates are harvested (occurring when the tree is damaged or infected) but the majority of gum is gathered by tapping the trees (Verbeken et al., 2003). Further processing involves dissolving the gum in water. While in solution, the substance is purified by filtration or centrifugation, and subsequent spray drying yields the powdered gum arabic (Verbeken et al., 2003).

2.9.2.1. *Structure of gum arabic*

GA is a glycoprotein and consists of calcium, magnesium and potassium salts of arabic acid, which is a polysaccharidic acid (Thomas and Murray Jr, 1928, Islam et al., 1997). Its acidity may be the reason for GA occurring either in neutral or slightly acidic form (Verbeken et al., 2003, Belitz et al., 2004). Monosaccharides occurring in gum arabic are L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid (Islam et al., 1997, Belitz et al., 2004, Ali et al., 2009).

The major structure of GA is a chain of 1→3 linked β-D-galactopyranosyl molecules (Verbeken et al., 2003, Belitz et al., 2004). The side chains attached to the backbone also contain 1→3 linked β-D-galactopyranosyl molecules attached to the main structure by 1→6 bonds. However, GA derived from trees of different species and from different geographical origins varies slightly in composition (Belitz et al., 2004).

2.9.2.2. *Applications of gum arabic*

As mentioned previously, GA is the oldest hydrocolloid of commercial meaning and technological applications. Once used abundantly in pharmaceutical industry, currently gum arabic has been all but replaced by cellulose derivatives and modified starches (Verbeken et al., 2003). However, there are still applications for GA in drug formulations and cosmetics. It is used also in lithography, in paints, pesticides and in textile industry (Verbeken et al., 2003).

Regarding the current research, the application of GA in the food industry is of interest. GA is used widely in the confectionery branch of the industry, as a stabilizer, texturizer, whipping agent and emulsifier (Verbeken et al., 2003). In the brewing industry it is also used as a foaming agent (Verbeken et al., 2003) and for the encapsulation of gaseous, liquid and solid substances. GA is used in low-calorie drinks as a soluble fibre source (Phillips, 1998, Verbeken et al., 2003).

The presence of approximately 2% protein in the structure of gum arabic guarantees its emulsifying properties (Belitz et al., 2004). The interfacial (oil-water) activity of GA arises from the adsorption of the hydrophobic polypeptide structure on the

surface of contact between the two phases (Verbeken et al., 2003). GA is very soluble in water, producing solutions of relatively low viscosity, which rises steeply in solutions over 50% (Belitz et al., 2004). Verbeken et al. (2003) stress the Newtonian behaviour of the solution of up to 40% of GA, and the pseudoplastic properties of solutions of concentrations higher than 40%. This low viscosity limits the use of gum arabic as a thickener in food industry (Islam et al., 1997). However, it does offer the possibility to use relatively high gum concentrations (Dziezak, 1991).

2.9.2.3.   *Health effects of gum arabic*

GA may exert hepato-, cardio- and nephro-protective effects through an antioxidant mode of action (Abd-Allah et al., 2002, Al-Majed et al., 2002, Al-Majed et al., 2003). However, Ali's (2004) study of concentration of reduced glutathione (GSH) and ascorbic acid, activity of superoxide dismutase (SOD) and the rate of lipid peroxidation following the administration of various concentrations of GA in drinking water, found no apparent evidence of this substance working this way. Noteworthy for the current study is the fact that GA seems to be linked to increased satiety (Calame et al., 2011) through an, as yet, unknown mechanism.

2.9.3. **Pectins**

Pectins are a group of polysaccharides associated with the plant cell wall, the function of which is to bind the cellulose elements of cell walls (Gulfi et al., 2006). Pectins' characteristic properties are the formation of a gel and high water binding capacity (Anderson and Chen, 1979). Pectins figure on the list of EU approved food additives under the number E 440 (FSA, 2010a).

2.9.3.1.   *Structure and properties of pectins*

Previously it was assumed that the basic element of the polymer structure of pectins is galacturonic acid (GalA) which has been esterified to a variable degree by methyl and acetyl groups (Anderson and Chen, 1979). However, more recent knowledge suggests different models of pectins' chemical structure (Willats et al., 2006). Currently accepted structure of pectin is presented on Figure 3.
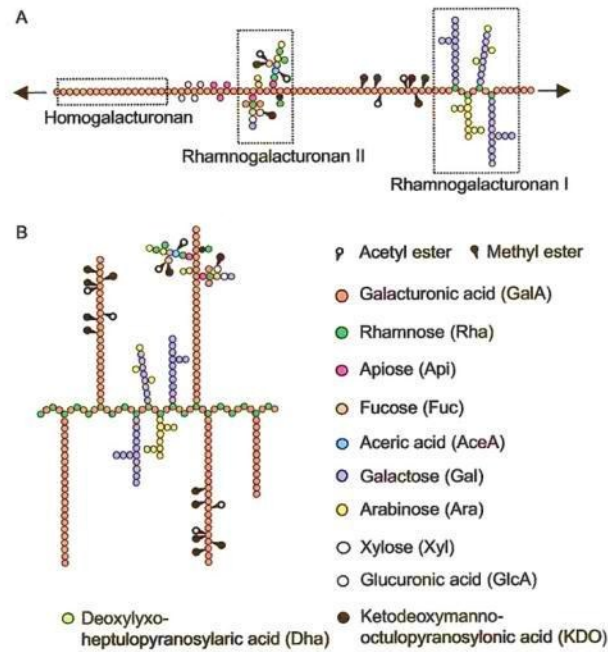
Figure 3. Structure of pectin (source: Willats et al., 2010)

Pectins are rich in galacturonic acid and, according to FAO and the EU, a polysaccharide has to consist in at least 65% of GalA which can be bound either as homogalacturonan (HG) or rhamnogalacturonan (RG). Additionally, HG might be esterified. The degree of esterification (DE) and the degree of acetylation (DA) are responsible for the set of properties possessed by different pectins. The classification of pectins based on the DE divides them into two groups: pectins of DE < 50% and pectins of DE > 50% (Voragen et al., 1995, Willats et al., 2006).

One of the most commonly known properties of pectins is their ability to form gels in aqueous solutions (Willats et al., 2006). Gel formation occurs between chains of HG which form a 3-D network entrapping water and dissolved substances. In addition to the DE and the DA, other factors, such as pH, type of pectin, presence of calcium and other solutes, also determine gel-forming properties of pectins (Willats et al., 2006).

2.9.3.2. *Health effects of pectins*

The fact that pectins are not digestible in the small intestine of humans has been known for a number of years. Anderson and Chen (1979) cite the high fermentability of pectins (95%) by microorganisms naturally inhabiting large intestine. The hypocholesterolaemic action of pectins is also their well-known property. The fact

that pectins are soluble in water determines their classification as a fraction of soluble dietary fibre. Pectin was demonstrated to promote satiety through modification of gastric emptying (Di Lorenzo et al., 1988). Pectins and pectic oligosaccharides possess prebiotic potential (Olano-Martin et al., 2002) and can offer the inhibition of Shiga-like toxin produced by *Escherichia coli* (Olano-Martin et al., 2003b). A study demonstrated anti-carcinogenic properties of pectins (Yamada, 1996). Pectins and pectic oligosaccharides were shown to induce the apoptosis of colonic adenocarcinoma cells (Olano-Martin et al., 2003a).

2.9.4. Technological considerations of application of soluble fibre in breadmaking

A recent trend in food product development is the production of high-fibre foodstuffs. This trend is dictated by the growing demand for healthier food products (Gómez et al., 2003). The function of this kind of product is to help maintain a healthy diet and to contribute to the prevention of obesity-related diseases troubling modern societies: hypertension, diabetes and colon cancer (Sudha et al., 2007). In section 2.7.1 it was stated that the recommended daily intake (RDI) of fibre in the UK is 18g/day for an adult. Still, the daily intake of fibre for an average adult is as low as 14g/day (Nelson et al., 2007, Bates et al., 2011). Hence, the current research explores the trend of producing foodstuffs that could contribute to the achievement of RDI of fibre by containing high amounts of fibre in relatively small portions. Additionally, the low-GI and rich in fibre foods consumption is recommended as a prevention against, amongst other diseases, insulin resistance leading to type 2 diabetes (De Angelis et al., 2009)

Functional food produced nowadays should offer the consumer the benefit of maintenance of good health (Falguera et al., 2012). In addition, it should be characterised by excellent sensory characteristics (Gómez et al., 2003). The main challenge that food scientists come across when adding fibre to bread is the detriment to dough handling properties, loaf volume and crumb texture (Pomeranz et al., 1977, Gómez et al., 2003). The influence on dough properties was suggested to come from the dilution and disruption of the gluten network and expansion of the gas cells in particular directions forced upon them by the presence of bran particles (Gan et al., 1992, Katina et al., 2006a). Nonetheless, these difficulties might be

overcome by the use of sourdough technology. Improved retention of leavening gas by gluten proteins in an acidic environment was previously reported by Gobbetti et al. (1995a), which in conjunction with the increased metabolic activity of yeast in the presence of heterofermentative LAB (Gobbetti et al., 1995a), offers a technological benefit.

2.9.4.1. *The influence of dietary fibre on rheological properties of the dough and the quality of fibre-enriched bakery products*

The stickiness of bread dough, as observed in a laboratory, characterises the internal rheological properties of dough. The balance between the viscous (flowing) and the elastic (stretching) behaviour of the dough, specific to the variety of flour used, is responsible for the force of adhesion occuring during contact between a dough and a surface. Whenever a dough is "strong", it is elastic and easy to remove from the surface. On the contrary, if a dough is "weak", it is slack and viscous (sticky) (Huang and Hoseney, 1999, Grausgruber et al., 2003). Increased dough viscosity poses a disadvantage in the bakery environment. The properties of viscous doughs are undesirable because the handling (during mixing or moulding) of such doughs may be difficult as they stick to utensils and mixing machines. Thus, from a technological point of view, the measurement of dough stickiness is essential as it might help to predict the handling properties of dough. Various factors have been identified as responsible for dough stickiness. The increase of dough stickiness is dependent upon flour extraction, the presence of soluble pentosans, protein composition, activity of alpha-amylase and proteolytic enzymes (Chen and Hoseney, 1995).

Research by Pomeranz et al. (1977) constitutes a fundamental study of the breadmaking properties of flour as affected by admixture of various fibres. The influence of the addition of different celluloses, wheat brans and oat hulls on the performance of dough during fermentation and on the quality of baked products was assessed versus control doughs. The findings showed variable influence of fibre addition on dough performance during the mixing. Increased flour water absorption was reported in the presence of dietary fibres. The behaviour of fibre-enriched doughs during fermentation was studied and poor gas retention, rather than the loss of gassing power, was revealed as a reason for the diminished loaf volume (Pomeranz et al., 1977). The degradation of texture, darker crumb colour and

increased crust firmness were also reported. The taste and mouthfeel of the bread were unaffected by the addition of celluloses but the addition of wheat bran had a non-objectionable effect on sensory properties. However, the addition of oat hulls gave the crumb an unacceptable texture. Some of the results obtained (mixing and breadmaking properties of doughs with added coarse brans and hulls, crumb firmness data) were not given and discussed without relation to control breads. Therefore, the findings of Pomeranz et al. (1977) may be difficult to discuss with respect of current literature. However, the nature of the study by Pomeranz et al. (1977) and its findings were of fundamental nature and constituted the base for further studies (*e.g.* Rosell et al., 2001) on the influence of fibre addition on breadmaking properties of flour.

The use of soluble fibres in breadmaking and their influence on the rheological properties of wheat doughs were studied by Rosell, Rojas, and Benedito de Barber (2001). Hydrocolloids such as sodium alginate, κ-carrageenan, xanthan gum and hydroxypropylmethylcellulose (HPMC, hypromellose) are widely recognized food additives. The authors performed a comprehensive study of the rheological parameters and the baking behaviour of wheat flour doughs with the addition of 0.5% of the above mentioned fibres. They reported an increase in water absorption by the flour in case of all of the added hydrocolloids, with the alginate and xanthan gum having the most effect. κ-carrageenan and HPMC had no influence on dough development time (DDT) and decreased the stability of dough, while sodium alginate and xanthan gum increased DDT and stability of dough (strengthening of the dough). All of the fibres lowered the elasticity of the dough. The gas retaining properties of dough were improved by the addition of all of the soluble fibres, while the deterioration of dough handling properties was observed. Rosell et al. (2001) suggested that their observations were due to the interaction between the gums and the flour proteins. This kind of interactions was also mentioned in the literature previously (Jones and Erlander, 1967, Huebner and Wal, 1979). The loss of volume was reduced by all of the soluble fibres; hence Rosell et al. (2001) concluded that the use of gums improved stability of the doughs during fermentation. However, all of the gums used, except for HPMC, decreased the time at which gas starts to permeate dough.

The baking properties of doughs were also changed by the addition of gums (Rosell et al., 2001). Except for HPMC, all of the substances reduced ovenspring (oven rise). This finding was discussed as being in opposition to an earlier study (Mettler and Seibel, 1995). The previous research used a lower level of different hydrocolloids (0.3% guar gum and carboxymethylcellulose) and in different type of dough, namely rye. This fact may account for the difference in findings of the two studies. Finally, the properties of the resultant breads were influenced by the addition of soluble fibres (Rosell et al., 2001). κ-carrageenan and HPMC promoted increase of the specific volume of bread. The use of gums resulted in an increase in water activity and moisture content of the crumb. This finding was attributed to the increased water absorption of doughs with the hydrocolloid added. Similar findings regarding the water absorption of flour with added fibre were reported in other studies (Pomeranz et al., 1977, Huebner and Wal, 1979, Wang et al., 2002, Sudha et al., 2007). Crumb firmness was reduced by the κ-carrageenan and HPMC, and increased by XG (Rosell et al., 2001).

Rojas et al. (1999) tested the effect of the addition of 0.5% and 1% of guar gum, pectin, κ-carrageenan, xanthan gum and hydroxypropylmethylcellulose on the pasting properties of flour using amylograph and differential scanning calorimetry. The addition of soluble fibres changes the parameters relating to the starch gelatinisation process to a varying degree (depending on the type of fibre used), with κ-carrageenan and xanthan gum having the largest effect on flour pastes' properties.

It was shown that working with bran can have a negative influence on dough rheology (Schmiele et al., 2012) and that the addition of gums can reduce dough stickiness and increase its extensibility (Bhattacharya, 2012). Accordingly, hypotheses were developed for the curent study, that inclusion of soluble fibre mixtures does not influence the stickiness of sourdoughs during fermentation; there is no correlation between the pH of sourdoughs and their stickiness; addition of different soluble fibres and bran does not alter the gelatinisation parameters of flour pastes; inclusion of soluble fibres and bran does not alter physico-chemical properties of sourdough breads; there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads (p. 62).

2.10. Origin of the concept and the definition of glycaemic index

GI can be defined as the hyperglycaemic potency of a given food (Bornet et al., 1997). In a 1998 report, the joint committee of WHO and FAO defined GI as the incremental area under the glucose response curve (iAUC) generated by a test food containing 50g of dietary carbohydrates and expressed as the percentage of the response generated by 50g of carbohydrate contained in a reference food (FAO, 1998, Wolever, 2004). It is essential that both measurements are compared in the same subject (FAO, 1998). Wolever (2004) defined GI as a classification of carbohydrates according to the acute blood glucose response they generate. The classification of foods according to their glycaemic response is: low GI (<55%), medium GI (55%-70%), and high GI (>70%) (Wolever, 2004, Venn & Green, 2007).

In the response to the fact that the food form, its content of dietary fibre and carbohydrates have an influence upon postprandial glycaemia, Jenkins et al. (1981) developed a study in order to rank foods according to their postprandial glucose response. The intended outcome was the ability to control glycaemia in people with diabetes based on the physiological effect that foods have on the human body rather than on the basis of these foods' chemical composition.

Wolever (2004) argued that in order for the concept of GI to be valid, GI values of the same foods should be the same across subjects. Inevitably, however, between- and within-subjects variations in GI values occur. The author recommended repeated trials of test and standard meals. In a big, inter-laboratory study, Wolever et al. (2003) showed that GI was not dependent upon age, gender, BMI or ethnic origin of the subjects, thus characterising a food product rather than the physiological state of the subjects.

2.11. Glycaemic index, glycaemic load and health

GI is a product of glycaemic response generated by carbohydrate in food and does not specifically consider the chemical composition of the carbohydrate involved. Currently, GI is internationally accepted as a measure of the physiological response generated by carbohydrate containing foods (Brand-Miller, 2003) and as such it is more useful than the chemical classification of the carbohydrate.

Glycaemic load (GL) is a product of GI of a test food and the content of carbohydrate in the portion of this food (Liu et al., 2000, Brand-Miller, 2003). GL links the quantity of carbohydrate with its glycaemic properties providing another way of classification dietary carbohydrate. The notion of GL appeared in in the work of Salmerón et al. (1997a, 1997b). The value of GL relates to the portion size of carbohydrate-containing food and can be calculated for a meal or a whole diet (Venn and Green, 2007) using the following formula:

$$GL = (GI \times dietary\ carbohydrate\ content)/100.$$

According to Björck et al. (2000), a low-GI diet is a tool in the fight against disease linked to metabolic syndrome. The majority of carbohydrate-rich foods in western diets are ranked as having a high GI. This includes potatoes, white bread and cereal products (Björck et al., 2000). It was found that glycaemic properties do not depend on the molecular size of the carbohydrate and that the dietary fibre content does not guarantee lower GI properties of food (Björck et al., 2000). Type 2 diabetes, hyperlipidaemia, coronary heart disease, obesity and cancer have been linked to the glycaemic carbohydrate through numerous studies spanning the last three decades (Jenkins et al., 1987b, Jenkins et al., 1987c, Salmerón et al., 1997a, Salmerón et al., 1997b, Jenkins et al., 2000, Liu et al., 2000, Ford and Liu, 2001, Liu et al., 2001, Brand-Miller, 2003, McMillan-Price and Brand-Miller, 2006, Barclay et al., 2008, Lin et al., 2012).

The health implications of high-GI diets are presented in Figure 4. The metabolic benefits of low-GI diet are lower postprandial glucose concentration in blood, reduced amounts of glycated haemoglobin (HbA1c) and improved insulin sensitivity (Björck et al., 2000). Some evidence exists to suggest an association between low-GL diet and reduced risk of type 2 diabetes in both men (Salmerón et al., 1997a) and women (Salmerón et al., 1997b).
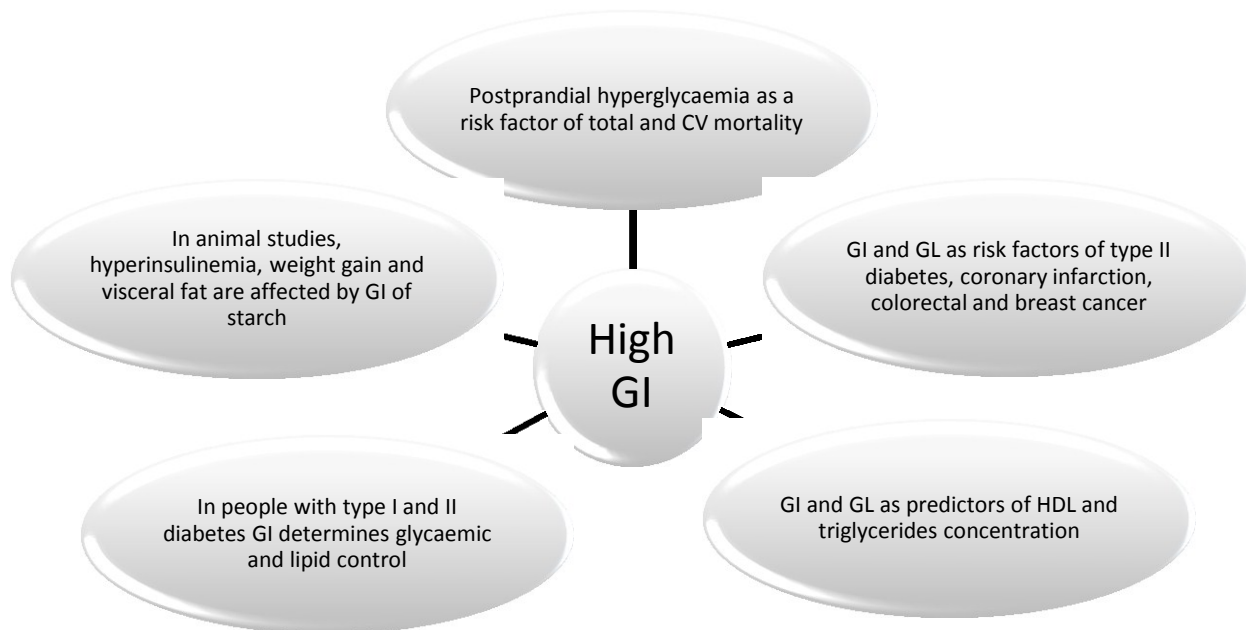
Figure 4. Evidence that high GI increases the risk of chronic disease (after Brand-Miller, 2003)

GI measurement is of importance in subjects with impaired glucose metabolism (type 2 diabetes) as it might help to control glycaemia. Also, in people with hyperlipidaemia, low-GI foods were shown to improve outcomes. In a study by Jenkins et al. (1987c), in the group of subjects with elevated triglyceride levels after consuming a low-GI diet for three months, a decrease in triglycerides, LDL and total cholesterol was noted. However, no change in the HDL fraction of lipoproteins was observed (Jenkins et al., 1987c). On the other hand, an increase in HDL as a result of the decrease in the overall GI of the diet was demonstrated in several epidemiological studies (Frost et al., 1999, Ford and Liu, 2001, Liu et al., 2001). It was later argued that the finding of no increase in the fraction of HDL by Jenkins et al. (1987c) was a result of a low statistical strength of the study (Pelkman, 2001). The recently published results of a large randomized trial (n = 756) seem to suggest that over the period of 18 months, the change in GI and GL of a diet had an effect on total cholesterol and low-density lipoprotein concentration (Lin et al., 2012). Conversely, the same study found no clear association between the GI and GL, and HDL.

Brand-Miller (2003) argued the importance of the control of postprandial hyperglycaemia. The risk of cardiovascular death was linked to postprandial glucose

concentration in blood in subjects with normal baseline glucose level (De Vegt et al., 1998). Various mechanisms were suggested as an explanation for high-GI diets' influence on human health (Figure 5). High glucose concentrations inhibit nitrous oxide synthase and therefore hinder vasodilation. Additionally, increased protein glycation, oxidative stress and impaired function of endothelium are the mechanisms of toxicity of postprandial hyperglycaemia to the blood vessels (Ceriello et al., 1999). Hyperinsulinaemia following hyperglycaemia and the resulting insulin resistance are risk factors of dyslipidaemia and other disorders leading to an increased risk of coronary heart disease (Stout, 1996). Diets with reduced fat content and increased carbohydrate content are recommended for management and prevention of obesity and overweight (McMillan-Price and Brand-Miller, 2006).
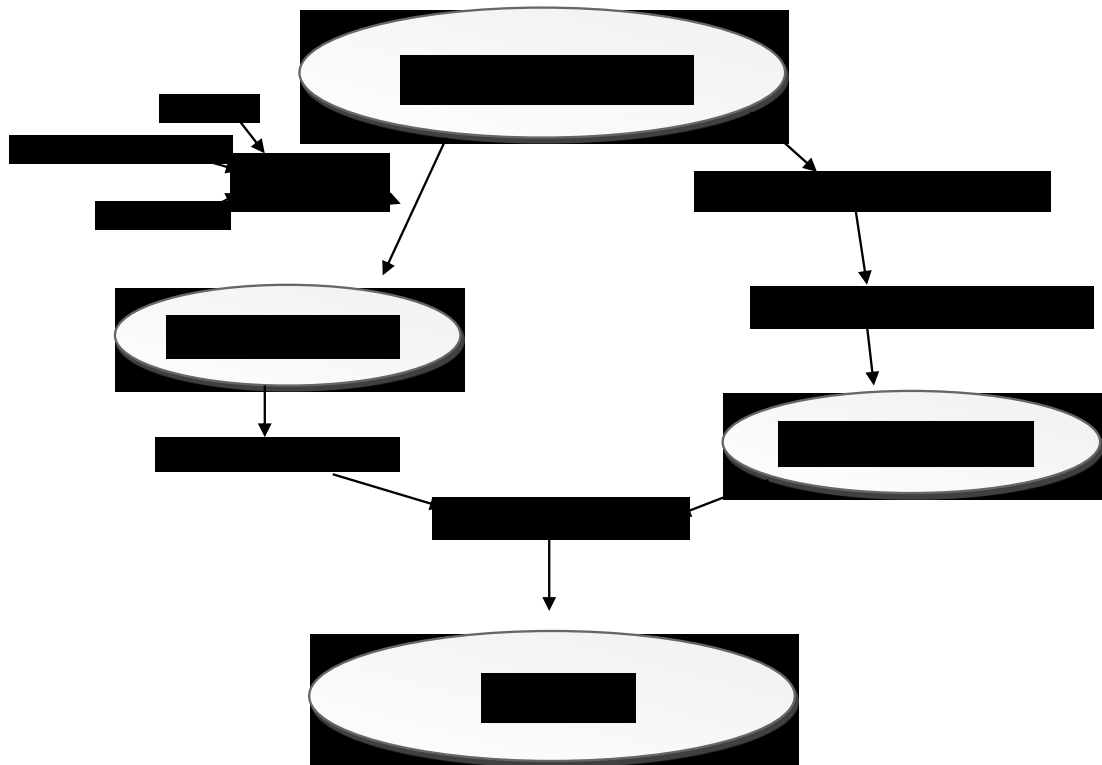
Figure 5. Relationship between long-term high-glycaemic load carbohydrate intake and type II diabetes (source: Willet et al. 2002)

2.12. The determinants of GI of bread

The concentration of fibre in white bread is normally too small (2-3%) to influence its GI (Fardet et al., 2006). However, the presence of wholemeal flour or its elements (bran) in bread is not a prerequisite for obtaining low GI (Björck et al., 2000). Fardet

et al (2006) stated that the processing rather than composition of bread played a role in glycaemic response regulation. This statement, however, was not supported directly by any evidence from empirical research.

Nevertheless, fibre may be added to bread which, in turn, can lead to reduced postprandial glycaemia. It was found that the presence of intact cereal kernels can produce lower postprandial glycaemia (Liljeberg and Björck, 1994). Also, viscous fibres (mainly β-glucan but also arabinoxylan) or cereal flours rich in soluble fibres have been applied in breadmaking in order to reduce the GI of breads (Fardet et al., 2006).

Due to its linear structure amylose, which comprises about 30% of total starch in wheat flour, is hydrolysed slower than amylopectin (Fardet et al., 2006). The content of amylose in the flour used for breadmaking matters in terms of resistant starch formation and GI (Åkerberg et al., 1998). Åkerberg et al. (1998) showed that by selecting high-amylose flours for breadmaking, the production of high-RS bread with a reduced hydrolytic index and GI becomes feasible.

Reduced bread volume was shown to elicit lower glycaemic response as well as higher satiety (Burton and Lightowler, 2006). Also, the storage of white bread in frozen form and toasting were both shown to influence the GI (Burton and Lightowler, 2008). Sourdough technology has been shown to be appropriate in the production of breads characterised by reduced glycaemic index. This aspect of sourdough technology was discussed in section 2.6.3.

2.13. Measurement of glycaemic index
2.13.1. Amount of test carbohydrates

Many methodological journal articles exist that discuss the amount of test carbohydrates that should be given to subjects participating in a glycaemic index trial (Wolever et al., 1991, Wolever et al., 2003, Wolever, 2004, Brouns et al., 2005, Wolever et al., 2008). The amount of carbohydrates in a test meal is between 25g and 50g of available CHO (Bornet et al., 1997). According to Wolever et al. (1991), the test food should contain 50g of available carbohydrates. However, more recently, Brouns et al. (2005) recommended that for foods with lower carbohydrate density,

the test carbohydrate content can be reduced to 25g of available CHO. However, the researchers need to be reasonable when choosing the amount of test food. Bornet et al. (1997) gave an example in which a test meal of carrots that would contain 25g of CHO is 500g.

Carbohydrates can be classified as available if they are digestible and can be absorbed in the intestine and enter metabolic pathways and cycles (FAO, 2003). This definition excludes dietary fibre and resistant starch, both of which contribute towards total carbohydrate count. Resistant starch has to be accounted for when assessing the available carbohydrate content of a product to be tested. Failure to account for resistant starch in the sample may lead to the underestimation of the size of the test food portion, therefore resulting in erroneous GI values (Brouns et al., 2005).

2.13.2. Sample size and screening

Based on an interlaboratory study (Wolever et al., 2003), Brouns et al. (2005) recommended that no fewer than 10 subjects should be used for GI determination and that the statistical strength of studies based on 10 subjects was sufficient. However, if smaller differences in the GI are sought by researchers, or greater precision is needed, the size of the sample may be increased. It needs to be remembered that using a larger sample size for GI determination increases the cost of the study and may cause problems with the recruitment of subjects (Brouns et al., 2005).

The physiological state of subjects should be assessed by questionnaire and initial screening of subjects' body mass index. In certain participants, especially those suffering from insulin-dependent diabetes (type 1 diabetes), GI values may show greater variation (Brouns et al., 2005). Thus, it was recommended that healthy volunteers should be used for routine testing of GI.

2.13.3. Subject-related factors which may influence GI

2.13.3.1. *Physical activity level*

Although GI does not depend upon subject-specific factors (Wolever et al., 2003), it was found by Mettler et al. (2006) that some differences existed in the GI values between sedentary and endurance-trained subjects. Further work by Mettler et al. (2007) found that the GI of breakfast cereal in sedentary young male subjects was higher than that measured in endurance-trained males ($p = 0.02$), and that the GI values obtained from moderately trained subjects were in between the values of the other groups of subjects. On the contrary, the same study repeated in sedentary and endurance-trained female subjects revealed no differences in either GI or insulinaemic response (Mettler et al., 2008), adding to the debate on subject–specific factors influencing the GI. Nonetheless, the recommended view on GI testing is that no strict control of the physical activity is necessary but subject should be urged to avoid unusual activity immediately prior to the GI study (Brouns et al., 2005).

2.13.3.2. *Alcohol consumption and smoking*

The effects of alcohol consumption immediately prior to, or during, glycaemic index determination have been shown to be profound. In the study by Brand-Miller et al. (2007), it was found that a significant reduction of glycaemia (up to 37%) was achieved by the ingestion of alcohol; at the same time, insulin concentrations remained unchanged. Therefore, it was concluded that alcohol enhanced glucose metabolism, probably due to its inhibitory effect on gluconeogenesis (Brand-Miller et al., 2007). Despite the acute effect of alcohol consumption on glucose homeostasis, in a more recent study it was shown that moderate consumption of alcohol in the evening preceding the GI study did not have an influence upon glucose determination or GI itself (Godley et al., 2009).

As well as producing an increase in blood pressure, hyperinsulinaemia, and hypertriglicerydaemia, smoking was demonstrated to result in hyperglycaemia (Frati et al., 1996). Aside from the obvious health risks, the inhalation of tobacco smoke can influence the glycaemic response during GI tests. Therefore it is recommended that subjects who smoke are asked to refrain from smoking prior to and during the measurement (Brouns et al., 2005).

2.13.3.3. *Caffeine consumption*

In epidemiological studies, coffee consumption was associated with reduced incidence of type 2 diabetes (Huxley et al., 2009). However, recently Hätönen et al. (2011) showed that coffee did not alter the glycaemic response of carbohydrate-containing food. The researchers concluded that it was not necessary to avoid coffee as the drink during GI study.

2.13.3.4. *Diet*

Robertson et al. (2002) shown that the composition of the meal eaten by the subjects on the evening preceding the glycaemic index trial altered their responses to carbohydrates and dietary fat the following morning. Evening meals rich in carbohydrate reduced the tolerance to fats; evening meals rich in fats reduced the tolerance to carbohydrate the following morning (Robertson et al., 2002). However, this effect was more pronounced for fat tolerance rather than for carbohydrate tolerance (Robertson et al., 2002, Brouns et al., 2005). Because the control of the diet adds burden to both subjects and researchers, it is not practical to control the subjects' diet; however, testing should take place after 10-14 hours of overnight fasting (Brouns et al., 2005).

2.13.3.5. *Implication for the current research*

As a result of the review of the literature presented in this section, volunteers participating in the current study were asked to avoid unusual physical activity immediately prior to the GI test. The participants' smoking status was ascertained using the screening questionnaire. Participants were asked to fast from 9pm in the evening preceding the measurement; to refrain from alcohol consumption the night before the test but were allowed to drink tea or coffee without sugar in the mornings the study was taking place.

2.14. Satiety

The literature search enabled a definition of satiety. Three phenomena are interconnected within this definition: hunger, appetite and satiety. All three phenomena have objective (physiological) and subjective (behavioural or learned)

aspects (Stubbs et al., 2000). Blundell and Naslund (1999) defined post-ingestive satiety as a state in which subjective and behavioural changes take place. The feeling of fullness intensifies whilst the feeling of hunger is diminished and results in the suppression of eating. Blundell (1999) discussed appetite control phenomenon as a very complex cascade of psycho-biological events. In simplistic terms, Blundell (1999) distinguished satiation (the processes bringing the food consumption to a halt) from satiety (the inhibition of hunger). Additionally, early- and late-stage satiety responses were described. Three levels of satiety/appetite expression were identified: the psychological/behavioural level, the peripheral metabolism level, and, finally, the neurotransmitter level. This model, known as the satiety cascade, is shown in Figure 6 (Blundell, 1999, Bornet et al., 2007).
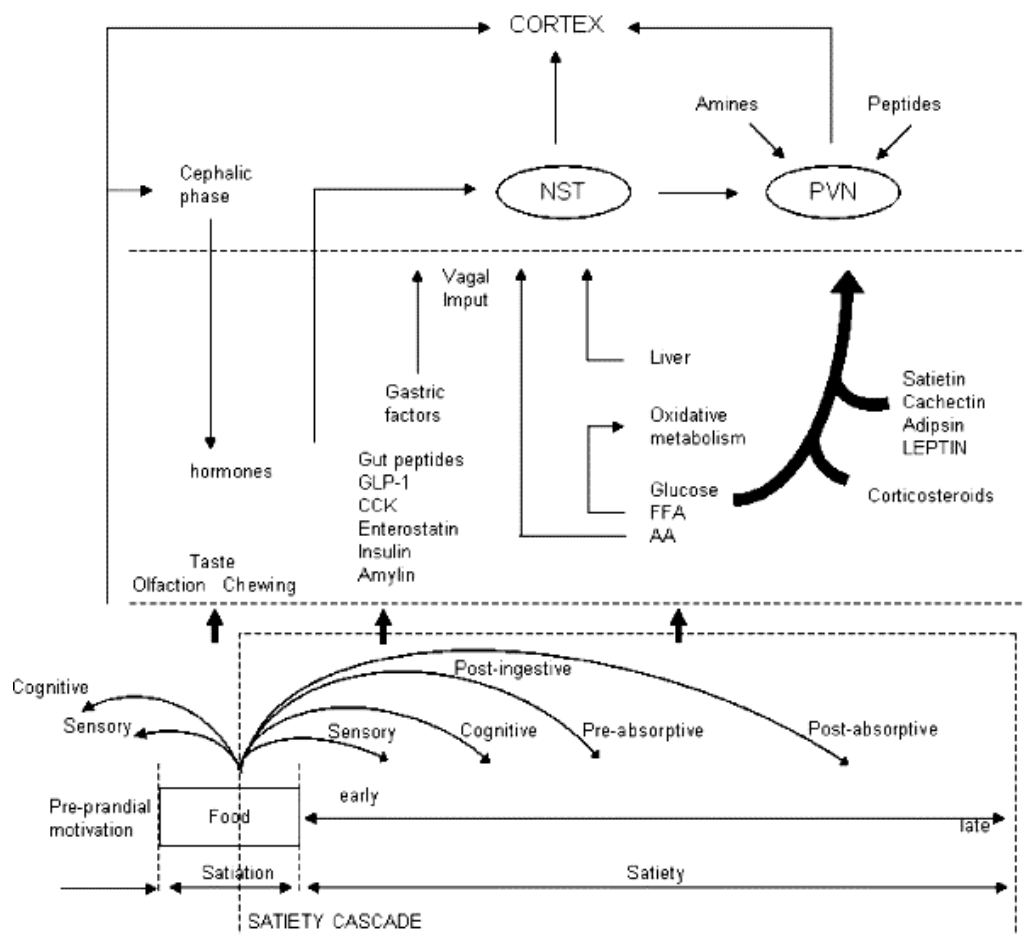
Figure 6. The satiety cascade (Blundell, 1999)

The satiety cascade shown in Figure 6 is a complex cascade of events taking place following the ingestion of a meal. Whilst food is ingested (satiation) and after ingestion (satiety), gastro-intestinal response to food takes place and comprises the

physiological stage of satiety response (Blundell and Naslund, 1999). In early-stage satiety response, information about the size of the meal is conveyed to the brain via *nervus vagus* from the chemo- and mechano-receptors in the gastro-intestinal tract. This process takes place before food is digested and absorbed into the bloodstream (pre-absorptive satiety). In the later stage, nutrients circulating in the blood either enter the brain or are peripherally metabolised (Blundell, 1999).

The gastro-entero-pancreatic system is the largest endocrine organ in human body (Chaudhri et al., 2006). Vast number of hormones have a role in satiety perception by acting upon the appetite centres located in hypothalamus and brain-stem (Chaudhri et al., 2006). The gut hormones shown to influence satiety are, amongst others: cholecystokinin (CCK), gastrin, motilin, pancreatic peptide (PP), glucagon-like peptides-1 and -2 (GLP-1 and -2), oxyntomodulin and glucose-dependent insulinotropic peptide (GIP) (Chaudhri et al., 2006). A protein (leptin) is expressed in adipose tissue after food and comprises a lipostatic message for the brain. As well as the control of appetite, leptin is also involved in an increased energy expenditure (Blundell, 1999). Recently, it was demonstrated that behavioural functions related to food consumption are of crucial meaning in satiety perception (de Graaf, 2012). It was demonstrated that slow eating increases concentrations of anorexigenic hormones PYY and GLP-1 (Kokkinos et al., 2010).

One of the hormones involved in satiety and feeding behaviour is ghrelin. In opposition to CKK, PP, GLP-1 and GIP, increased plasma concentration of ghrelin occurs before food and decreases postprandially, making this hormone an orexigenic signal (Date and Kangawa, 2012). A number of studies demonstrated variable influence of meals on plasma ghrelin concentration (Erdmann et al., 2004, Lee et al., 2006, Lioger et al., 2009, Rosén et al., 2011).

In the study by Erdmann et al. (2004), it was shown that postprandial concentration of ghrelin was reduced by carbohydrate-rich meal (bread) and that fat- and protein-rich meals increased ghrelin plasma concentration and resulted in increased feeding behaviour. In the same study, a reduction of ghrelin concentration was present after a fruit meal. However, a vegetable meal transiently increased the concentration of the hormone. Erdmann et al. (2004) observed no correlation between ghrelin plasma concentration and the ratings of hunger and satiety after each of the five meals. This

observation lead to the conclusion that ghrelin was an unlikely modulator of feeding behaviour. However, the same study showed that there was a correlation (r = 0.44, p < 0.001) between the concentration of ghrelin 4 hours after a meal and the amount of food consumed in a subsequent meal. Additionally, there seems to be an interrelationship between ghrelin and insulin plasma concentrations (Erdmann et al., 2004).

Following the study by Erdmann et al. (2004), further research into carbohydrate–rich foods and their effect on satiety and satiety mediators was performed. Lee et al. (2006) tested the postprandial glycaemic, metabolic and satiety response to the consumption of two breads: white wheat bread and bread made with addition of lupin kernel flour. Lee et al. (2006) found that the reduction of postprandial blood plasma concentration of ghrelin was more pronounced after ingestion of bread made with lupin kernel flour. Despite the fact that the results of both studies (Erdmann et al., 2004, Lee et al., 2006) were complementary, more research in this area might be needed to establish nutrients influence on ghrelin concentration.

Despite the interest of the researchers in carbohydrate-rich meals' influence on postprandial plasma ghrelin concentration, very little has been done so far to elucidate the effect of sourdough products upon this hormone's concentration. The study by Lioger et al. (2009) considered the effect of sourdough fermentation of crushed whole wheat prior to processing into breakfast cereal. Lioger et al. (2009) did not find statistically significant differences between the concentrations of ghrelin after the consumption of white bread, standard wheat flakes and modified wheat flakes (sourdough fermented), whilst the participants of the research felt more satiated after consumption of modified wheat flakes. GIs of the two types of wheat flakes were not statistically significantly different. However, the insulinaemic response generated by the modified wheat flakes was significantly lower than that of standard wheat flakes. It is methodologically unclear, however, whether or not the flakes were given to the participants with milk, the protein of which could influence metabolic response generated by wheat flakes.

Further study into carbohydrate-rich foods' (bread) influence on metabolic postprandial response was performed by Rosén et al. (2011). Participants in that study ingested a sample of boiled wheat kernel (WK), boiled rye kernel (RK), white

wheat bread (WWB), endosperm rye bread (ERB), lactic acid-enriched endosperm rye bread (lac-ERB), whole grain rye bread (WGRB) and lactic acid-enriched wholegrain rye bread (lac-WGRB). In addition to glycaemic and insulinaemic response, satiety and hunger scores, and the subsequent intake of an *ad libitum* meal, Rosén et al. (2011) assessed the influence of the seven products on postprandial levels of ghrelin. It was found that only plasma ghrelin concentration following ingestion of lac-WGRB was significantly lower than that following the ingestion of WK. There were no differences between ghrelin concentrations following the consumption of the remaining foods. This finding suggests that the presence of lactic acid was responsible for lowering the plasma concentration of ghrelin. The bread producing the lowest concentration of ghrelin postprandially was not obtained using sourdough fermentation but, instead, lactic acid was added to the formulation (1.8 g/100g flour). Therefore, it remains to be explored whether breads containing lactic acid as a result of microbial activity would produce similar effect. Additionally, the most satiating food product was RK, which did not produce a significantly lower ghrelin response. The role of foods of various compositions on regulation of ghrelin release and production of satiety may be explored in more depth in future studies.

2.14.1. Carbohydrate consumption and satiety

According to the glucostatic model, the hunger returns faster after the ingestion of high-GI CHO because of the extreme insulinaemic reaction triggered by the elevated concentration of glucose in the blood leads to hypoglycaemia. On the other hand, low-GI carbohydrates do not elicit high concentrations of glucose in blood plasma, therefore the insulinaemic response is not exacerbated and the feeling of satiety is present for longer (Bornet et al., 2007).

However, it needs to be emphasised that there is no unanimous view on both the glucostatic model and the influence of carbohydrate on satiety or general health. Sloth and Astrup (2006) argue against McMillan-Price and Brand-Miller (2006) who, in essence, concluded that low-fat, high-fibre diets were unpopular in public and that low-GI diets have more popular appeal and accidentally, are healthier.

As useful as the glucostatic model is, the findings of Anderson and Woodend (2003) seem to contest the glucostatic model. Based on work by Ludwig et al. (1999) and

Ludwig et al. (2001), Anderson and Woodend (2003) built their argument on the premise that high-GI CHO do not promote satiety but instead increase hunger, therefore leading to overeating and obesity. However, Anderson and Woodend (2003) remind that the influence of carbohydrate on satiety remains unclear because few studies measure appetite, food intake and plasma glucose concentration concurrently, and that the findings of the studies that can be found in the published literature often contradict one another. Finally, Anderson and Woodend (2003) caution that very often GI is used as an explanation for prolonged satiety. In the previous study (Anderson et al., 2002) with the use of an objective method (pre-load), it was shown that the higher the glycaemic index of carbohydrate, the greater the satiety. Anderson et al. (2002) offer a conclusion that the absorption properties of carbohydrates and not their satiety promoting characteristics are observed through the glycaemic studies. Additionally, both high- and low-glycaemic carbohydrate promote the suppression of appetite (Anderson and Woodend, 2003).

The findings of the studies concerning the influence of dietary fibre on satiety are largely inconclusive. In a short-term study by Hlebowicz et al. (2007) it was shown that breakfast cereals characterised by higher fibre (bran, whole wheat) content did not produce satiety different from cornflakes. On the contrary, in a similar study by Willis et al. (2009), where effects of various dietary fibres on short-term satiety were analysed, it was found that RS and corn bran produced greater satiety than polydextrose and low-fibre control. It is plausible, then, that the type of fibre used in the study influences the satiety. Previously it was shown that colonic fermentation induced by RS was associated with increased satiety and slower gastric emptying (Nilsson et al., 2008).

2.14.2. Satiety measurement methodologies

As argued before, the physiological satiety response takes place on three levels (behavioural, nutrient and neurohormonal). As such, it is possible to measure the effect that foods have on the appetite in two general ways, objective and subjective (Blundell et al., 2010). Within the objective methodologies, the pre-load test meal paradigm using double-blind randomised trials has been named as the most influential in the measurement of short-term effects of test meals (Blundell et al., 2010). However, except for high probability of type II error, the design of this type

of study is very complicated and leads to many problematic issues such as type and nutrient composition of the pre-load meal, and timing of the pre-load meal (Blundell et al., 2010).

The subjective perception of hunger/fullness relies on the use of self-reporting scales (Blundell et al., 2010). A number of visual analogue scales (VAS) are available for use in research concerning satiety. Recently, the study by Sadoul et al. (2012) found that using pictures of foods instead of VAS in satiety measurement gave more sensitive results. However, it can be expected that the reproducibility of this kind of study might be difficult because foods vary not only from country to country but also from region to region. A study by Monello and Mayer (1967) did not find any patterns of hunger and satiety that could construct a "typical" picture of hunger and satiety perception (based on 603 participants) (Monello and Mayer, 1967, in Stubbs et al., 2000). Therefore, personal feelings of hunger and satiety are subjective and unique to every considered subject. Stubbs et al. (2000) suggested that measurement of perceptions is so variable from person to person that it should be performed as a within-subject, repeated measure design. The use of scales based on subjective perception of every subject participating in a study can be justified by the following statement:

"As hunger is a subjectively expressed construct that is used to express a motivation to eat, the most appropriate measure of hunger is its subjective expression at a given time. The same is true for other aspects of motivation to eat" (Stubbs et al., 2000, p. 407).

2.14.3. Visual analogue scales (VAS) and satiety labelled intensity magnitude scale (SLIM)

One of the most frequently used ways of measuring the subjective perceptions is visual analogue scales (VAS). VAS have been applied to appetite and pain research (Stubbs et al., 2000). According to Stubbs et al. (2000), VAS is normally a straight line with anchor words representing the two opposing extremes at each of the ends. Additionally, VAS can be either unidirectional (to measure hunger or fullness/satiety) or bidirectional (to measure hunger and fullness/satiety). VAS are quick and easy to use, easy to interpret and allow for a certain level of

discrimination. It is difficult to objectively validate the results obtained this way (Stubbs et al., 2000). However, VAS's predictive power, sensitivity to experimental manipulation and testing/re-testing, can be used as validation measures (Stubbs et al., 2000).

Cardello et al. (2005) developed a modification of a bidirectional VAS. This new type of scale was based on the fact that normal VAS, even labelled or categorised (seven- or nine-points), rarely explored the quantitative data according to the magnitudes expressed using these scales (which allow ordinal data to be obtained). This, according to Cardello et al. (2005), resulted in many researchers treating the data obtained through the use of VAS as ratio-type data, a mathematically incorrect manner. In addition, the use of the categorised scales leads to central tendency, with the subjects avoiding the end points of the scale, resulting in diminished sensitivity of the measurement (Cardello et al., 2005). A group of subjects was used to assign the magnitude to 47 English language phrases that could be used to describe hunger and fullness. From 47 only 11 phrases with magnitude values distributed symmetrically on bidirectional axis were chosen to construct their satiety labelled intensity magnitude (SLIM) scale (Cardello et al, 2005). The scale devised by Cardello et al. (2005) is easy to use, reliable and sensitive to the degree comparable with VAS, and enables the gathering of ratio data. Additionally, it is notable that Cardello et al. (2005) excluded wording that could have any hedonistic connotation, *e.g.* "satisfied", "unsatisfied", "content", "discontent" and "stuffed" choosing, instead, objective labels (Table 6). Zalifah et al. (2008) tested the SLIM scale in a linguistically diverse group of subjects to the conclusion that as long as any ambiguous wording is avoided, the labelled magnitude scale is appropriate for use in a population where the first language is not uniform. However, the principle of using of any scale is that the phrasing used must be simple, understandable and meaningful to subjects (Stone and Sidel, 1993).

Table 6. The magnitudes of phrases selected for SLIM scale

Greatest imaginable fullness	100
Extremely full	79.4
Very full	74.3
Moderately full	46.7
Slightly full	31.9
Neither hungry nor full	0
Slightly hungry	-18.6
Moderately hungry	-38.2
Very hungry	-56.2
Extremely hungry	-67.4
Greatest imaginable hunger	-100

Source: Cardello et al. (2005)

2.15. Summary

In this chapter it was demonstrated that the use of sourdough in breadmaking can result in a number of technological benefits. Sourdough can offer an improvement to the texture and flavour of the bread. From the perspective of this thesis, the nutritional benefits brought about by the application of sourdough can make it a functional food. Sourdough technology offers a way to fortify bread with some of the key nutrients (folic acid, minerals). LAB fermentation of the dough may also reduce the digestibility of the starch contained in the resultant bread and, in effect, reduce the glycaemic index of the bread.

Dietary fibre is a functional food ingredient. It was demonstrated that inadequate consumption of dietary fibre may result in illnesses. The main mechanism of DF's benefits to human health is the production of SCFAs in the large intestine. Additionally, viscous soluble fibre might contribute towards reduced postprandial glycaemia generated by bread and induce greater satiety.

Glycaemic index is an important research tool. Within this chapter it was discussed that the measurement of the GI was developed in order to enable glycaemic control in people with impaired glucose tolerance. However, long-term ramifications of the consumption of high-GI diets include insulin resistance (metabolic syndrome), type 2 diabetes, increased risk of coronary heart disease, increased body weight, and certain types of cancer. Therefore, lowering the glycaemic response of the most common foods (staple food commodities) may contribute to improved health outcomes in the general population.

Based on the literature review presented in this chapter, a number of hypotheses were developed (Table 7). In addition to hypotheses presented on p.44, the following hypotheses were developed: there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads; there is no difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread; there is no difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread.

The principles behind the methodology of measuring the GI and satiety were critically evaluated in this chapter. These principles enabled the reliable design of the glycaemic index and satiety studies which is described in detail in chapter 5. Chapters 3 and 4 document product development and the analyses of nutrient content, physico-chemical properties of sourdoughs and sourdough breads enriched with soluble fibres, and their acceptability to the taste panel. The experiments presented in chapters 3, 4 and 5 allowed for the testing of the hypotheses presented below.

Table 7. Hypotheses developed as a result of literature review and statistical tests allowing their testing

	Null hypothesis	Alternative hypothesis	Source	Statistical test
1)	H_0: inclusion of soluble fibre mixtures does not influence the stickiness of sourdoughs during fermentation	H_1: inclusion of soluble fibre mixtures influences the stickiness of sourdoughs during fermentation	Bhattacharya (2012), Schmiele et al. (2012)	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one-way ANOVA *post-hoc*
2)	H_0: there is no correlation between the pH of sourdoughs and their stickiness	H_1: there is correlation between pH of sourdoughs and their stickiness		Pearson's correlation
3)	H_0: addition of different soluble fibres and bran does not alter the gelatinisation parameters of flour pastes	H_1: addition of different soluble fibres and bran alters the gelatinisation parameters of flour pastes	Rojas et al. (1999)	ANOVA
4)	H_0: inclusion of soluble fibres and bran does not alter physico-chemical properties of sourdough breads	H_1: inclusion of soluble fibre and bran alters the physico-chemical characteristics of soluble fibres	Rosell et al. (2001), Guarda et al. (2004), Kopeć et al. (2011)	ANOVA
5)	H_0: there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads	H_1: there is difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads		MANOVA
6)	H_0: there is no difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread	H_1: there is difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread	De Angelis et al. (2007, 2009), Frati Munari et al.(1998), Lu et al. (2000), Östman et al. (2002), Östman (2003) Brennan et al. (2012)	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one way ANOVA *post-hoc*
7)	H_0: there is no difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread	H_1: there is difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread	Di Lorenzo et al. (1988), Östman et al. (2005), Venn and Green (2007), Slavin and Green (2007), Calame et al. (2011),	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one-way ANOVA *post-hoc*

Chapter 3 Product development

3.1. Introduction

This PhD study comprised of three key stages: firstly, product development; secondly, the analysis of nutrient content and consumer acceptability; and finally, the analysis of nutritional goodness of the sourdough bread enriched with soluble fibre. The used methods are going to be introduced in this chapter in chronological order following the key stages of the research.

The flowchart in Figure 7 demonstrates the experimental stages of this PhD project. Initially, the 'benchmark' sourdough bread for the entire study was developed (control sourdough bread). The experiments using GA, followed by experiments with XG, pectin, bran and their mixtures led to the development of five sourdough and sourdough breads. Three of these breads were enriched with soluble and insoluble fibre. All of these breads were subject to consumer acceptability test, and as a result, one sourdough bread enriched with soluble fibre(XG/GA/Pec/bran) was chosen for the glycaemic index trial. More detailed explanation of the steps taken and decision made during the product development stage of this project is given in the subsequent sections.
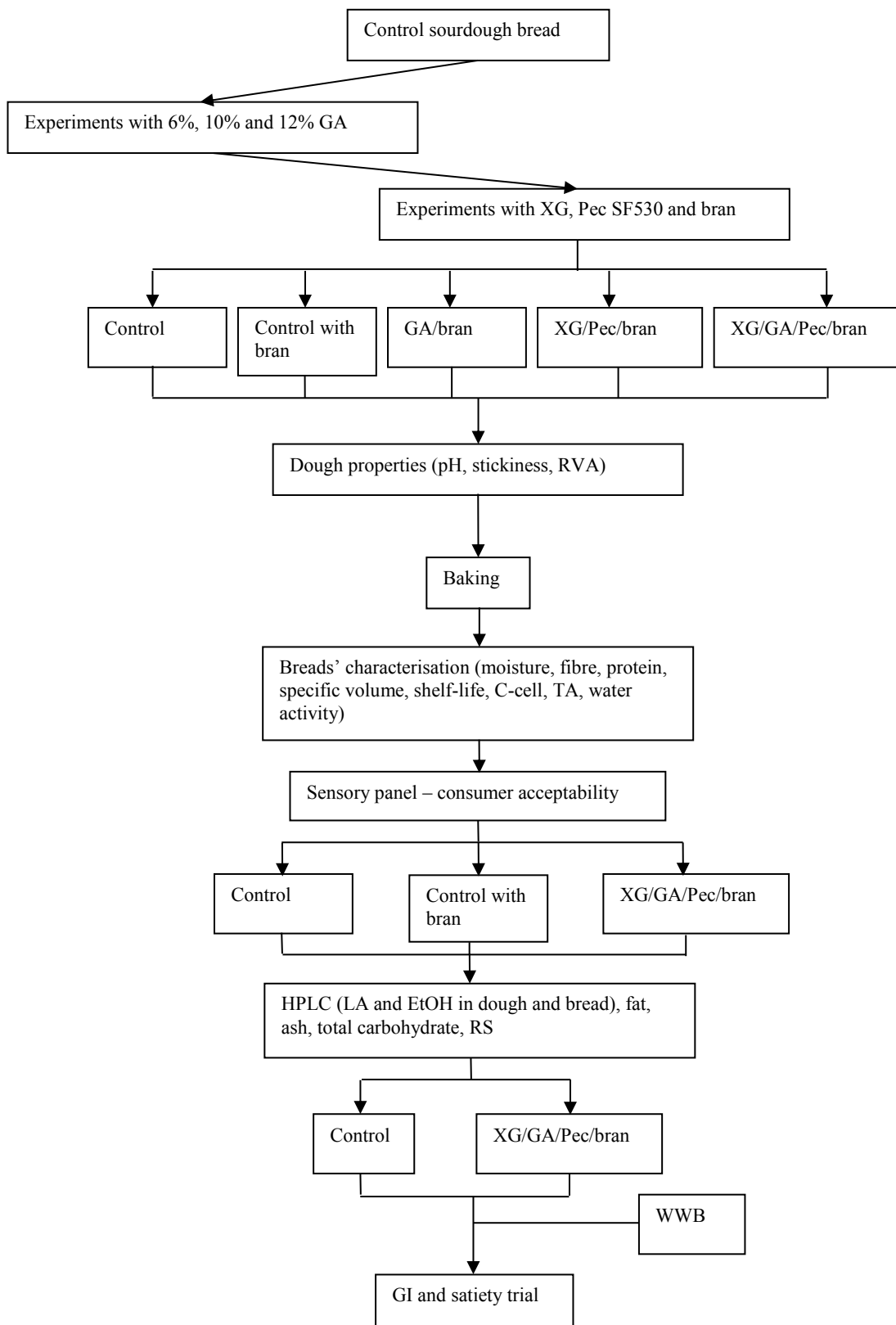
Figure 7. Flowchart showing the experimental phases of this PhD study

3.2. Methodology

3.2.1. Product development

The ingredients used for breadmaking were: strong flour (Smith's Flour Mills Worksopp, UK), fresh yeast (DCL Yeast Ltd., Sutton, UK), Super Gum EM10 (San-Ei Gen F.F.I., Inc., Osaka, Japan), xanthan gum (Sigma-Aldrich, St Louis, MO, USA), Grinsted Pectin SF 530 (Danisco, Kettering, UK), wheat bran (Jordans, Biggleswade, UK). Sourdough was initiated with the use of dried selected starter culture LV2 provided kindly by Fermex Ltd. According to the specification supplied by the manufacturer, the culture consisted of two species of LAB (*L. brevis* and *L.cassei*) and sourdough yeast (species not specified). Two-stage type II sourdough fermentation was selected. The sourdough starter was prepared according to Table 8:

Table 8. Preparation of sourdough starter

Water (hand warm)	100 parts
Strong flour	100 parts
Salt	1 part
Starter culture LV2	0.5 part

All the ingredients were mixed together in a bowl and allowed to ferment for 24 hours in a manual prover (humidity: 97%, temperature: 30 °C). The starter obtained this way had a pH 4.1. The next day the starter was used to prepare the sourdoughs, which were subject to further four hours of fermentation in a manual prover (humidity: 97%, temperature: 30 °C). This procedure was adopted in the production of all sourdoughs described within this thesis.

3.2.1.1. *Development of breads enriched with GA*

During the early stages of the study, the formula for breads, which would contain 15%, 25% and 30% RDI of fibre per portion of bread after baking, was developed. The portion was defined as 60g (two slices) of bread and RDI of fibre as 25g/day. The source of soluble fibre used at that stage was GA. The preparation of that kind of bread has been summarised in Table 9. The substitution of 12, 20 and 24% of flour with the source of soluble fibre would lead to doughs containing 6, 10 and 12% of GA respectively.

Table 9. Preparation of sourdough breads in which 12, 20 and 24% of flour was substituted for GA

	Strong flour	GA	Water	Starter	Salt	Yeast
Control	632.60g	0g	316.3g	316.3g	9.5g	25.3g
6% GA	556.70g	75.9g	316.3g	316.3g	9.5g	25.3g
10% GA	506.08g	126.52g	316.3g	316.3g	9.5g	25.3g
12% GA	480.78g	151.82g	316.3g	316.3g	9.5g	25.3g

Figure 8. Breads made from doughs containing 0, 6, 10 and 12% gum arabic

The incorporation of high amounts of GA into dough posed several challenges – the dough was runny, sticky and the product obtained after the bake-off had unsatisfactory properties such as low volume and sticky, doughy crumb (Figure 8). The water content was reduced and the procedure of admixing GA to the rest of the ingredients was developed at that stage. Addition of 5%, 8% and 10% of fat to sourdough with the medium content of GA was tried. The dough had GA added after pasting it with the shortening and 2% of emulsifier COLCO. The best appearance was obtained with the highest addition of fat. The formula for this bread is presented in Table 10.

Table 10. Preparation of sourdough containing 10% GA

	Control	10% GA
Strong flour	360g	360g
GA	-	80.7g
Water	180g	112.5g
Starter	180g	180g
Salt	5.4g	5.4g
Yeast	14.4g	14.4g
Fat (10%)	45g	45g
Emulsifier (2%)	9g	9g

The principle of this procedure was mixing powdered gum arabic with melted vegetable fat and emulsifier in order to coat the molecules of GA with fat and to prevent their solubilisation after addition of water and starter.

3.2.1.2. *Experiments with XG pectin and bran*

At the next stage, alternative gums or gum-like substances were tried as a source of soluble fibre for use in sourdough bread. These were xanthan gum and Grinsted Pectin SF 530, and the formula applied was as in Table 10. The rationale behind the choice of alternative gums was the fact that both of the substances are approved within the EU as food additives. Additionally, both hydrocolloids have specific health effects (sections 2.8.1.3 and 2.8.3.2). The concentration of 25% RDI of fibre per portion of bread (allowing for the filing of a health claim) was chosen at this stage.

Figure 9. Sourdough bread made with from dough enriched with soluble fibre (from left: 10% xanthan gum; 10% pectin SF 530; control sourdough bread)

Xanthan gum (XG) and Pectin SF 530 yielded more promising results than GA previously (Figure 9). Still, possibly due to high soluble fibre content, the properties of both doughs and resultant breads proved unsatisfactory.

The substitution of 50% of soluble fibre added with a source of mainly insoluble fibre (wheat bran) followed at this stage (Figure 10). The rationale behind this strategy was to improve the handling properties of dough. Control dough containing the admixture of equal portion of wheat bran was developed in parallel. Sugar was added in order to improve the loaves volume.

Figure 10. Sourdough bread enriched with soluble and insoluble fibre (from left: bread with xanthan gum and bran; bread with pectin SF 530 and bran; control sourdough bread)

3.2.1.3. *Development of sourdough breads containing mixtures of soluble fibres*

In order to reduce the undesired sensory properties arising from the use of high levels of gum-like sources of soluble fibre, combinations of the fibres were used. It was observed that when GA was used in combination with pectin and XG, it was possible to omit the coating of GA with fat without deterioration of the handling properties of dough. All doughs were fermented for four hours in a manual prover (humidity: 97%, temperature: 30 °C).

At this point of the experiment, the formulae for soluble fibre-enriched sourdough breads were standardised (Table 11). Sourdough GA contained 10% GA (based on flour), sourdough XG/Pec contained 5% pectin and 5% XG. Bread XG/GA/Pec contained 5% pectin, 2.5% XG and 2.5% GA. Breads with pectin, and pectin and bran were characterised by similar appearance to control sourdough breads (Figures 10 and 11). This dictated the choice of pectin as a dominant source of soluble fibre in bread XG/GA/Pec.

Figure 11. From left: control sourdough bread, control with bran, GA and bran, bread with XG/Pec and bran, XG/GA/Pec and bran

Table 11. Preparation of sourdoughs enriched with soluble and insoluble fibre

	Control	Control + bran	GA and bran	XG/Pec and bran	XG/GA/Pec and bran
Strong flour	100g	90g	90g	90g	90g
Bran	-	10g	10g	10g	10g
Xanthan gum	-	-	-	5g	2.5g
Pectin SF 530	-	-	-	5g	5g
Gum arabic	-	-	10g	-	2.5g
Water	37.5g	41.25g	32.5g	65g	65g
Starter	50g	50g	50g	50g	50g
Salt	1.875g	1.875g	1.875g	1.875g	1.875g
Yeast	4g	4g	4g	4g	4g
Sugar	5g	5g	5g	5g	5g
Fat	-	-	11.25g*	5g**	-
Emulsifier	-	-	2.25g	-	-

* White shortening. ** Oil; added for smooth appearance of the crust.

Trial and error modification of the dough appearance led to the following water content of the sourdoughs: 62.5% for control sourdough; 66.25% for control sourdough with added wheat bran; 57.5% for sourdough containing gum arabic and wheat bran; and 90% for both sourdough with XG and pectin and bran, and for sourdough with XG, GA, pectin and wheat bran. The water content is expressed in 'bakers' maths' and refers to flour as 100%. Bran is treated as flour.

The improvement of handling properties of the doughs containing gum-like soluble fibres as compared to the properties of control doughs (both with and without the admixture of wheat bran) was observed. This determined the use of a dough stickiness rig as a tool for quantification of the dough properties.

3.2.2. Measurement of the pH of sourdoughs

The experiments described in this and the next sections were designed to test hypotheses 1 (H_0: inclusion of soluble fibre mixtures does not change the stickiness of sourdoughs during fermentation) and 2 (H_0: there is no correlation between the pH of sourdoughs and their stickiness) presented previously in Table 7 (section 2.15). The sourdoughs were prepared according to Table 11, and allowed a 4 hour fermentation time (manual prover/retarder) (30°C/ humidity 97%). At the beginning of fermentation and at 1 hour intervals, a sample of dough was taken for pH determination. The pH of sourdoughs was measured directly from the sample with the use of glass electrode coupled with a pH-meter. For each sample determination was repeated three times. The samples of sourdough were subsequently frozen in the blast freezer and stored for further analysis.

As described by Miller et al. (1994), the method of measuring pH of dough in 10% aqueous slurry overestimates the pH due to dilution of acid and loss of carbon dioxide during the sample preparation. It was concluded that direct measurement was effective for the true pH of dough (Miller et al., 1994). Additionally, the change of pH during sourdough fermentation takes place continuously; direct measurement could solve the problem of changing properties of dough which would be unavoidable if the sample was processed into slurry.

3.2.3. Dough stickiness test

The principle of dough stickiness determination is an application of compression force to the dough sample, and measurement of the force required to detach the probe from the surface of the sample (Grausgruber et al., 2003).

Wheat dough poses a challenge with regards to stickiness determination. Application of compression to the sample of dough causes the change in its stickiness. Hence, it is vital to ensure that for every determination of dough stickiness the compression applied is constant, so the change in stickiness during the determination is constant for each sample (and can be accounted) (Chen and Hoseney, 1995). This can be achieved with the use of Texture Analyser (Stable Micro Systems, UK). For the

purpose of this experiment a Texture Analyser equipped with Chen/Hoseney dough stickiness rig and a 32kg cell was used.

A small amount of dough was placed in the sample space of the Chen/Hoseney dough stickiness cell. An amount of dough was extruded through the holes in the extrusion lid, and discarded. For stickiness determination, a further 1mm of sample dough was extruded, covered with a perspex cap to prevent moisture loss and allowed to rest for 30s in order to release the tensile pressure caused by the extrusion of dough. After the resting time, the perspex cap was removed and a compression force of 40g was applied to the extruded sample. At least six determinations (each preceeded by extrusion and 30s resting time) from each sample were performed in order to obtain a sufficient number of repeatable results.

The dough stickiness was recorded as a plot of force in time with the use of Exponent software (Stable Micro Systems, UK). An example of such plot is shown in Figure 12.
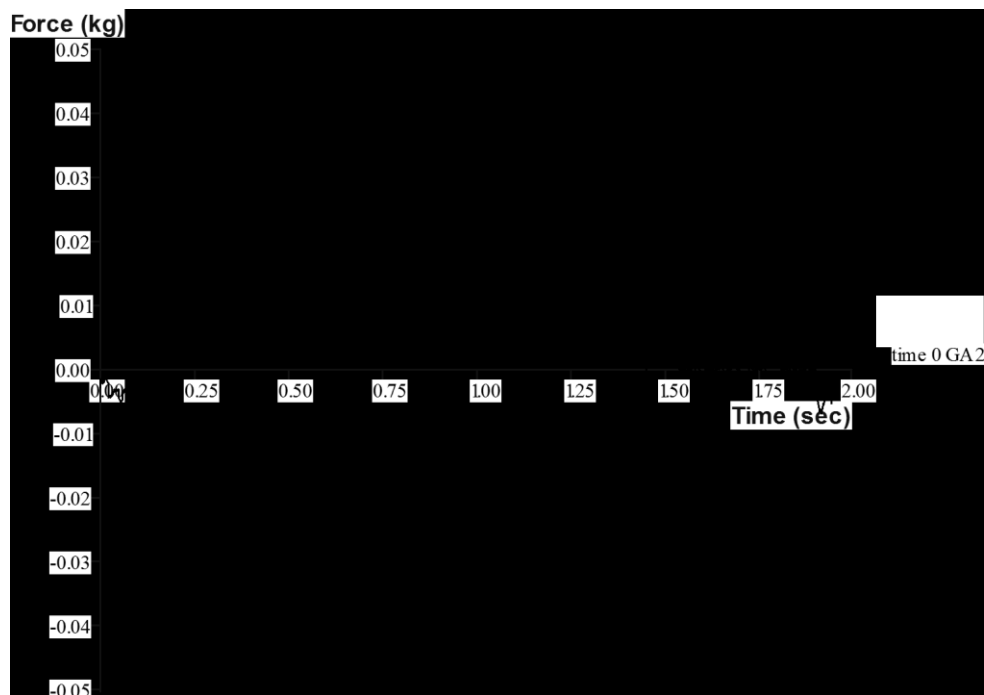
Figure 12. An example of a graph of the dough stickiness (as force, g) measured with the use of Chen/Hoseney dough stickiness rig (source: primary research)

71

3.2.4. Rapid viscosity analysis (RVA) of the pasting properties of mixtures of wheat flour and soluble fibres

The rapid viscosity analysis was applied to test hypothesis 3 (H$_0$: addition of different soluble fibres and bran does not alter the gelatinisation parameters of flour pastes) presented previously in Table 7 (section 2.15).

3.2.4.1. *Materials and equipment*

The composition of tested blends is presented in Table 12. Flour was fortified with 10% soluble fibres (based on flour). Where bran was used, it substituted 10% of the flour and the addition of 10% of soluble fibre was based on the weight of flour with bran. The equipment used was RVA TecMaster (Perten, Warrington, UK).

Table 12. Composition of flour blends subject to RVA

Mixture	Flour (g)	Bran (g)	XG (g)	GA (g)	Pec (g)
Flour	100	-	-	-	-
FBr	90	10	-	-	-
FPec	100	-	-	-	10
FBrPec	90	10	-	-	10
FXG	100	-	10	-	-
FBrXG	90	10	10	-	-
FGA	100	-	-	10	-
FBrGA	90	10	-	10	-
FBrXGPec	90	10	5	-	5
FBrXGGAPec	90	10	2.5	2.5	5

3.2.4.2. *Method*

Pasting properties of flour enriched with soluble fibres were measured according to AACC Approved Method 76.21-01 (AACC, 2000). 25g of water was weighed accurately into the RVA sample can, followed by 3.5g of the sample. The mixing paddle was added and the samples subjected to RVA general pasting method procedure presented in Table 13. All determinations were done in triplicates. The values recorded by RVA were: pasting temperature (PT), peak viscosity (PV), time

to peak (PTi), breakdown (BD), trough viscosity (TV), setback (SB), final viscosity (FV).

Table 13. Temperature and paddle speed profile of the RVA general pasting method (from Perten RVA Application Note)

Time	Type	Value
00:00:00	Temperature	50 °C
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:01:00	Temperature	50 °C
00:04:42	Temperature	95 °C
00:07:12	Temperature	95 °C
00:11:00	Temperature	50 °C
00:13:00	End	
Idle temperature: 50 ± 1 °C; Time between readings: 4s		

3.2.5. Moisture content

Experiments presented in this and the following sections allowed the testing of hypothesis 4 (H_0: inclusion of soluble fibres and bran does not alter physico-chemical properties of sourdough breads) developed as a result of literature review (section 3.6). The moisture content of breads was determined gravimetrically. Approximately 3g of bread milled in a blender into breadcrumbs was accurately weighed in a weighing tin. Samples were left in an oven (65 °C) overnight and dried to constant weight.

3.2.6. Fibre determination

The content of fibre in all of the bread samples was determined using the combination of enzymatic and gravimetric methods as described by Asp et al. (1983). It is a combination of AACC approved methods (32-05.01 total fibre, 32-21.01 insoluble and soluble fibre) (AACC, 2000). The principle of this assay is the enzymatic removal of proteins and starch from the sample (Figure 13). Total dietary fibre assay kit (SIGMA-Aldrich) was used. The samples (half a loaf of each type) were blended into breadcrumbs in a food processor and frozen in sealed bags until further analysis.

- Thermostable α-amylase, pH 6.0, 95 °C, 15 min.

- Pepsin pH 7.5, 60 °C, 30 min.

- Pancreatin, pH 4.5, 60°C, 30 min.

- Filtration of insoluble fibre, washing with ethanol and acetone; drying

- Precipitation of soluble fibre with ethanol; filtration; washing with ethanol and acetone; drying

- Protein determination (for indigestible proteins, Kjeldahl method)
- Ash determination
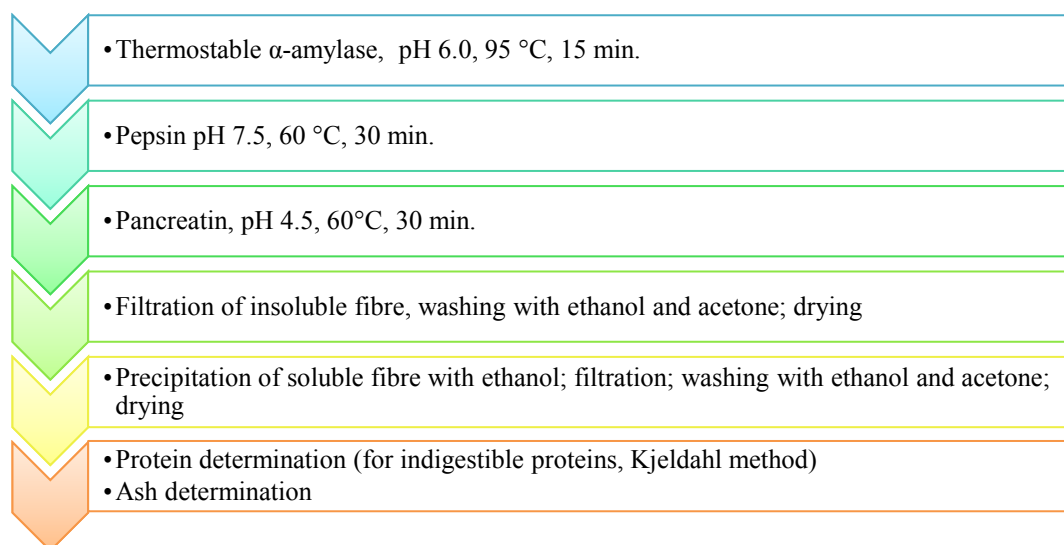
Figure 13. Determination of total dietary fibre using total dietary fibre assay kit

3.2.7. Protein determination

The protein content of sourdough breads enriched with soluble fibre was determined as crude protein with the use of Kjeldahl method (N x 6.25). The principle of this method is the liberation of nitrogen from its organic compounds. This is achieved in the environment of concentrated sulphuric acid (H_2SO_4) by boiling the sample in the presence of a catalyst (copper sulphate, $CuSO_4$) (1). The liberated nitrogen is present as ammonia, which can be extracted from the highly acidic environment by alkalisation of the sample and subsequent distillation of the sample with steam:

$$(1)\ NH_4^+ + OH^- \rightarrow NH_3 + H_2O$$
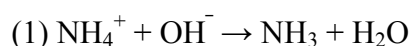
The distillate is gathered in a conical flask containing boric acid and then titrated with hydrochloric acid (HCl) (2).

$$(2)\ 3NH_4^+ + BO_3^{3-} + 3H^+ + 3Cl^- \rightarrow 3NH_4Cl + H_3BO_3.$$
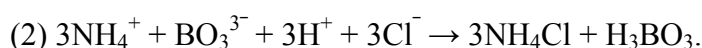
1ml of 1M HCl is equivalent to 0.0014g of protein. Thus protein content was calculated as follows:

$$Protein\ (g) = 0.0014 * titre\ * N;$$

where N is nitrogen conversion factor. For wheat bread $N = 6.25$ (Kirk and Sawyer, 1991).

3.2.8. Specific volume

The specific volume of the breads was assessed as follows. Baked loaves were left to cool down, wrapped in polyethylene bags and left for 24 hours until the analysis. Breads were subject to volume measurement with the use of rapeseed displacement method (AACC approved method, 10-05.01) (AACC, 2000) and weighing. Specific volume was calculated by division of loaf volume by its mass. The determination was performed in triplicate and the final value calculated as an average.

3.2.9. Water migration between the crumb and crust of sourdough breads enriched with soluble fibres.

Water migration between crumb and crust can provide information regarding the staling process. In order to observe the water migration between the crumb and crust of resultant breads, the water activity of crumb and the water activity of crust were measured separately after 24 and 144 hours of storage (1 and 6 days). To make this feasible, the crusts (0.5 cm) were removed immediately prior to the measurement. Crust and crumbs were milled separately in a blender until fine breadcrumbs were obtained. The water activity (a_w) was measured as equilibrium humidity of a sample with the use of laboratory hygroscope (Hygroskop).

3.2.10. Crumb firmness – texture analysis and shelf-life

The principle of crumb firmness analysis is the measurement of the force in compression. The method used for the purpose of this study was the AACC Standard Method (74-10.02) (AACC, 2000) in which a force of compression is applied to the sample with a 36 mm diameter probe until the crumb is compressed by 40% of its initial thickness. The force required to achieve this level of compression is then measured and recorded by a texture analyser (Stable Micro Systems, UK) and associated software (Exponent Software, Stable Micro Systems, UK), which plots a crumb firmness plot (Figure 14). Crumb firmness is reported as the force measured at 25% (6.25 mm) sample compression.

Determination was conducted in following manner. A sample of two slices of bread (12.5 mm each) was compressed with the use of TA. Determination was run in triplicates.
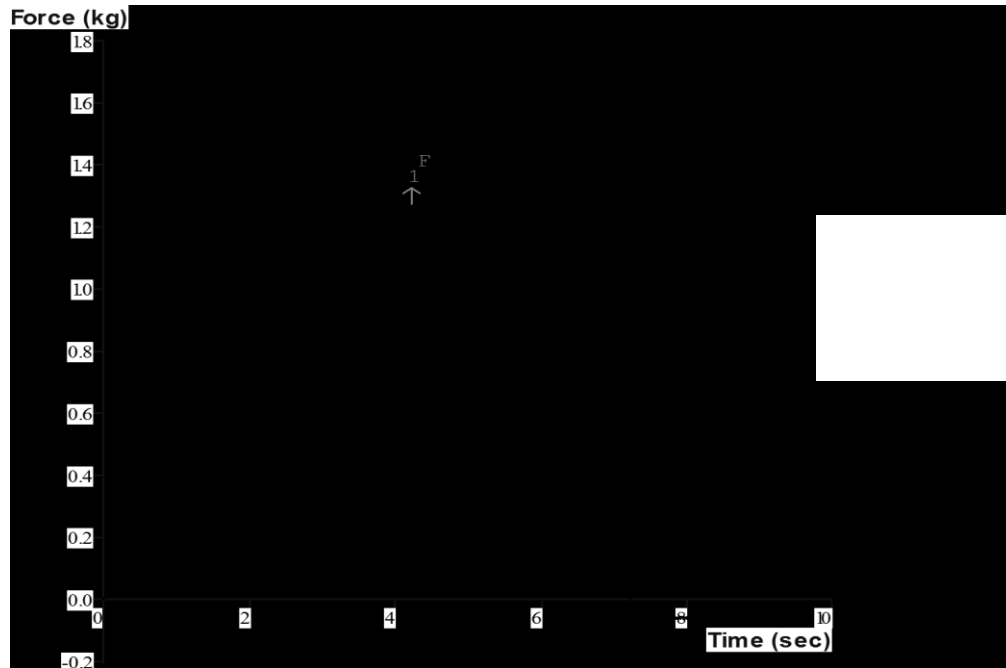
Figure 14. An example of a plot of crumb firmness (measured as compression force, g) with the use of texture analyser

Potential shelf-life of sourdough breads enriched with soluble fibre was assessed with the use of crumb compression test performed after 24, 72 and 144 hours (1, 3 and 6 days) of storage of bread. All the breads were stored under the same conditions, wrapped in polyethylene bags.

3.2.11. HPLC determination of organic acids and ethanol in sourdoughs and sourdough breads

As presented in Figure 7 (section 3.1), more analyses followed the sensory evaluation of the sourdough breads enriched with soluble fibres. This section discusses the HPLC analysis of organic acids and ethanol content in selected sourdoughs, which was performed to establish whether substances which may influence the glycaemic response (organic acids) were present in developed sourdoughs.

3.2.11.1. *HPLC system*

All HPLC separations were conducted on HPLC system consisting of: degasser Shimadzu Prominence DGU-20A3 (Shimadzu, Milton Keynes, UK), two LC-10 ADVP solvent delivery modules (Shimadzu, Milton Keynes, UK), mixer SUS-L (Shimadzu, Milton Keynes, UK), and SIL-HTC autosampler (Shimadzu, Milton Keynes, UK).

The temperature of the column was kept at constant values by Phenomenex Thermashpere™ TS-130 column thermostat (Phenomenex, Macclesfield, UK). The HPLC system was connected to refractive index detector RI-2031 (JASCO, Great Dunmow, UK)

3.2.11.2. *Preparation of the calibration curves*

Five point calibration curves have been prepared according to Table 14. Final concentrations of mixed standard solutions are given in bold writing.

Table 14. Concentrations used for calibration curves for HPLC quantification of organic acids and ethanol

		acetic acid (g/L)	propionic acid (g/L)	butyric acid (g/L)	lactic acid (g/L)	ethanol (g/L)
STANDARD SOLUTION		2.50	2.50	2.50	10.00	10.00
CALIBRATION CURVE CONCENTRATION	1	**0.05**	**0.05**	**0.05**	**0.10**	**0.10**
	2	**0.10**	**0.10**	**0.10**	**0.20**	**0.20**
	3	**0.15**	**0.15**	**0.15**	**0.30**	**0.30**
	4	**0.20**	**0.20**	**0.20**	**0.40**	**0.40**
	5	**0.25**	**0.25**	**0.25**	**0.50**	**0.50**

Due to the use of the refractive index detector, based on the measurement of refractive index of the sample components in relation to the refractive index of the mobile phase, all of the standards and samples were prepared by dissolving the HPLC grade substances in the mobile phase ($0.005N$ H_2SO_4).

3.2.11.3. *Extraction of dough samples*

Sourdoughs were prepared and subject to fermentation as described in section 3.2.1. Extraction of the organic acids and ethanol was performed with water using a

modification of method described by Lefebvre et al. (2002). 10 g of dough was sampled at the beginning of the fermentation and every hour for four hours. The dough was homogenized with 90 ml water in a Kenwood blender (speed 1, 30 s). To deproteinise the samples and stop the fermentation, 50ml of 1mol/L $HClO_4$ was added to homogenates before centrifuging at 2800 x g (Rotanta 460R, Hettich Zentrifugen, UK; 15 min, 15 °C). The supernatant was frozen until further analysis.

Samples were defrosted overnight, transferred to glass beakers and neutralised with 4M NaOH (approximately 7ml). The volume of the samples was adjusted to 110ml with water in a measuring cylinder, followed by the addition of 0.552ml of 1N H_2SO_4. From so prepared samples, 1ml was mixed with1 ml of 0.005N H_2SO_4 and filtered through a syringe filter (Whatman, 13mm, ϕ 0.45μm) into a glass vial which was placed in autosampler for HPLC analysis.

3.2.11.4.   *Extraction of bread samples*

The bread samples were extracted in two ways, referred here as Methods A and B. All samples were prepared in duplicates.

Method A (single extraction): Half of each loaf was ground with the use of Kenwood blender (speed 2, 1 min). 10g of breadcrumbs was weighed into a bottle, mixed with 100ml of water and stirred with a magnetic stirrer for 10 minutes. Samples were then centrifuged at 2800 x g (Rotanta 460R, Hettich Zentrifugen, UK; 20 min, 15 °C). Supernatant was reserved for further HPLC analysis. In volumetric flasks (50ml), 0.25ml of 1N H_2SO_4 was made up to the volume with the supernatant. At this stage, samples were frozen until further analysis.

When needed, samples were defrosted overnight. 0.5ml of a sample was mixed with 1.5ml 0.005N H_2SO_4 and filtered through a syringe filter (Whatman, 13mm, ϕ 0.45 μm) into a glass vial which was placed in the autosampler for HPLC analysis.

Method B (double extraction): Half of each loaf was ground with the use of Kenwood blender (speed 2, 1 min). 10g of breadcrumbs was weighed into a bottle, mixed with 50ml of water and stirred with a magnetic stirrer for 10 minutes. Samples were then centrifuged at 2800 x g (Rotanta 460R, Hettich Zentrifugen, UK; 20 min, 15 °C). Supernatant was reserved and the residue was mixed with 50ml of water and

stirred with the magnetic stirrer for 10 min before again centrifuging at 2800 x g (20 min, 15 °C). The supernatant was mixed with the supernatant from the first extraction. In volumetric flasks (50ml), 0.25ml of 1N H_2SO_4 was made up to the volume with the extract. At this stage, samples were frozen until further analysis.

When needed, samples were defrosted overnight. 0.5ml of a sample was mixed with 1.5ml 0.005N H_2SO_4 and filtered through a syringe filter (Whatman, 13mm, ϕ 0.45 µm) into a glass vial which was placed in the autosampler for HPLC analysis.

3.2.11.5.   *Conditions of separation*

Shimadzu HPLC system as described in section 4.5.2 was used for analyses. The use of refractive index detector dictated the use of isocratic elution. The column applied was a Rezex-ROA (Phenomenex, Macclesfield, UK) coupled with SecurityGuard cartridge. The dimensions of the column were 300 x 7.8 mm. The mobile phase was 0.005N H_2SO_4. The column was thermostated at 40 °C. The temperature of the refractive index detector was also kept constant at 40 °C. The flow rate was 0.25 ml/min. The chromatographs were registered and integrated by LC Solution software (Shimadzu, UK). The integration was based on five-point calibration curves prepared as described in section 4.5.3. The dough samples were prepared in three batches of ten and calibration solutions were processed in between batches of samples. Each sample and each standard was run in duplicate. Bread samples were prepared in three batches of eight. The calibration curves were run between each batch of samples.

3.2.12. Anthrone method for total carbohydrate content

The anthrone method was used to determine the total carbohydrate content of the sourdough breads. In a strongly acidic environment, glucose is dehydrated into hydroxymethyl furfural which forms a green-coloured compound with anthrone. The product of the reaction has maximum absorbance at wavelength 630 nm.

In order to estimate the total carbohydrate content of breads obtained in the course of product development, the anthrone method was used as described by Hedge and Hofreiter (1962). The sample preparation procedure was as follows: 0.1000g of sample was weighed into a boiling tube and after the addition of 5ml of 2.5N HCl hydrolysed by heating in boiling in water bath for three hours. After that time,

crystalline Na_2CO_3 was added to neutralise the samples (against glass electrode) and samples were made up to 100ml in volumetric flasks. 2ml aliquot from the samples were centrifuged, and from the supernatant 0.1ml was mixed with 0.9 ml of water. 4ml of ice-cold anthrone reagent was added to the samples before heating for eight minutes in boiling water bath. After rapidly cooling, the absorbance of the samples (green to dark green) was read at 630 nm.

Calibration curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0ml of glucose standard solution (0.1 mg/ml) into boiling tubes, making the volume up to 1 ml with water, adding 4ml ice-cold anthrone reagent and heating in boiling water bath for eight minutes. The absorbance of the standards was read at 630 nm.

3.2.13. Ash content

Approximately 2g of homogenised sample was accurately weighed into an ignited, cooled and weighed crucible. Samples were initially ignited in the flame of a Bunsen burner and subsequently placed in a muffle furnace set at 550 °C for three hours. After cooling in the desiccator, the crucibles were weighed again. Ash was calculated as the difference of weight of crucibles containing samples after and before incineration. The test was run in triplicate for each sample.

3.2.14. Fat content by Soxhlet extraction

Fat content was determined using gravimetric method based on Soxhlet extraction of fat from food products with organic solvents (petroleum ether and chloroform). Homogenised samples of bread were dried overnight in 65 °C and 5 g of dried sample was weighed into cellulose thimbles. Dry extraction cups were weighed with dry antibumping granules. Each cellulose thimble was placed in an extraction cup which was half-filled with Soxhlet solvent (petroleum ether – chloroform, 50:50). The extraction was performed in a Soxtec apparatus (Foss UK, Warrington, UK). After extraction, the cups were placed in an oven for 30 minutes (65 °C) to evaporate any remaining extraction solvent. The cups were cooled in a desiccator and weighed. The fat content in dry matter was calculated according to the equation:

$$Fat\ content\ (g/100g) = \frac{a-b}{c} \times 100;$$

where a – cup weight after extraction, b – cup weight before extraction, c – sample weight.

3.2.15. Resistant starch measurement

In order to be able to accurately predict the size of bread sample containing 50g of available carbohydrates, the bread samples were subject to the analysis of resistant starch (RS) content. The method employed was AACC Method 32-40 for resistant starch, based on work of McCleary and Monaghan (2002). The method is suitable for samples containing in excess of 2% resistant starch.

The assay was carried out with the use of Megazyme Resistant Starch kit and as directed in the assay procedure supplied by the manufacturer. The breads were baked 24 hours prior to the analysis. Half of a loaf of bread was initially homogenised in a food blender. The moisture content of the samples was determined by drying to constant weight. Calculations of available, resistant and total starch were carried out according to equations given in Megazyme kit procedure.

3.3. Results

3.3.1. Change of pH of sourdoughs during fermentation
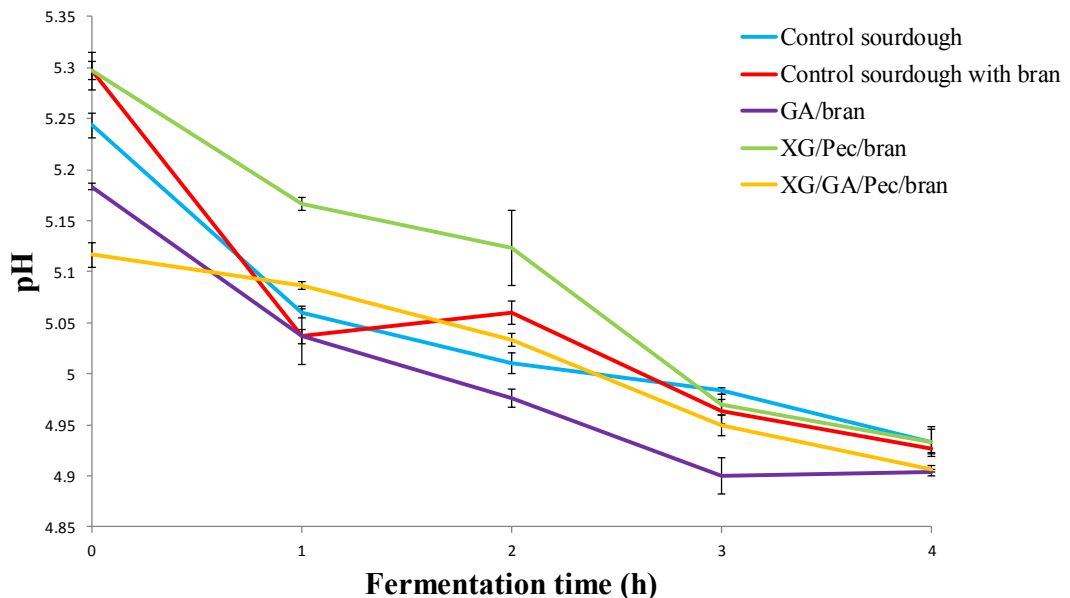
Figure 15. pH of sourdoughs enriched with soluble fibre during 4 hour fermentation; all values are means from three replications; error bars are +/- 1 SE

The results of the pH determination of sourdoughs are shown on Figure 15 and summarised in Table 15. The starter sourdough (first stage fermentation, 24 hours) used for preparation of the sourdoughs (second stage fermentation) destined for breadmaking had pH of 4.14.

At the initial stage of fermentation all of the doughs had a pH in a range of 5.12 − 5.30. After 1 hour of fermentation all of the doughs, except for the dough with xanthan gum, gum arabic, pectin and bran increased their acidity significantly ($p \leq$ 0.001). The pH after 4 hour fermentation of all the doughs was 4.90 − 4.93.

Table 15. pH of sourdoughs enriched with soluble fibre during 4 hour fermentation

| | pH | | | | | | | | | |
| | 0 h | | 1 h | | 2 h | | 3 h | | 4 h | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control sourdough	5.24	0.01	5.06[a]	0.01	5.01[a]	0.01	4.98[a]	0.01	4.93[a]	0.01
Control sourdough with bran	5.30	0.02	5.04[a]	0.03	5.06[a]	0.01	4.96[a]	0.01	4.93[a]	0.01
GA and bran	5.18	0.01	5.04[a]	0.01	4.98[a]	0.01	4.90[a]	0.02	4.90[a]	0.01
XG/Pec and bran	5.30	0.01	5.17[a]	0.01	5.12[a]	0.01	4.97[a]	0.01	4.93[a]	0.01
XG/GA/ Pec and bran	5.12	0.01	5.09	0.01	5.03[a]	0.01	4.95[a]	0.01	4.91[a]	0.01

Data read in rows; superscript 'a' denotes significant ($p \leq$ 0.001) difference in comparison to the initial pH; all values are means from three replications

3.3.2. Change of dough stickiness during fermentation

Stickiness data was analysed using two-factor repeated measures ANOVA to determine main effects of type of dough and fermentation time on the stickiness value. Two-factor repeated measures ANOVA was followed by one-way ANOVA and one-way repeated measures ANOVA to explore the differences if two-factor design pointed at the main effect.

Two factor ANOVA demonstrated the main effect of dough type ($p < 0.001$), fermentation time ($p < 0.05$) and interaction ($p < 0.05$). *Post-hoc* repeated measures ANOVA revealed that change of stickiness for all three sourdoughs enriched with soluble fibres was not statistically significant. In contrast, changes of stickiness of control sourdough and control sourdough with added wheat bran proved to be statistically significant.

Post-hoc one-way ANOVA showed that during fermentation stickiness of sourdoughs enriched with soluble fibre did not change significantly. Table 16 shows that the mean value of stickiness of the dough with added XG and Pec was significantly lower than the means of stickiness of both control doughs at the same stage of fermentation. The dough with xanthan gum, pectin and bran was the least sticky after 4 hours of fermentation when compared with the control doughs. This difference was statistically significant ($p \leq 0.001$). Also, in comparison with other sourdoughs enriched with soluble fibres, the mean value of stickiness of sourdough enriched with xanthan gum, pectin and bran (XG and Pec), proved to be significantly different after 1, 2, 3 and 4 hours of fermentation. The mean values show that the dough with XG and Pec was the least sticky throughout the period of fermentation.

Table 16. Stickiness of sourdoughs enriched with soluble and/or insoluble fibres during 4 hour fermentation; all values are means from *n*= 5 determinations

		Stickiness (g)								
		Control dough (a)		Control with bran (b)		XG/Pec and bran (c)		XG/GA/ Pec and bran (d)		GA and bran (e)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Time of fermentation (h)	0	23.06[d, e]	3.30	26.64	2.32	25.13[e]	1.01	35.33[a, e]	1.71	33.47[a, d]	4.54
	1	29.97[c]	1.42	33.31[c]	0.89	25.56[a, b, d, e]	0.36	32.25[c]	0.95	33.20[c]	1.06
	2	36.88[c]	1.89	38.90[c, d]	0.99	23.97[a, b, d, e]	0.98	32.75[b, c, e]	0.67	38.78[c, d]	1.23
	3	39.06[c]	1.83	43.71[c, d, e]	1.24	23.19[a, b, d, e]	1.19	34.80[b, c]	1.29	36.60[b, c]	0.74
	4	46.44[c, d, e]	3.06	42.16[c]	1.17	24.00[a, b, d, e]	0.65	36.65[a, c]	1.21	34.56[a, c]	2.96

Data read in rows; a – denotes the significance in comparison to control sourdough; b – denotes the significance in comparison to control sourdough with added wheat bran; c – denotes the significance in comparison to sourdough enriched with XG, Pec and bran; d – denotes the significance in comparison to sourdough enriched with XG, GA, Pec and bran; e – denotes the significance in comparison to sourdough enriched with GA and bran. All values are means of five replications

At the beginning of fermentation, the mean value of stickiness of sourdough enriched with XG/GA/Pec and wheat bran was higher than for any of the control sourdoughs. ANOVA revealed that this result had statistical significance ($p \leq 0.05$) only against the control dough without any fibre added.

The initial stickiness of dough enriched with gum arabic was higher than the stickiness of either of the control doughs. This difference holds statistical significance only when compared against the initial mean stickiness of control dough without added wheat bran ($p \leq 0.01$). The difference of the means measured after 1 hour and 2 hours of fermentation was not significant statistically. However, after 3 hours of fermentation, dough with GA and bran was significantly less sticky ($p \leq 0.01$) than control dough with wheat bran. Finally, after 4 hours of fermentation, the mean stickiness value of dough with GA was lower than the stickiness of control dough with added bran (not statistically significant) and significantly lower ($p \leq 0.01$) than the corresponding value measured for control dough without wheat bran. For clarity, these results are represented in graphic form in Figure 19. As a result of this analysis, the null hypothesis 'inclusion of soluble fibre mixtures does not influence the stickiness of sourdoughs during fermentation' was rejected and the alternative hypothesis (H_1: inclusion of soluble fibre mixtures influences the stickiness of sourdoughs during fermentation) accepted.
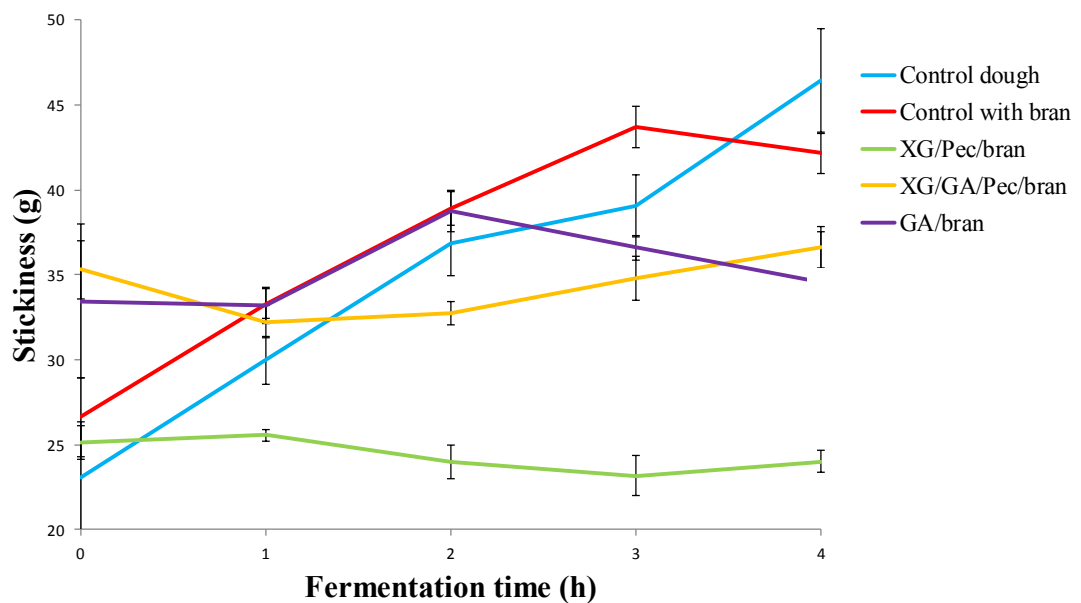
Figure 16. Change of stickiness of sourdoughs enriched with soluble fibres during the fermentation; values are means of *n* = 5 determinations; error bars +/- 1 SE

3.3.3. Correlation between pH and dough stickiness

The results of statistical analysis of correlation between the pH and stickiness of the sourdoughs in the experiment are presented in Table 17. The scatter plots are included in Appendix 11. A strong statistically significant correlation was observed between the pH and stickiness of control sourdough (r = -0.786, n = 15, p < 0.01, R^2 = 0.618) and control sourdough with added wheat bran (r = -0.729, n = 15, p < 0.01, R^2 = 0.532). However, there was no correlation between the pH and stickiness of any of the sourdoughs with added soluble fibre. Therefore, the null hypothesis 2 (H_0: there is no correlation between the pH of sourdoughs and their stickiness) was accepted for the sourdoughs with added soluble fibres. The same hypothesis was not true for both control sourdoughs, hence H_1 (there is correlation between pH of sourdoughs and their stickiness), was accepted for these sourdoughs.

Table 17. Correlations between pH and dough stickiness

	R	*N*	*P*	*R²*
Control	-0.786	15	0.001	0.618
Control with bran	-0.729	15	0.002	0.532
GA and bran	0.124	15	0.659	0.015
XG/Pec and bran	0.288	15	0.298	0.083
XG/GA/Pec and bran	- 0.251	15	0.367	0.063

3.3.4. Pasting properties

Analysis of ten different samples (mixtures of 10% of single soluble fibres with flour, with flour and bran, and selected combinations of the fibres with flour and bran) yielded a large amount of data. The pasting curves of the mixtures considered in this research are presented on Figures 17 and 18. The values of pasting temperature, pasting time, peak viscosity, trough viscosity, breakdown, final viscosity and setback are presented in Table 18.

The statistical analysis of the results revealed significant influences of the addition of soluble fibres on the pasting properties of flour or flour containing 10% of bran. The application of 10% of bran caused a statistically significant reduction of peak

viscosity of flour ($p < 0.05$). Similar trend was observed also in the mixture of flour with soluble fibres, although it was not statistically significant. Furthermore, the trough viscosity value was also affected by the addition of 10% of bran in all mixtures, but was statistically significant only for flour (no treatment) ($p < 0.001$) and FXG ($p < 0.001$). In general, the breakdown values in the pasting curve measured for all of the considered mixtures were not significantly different. However, the breakdown value for FBrXG was significantly higher than those of mixtures containing gum arabic: FGA ($p < 0.05$), FBrGA ($p < 0.01$) and FBrXGGAPec ($p < 0.05$).

The final viscosity of flour paste was significantly reduced by the addition of 10% of wheat bran ($p < 0.001$). The mixtures containing gum arabic were also characterised by statistically significant reduction in final viscosity ($p < 0.01$ for FGA, $p < 0.001$ FBrGA). The highest final viscosity was recorded for the mixtures containing xanthan gum. The mixture FXG had a final viscosity of 3364 cp which was reduced by admixture of 10% bran in mixture FBrXG to 2929 cp ($p < 0.05$). The final viscosities attained by these two mixtures were significantly different from the final viscosity values of the remaining flour-hydrocolloid pastes (Table 18).

Setback values measured in this experiment followed similar trend to the final viscosity values. The mixtures containing xanthan gum had the highest setback values (1446 cp for the FXG blend and 1442 cp for FBrXG) which were significantly different from those for the remaining flour and soluble fibre blends.

The statistical analysis of the test results of the pasting properties results did not reveal statistical differences in pasting time of the mixtures of flour with soluble fibres. In all cases, pasting took place during the sixth minute of the experiment. However, the soluble fibres' addition altered the pasting temperature (see Table 18). The lowest pasting temperatures, 50.38 °C and 51.87 °C were observed for the blends FBrXG and FXG respectively. These pasting temperatures were significantly lower ($p < 0.05$) than those of flour, flour with bran, flour blends containing pectin and flour blends containing gum arabic. Gelatinising at the highest temperature were the blends containing gum arabic with pasting temperature of 89.18 °C and 88.8 °C for FGA and FBrGA respectively. These two results were not significantly different from control (untreated flour), flour containing wheat bran (FBr) and blends

containing pectin (FPec, FBrPec). However, temperatures of gelatinisation of the remaining four samples were lower, and the difference of the mean values was statistically significant ($p < 0.05$).

The RVA experiment and subsequent data analysis allowed the testing of hypothesis 4 (section 2.15). As a result of these steps, the null hypothesis H_0: inclusion of soluble fibres and bran does not alter physico-chemical properties of sourdough breads was rejected and the hypothesis alternative to this null hypothesis was accepted.
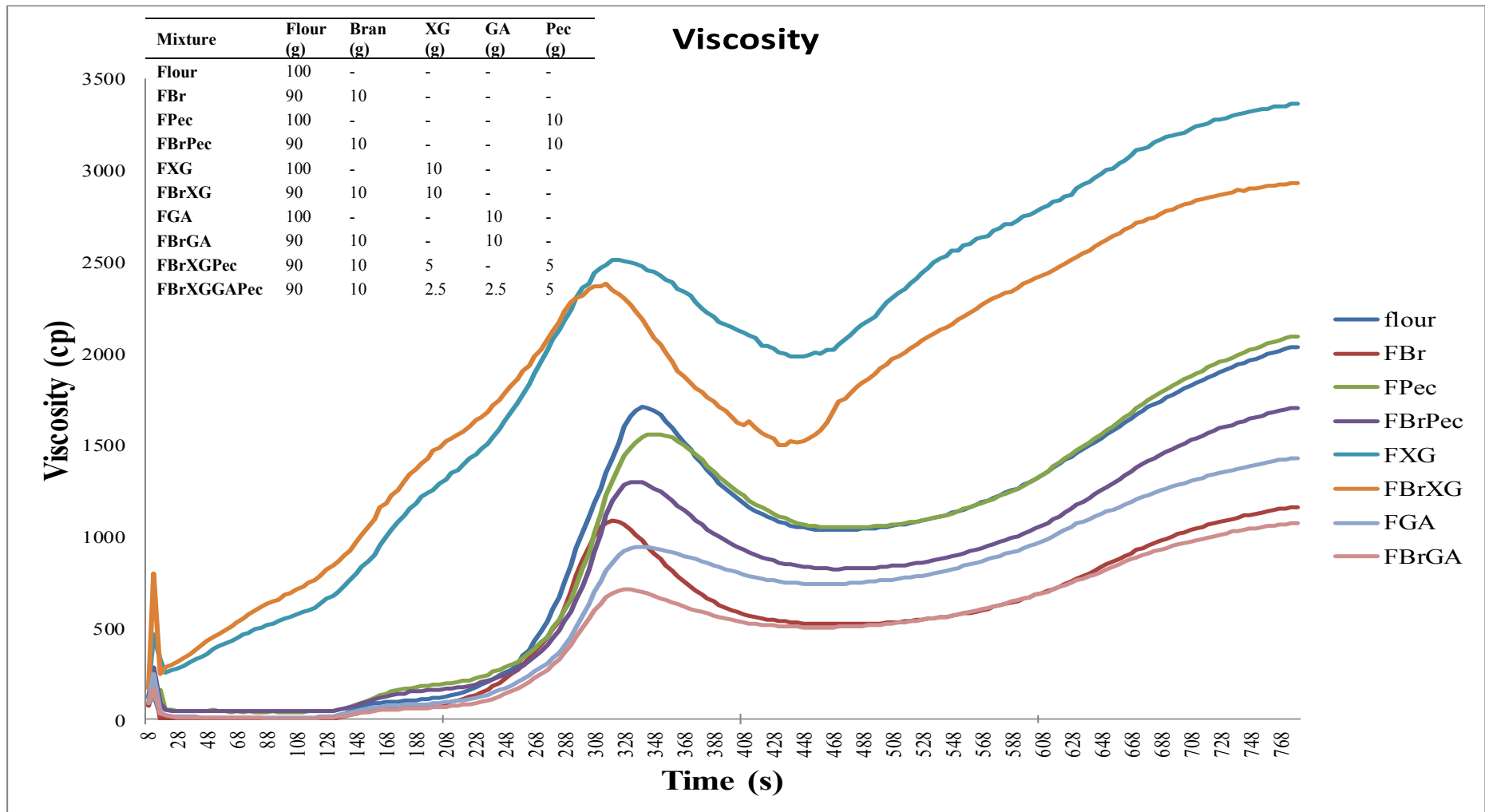
Figure 17. RVA results of flour and flour-bran pastes with addition of soluble fibres; table in the inset shows the composition of the analysed flour blends
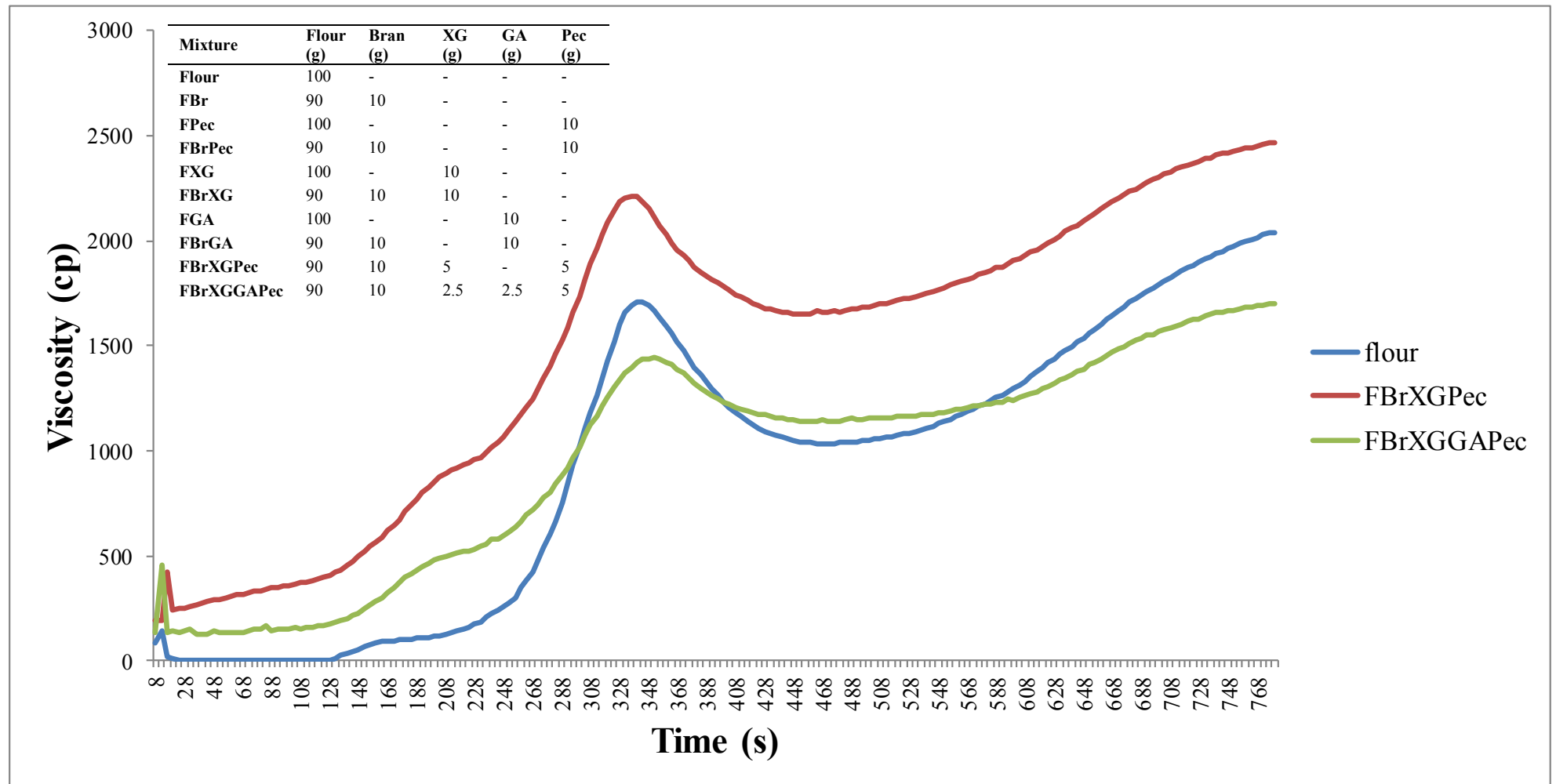
Figure 18. RVA results of flour pastes containing the mixture of soluble fibres used in breadmaking during the product development stage of the study. Table in the inset shows the composition of analysed flour blends

Table 18. The results of RVA analysis of various combinations of hydrocolloids and flour

	Flour type									
	Flour (a) (*n*=3)		**FBr (b) (*n*=3)**		**FPec (c) (*n*=3)**		**FBrPec (d) (*n*=4)**		**FXG (e) (*n*=3)**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PV (cp)	1714.00^{b,e,f,g,h}	13.58	1088.67^{a,e,f,ı}	9.91	1572.67^{e,f,h,ı}	110.97	1316.00^{e,h,ı}	35.67	2575.00^{a,b,c,d,g,h,j}	318.08
TV (cp)	1035.67^{b,e,f,h,i}	5.24	519.67^{a,c,d,e,f,i,j}	4.91	1047.00^{b,e,f,g,h,i,}	72.92	823.00^{b,c,e,f,h,i,j}	38.79	1917.33^{a,b,c,d,f,g,h,j}	75.55
BD (cp)	678.33	11.39	569.00	7.55	525.67	51.95	493.00	6.10	657.67	299.00
FV (cp)	2034.33^{b,e,f,g,h}	11.41	1160.33^{a,c,d,e,f,i,j}	14.84	2093.67^{b,e,f,g,h}	114.64	1702.75^{b,e,f,h,i}	63.45	3364.00^{a,b,c,d,f,g,h,i,j}	195.92
SB (cp)	998.67^{b,e,f,h,j}	6.17	640.67^{a,c,e,f}	10.11	1046.67^{b,e,f,g,h,j}	41.84	879.75^{e,f,h,j}	24.90	1446.67^{a,b,c,d,g,h,i,j}	150.52
PTi (min)	5.69	0.04	5.35	0.02	5.84	0.06	5.57	0.08	5.86	0.53
PT (°C)	78.82^{e,f}	7.21	85.33^{e,f,i,j}	0.29	75.83^{e,f}	6.51	83.51^{e,f,i,j}	5.33	51.87^{a,b,c,d,g,h}	1.45

	Flour type									
	FBrXG (f) (*n*=3)		**FGA (g) (*n*=2)**		**FBrGA (h) (*n*=3)**		**FBrXGPec (i) (*n*=3)**		**FBrXGGAPec (j) (*n*=3)**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PV (cp)	2390.67^{a,b,c,d,g,h,j}	54.01	955.50^{a,e,f,ı}	12.50	710.00^{a,c,d,e,f,ı,j}	10.15	2218.00^{c,d,g,h,j}	30.41	1455.00^{e,f,h,ı}	34.27
TV (cp)	1487.00^{a,b,c,d,e,g,h,j}	66.53	747.00^{c,e,f,i,j}	17.00	504.33^{a,c,d,e,f,i}	10.67	1644.00^{a,b,c,d,e,f,g,h,j}	56.89	1137.00^{b,d,e,f,g,h,i}	37.99
BD (cp)	903.67^{g,h,j}	33.65	208.50^{f}	4.50	205.67^{f}	1.86	574.00	86.64	318.00^{f}	24.17
FV (cp)	2929.33^{a,b,c,d,e,g,h,i,j}	29.36	1437.00^{a,c,e,f,i}	27.00	1070.33^{a,c,d,e,f,i,j}	10.91	2462.67^{b,d,e,f,g,h,j}	23.73	1699.00^{b,e,f,h,i}	52.46
SB (cp)	1442.33^{a,b,c,d,g,h,i,j}	78.19	690.00^{c,e,f,g}	10.00	566.00^{a,c,d,e,f}	2.31	818.67^{e,f}	34.64	562.00^{a,c,d,e,f}	19.47
PTi (min)	5.22	0.05	5.67	0.00	5.49	0.02	5.60	0.04	5.84	0.06
PT (°C)	50.38^{a,b,c,d,g,h}	0.16	89.18^{e,f,i,j}	0.42	88.80^{e,f,i,j}	0.43	60.95^{b,d,g,h}	2.65	61.38^{b,d,g,h}	5.10

Results are displayed in rows. The letters in superscript denote the samples in comparison to which the value is statistically significant and the symbol of the sample is given in brackets (*p* < 0.05). The number of replications for each sample given in brackets.

Key: PV – peak viscosity, TV – trough viscosity, BD – breakdown, FV – final viscosity, SB – setback, PTi – time to peak, PT – pasting temperature.

3.3.5. Physico-chemical analysis of the properties of breads

Table 19. Nutrient analysis of sourdough breads enriched with soluble fibre; all values are means, fibre was determined in duplicate, protein in triplicate

	Control (a)		Control + bran (b)		GA and bran (c)		XG /Pec and bran (d)		XG/GA/Pec and bran (e)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total fibre (g/100g)	2.73[c,d,e]	0.01	7.94	1.28	11.98[a]	0.23	12.25[a]	1.34	11.92[a]	0.20
Insoluble fibre (g/100g)	0.99[c,d,e]	0.08	4.10	0.32	4.91[a]	0.75	5.52[a]	0.62	5.13[a]	0.34
Soluble fibre (g/100g)	0.30[c,d,e]	0.11	0.69[c,d,e]	0.35	7.43[a,b,d]	0.47	4.52[a,b,c]	0.97	6.16[a,b]	0.03
Protein content (g/100g)	11.88[c,d,e]	0.34	11.42[c,d,e]	0.09	10.25[a,b]	0.37	9.99[a,b]	0.26	10.21[a,b]	0.03

Results are displayed in rows. Letters in superscript denote the samples in comparison to which the value is statistically significantly different ($p < 0.05$). The symbol of the sample is given in brackets. All values are means obtained from two replications, except for protein ($n = 3$).

Table 20. Physico-chemical assessment of the properties of sourdough breads enriched with soluble fibre; all values are mean from n=3 determinations

	Control (a)		Control + bran (b)		GA and bran (c)		XG/Pec and bran (d)		XG/GA/Pec and bran (e)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Specific volume (cm³/g)	2.86[b,d,e]	0.02	2.45[a,d,e]	0.01	2.70[d,e]	0.01	2.04[a,b,c]	0.08	1.92[a,b,c]	0.07
Crumb firmness (g)	932[e]	55	1142[c]	89	688[b,d,e]	36	1147[c]	112	1371[a,c]	87
Slice area (mm²)	7020[b,c,d,e]	179	6235[a,c]	109	5253[a,b,d]	201	6088[a,c]	87	5773[a]	100
Slice brightness	128[b,c,d,e]	1.0	87[a,c]	0.8	107[a,b,d,e]	0.6	88[a,c]	0.5	89[a,c]	1.2
Number of cells (1/slice)	4316[b,c]	81	3812[a,c,d]	69	2750[a,b,d,e]	8	4360[b,c]	80	4021[c]	154
Area of cells (% of slice area)	49.00[d,e]	0.1	49.20[d,e]	0.21	49.47[d,e]	0.53	46.70[a,b,c]	0.4	46.80[a,b,c]	0.36
Cell diameter (mm)	1.94[c]	0.02	1.88[c]	0.05	2.60[a,b,d,e]	0.15	1.62[c]	0.07	1.60[c]	0.08

Results are displayed in rows. Letters in superscript denote the samples in comparison to which the value is statistically significantly different ($p < 0.05$). The symbol of the sample is given in brackets. All values are means obtained from three replications.

The results of nutrients analysis are presented in summary Table 19 and physico-chemical assessment of sourdough breads enriched with soluble fibres is presented in summary Table 20.

3.3.6. Moisture content of breads

The moisture content of sourdough breads obtained in the course of the current study is presented in Figure 19. The moisture content of all the breads enriched with soluble fibre was significantly different ($p \leq 0.001$) from the both control breads. The breads XG/Pec/bran and XG/GA/Pec/bran had the highest moisture content which reflected high content of water in the dough. However, bread containing gum arabic and wheat bran, was characterized by lower moisture content than both control breads. This difference was also statistically significant ($p \leq 0.001$).

*** - $p \leq 0.001$ in comparison to control sourdough bread

Error bars: +/- 1 SE
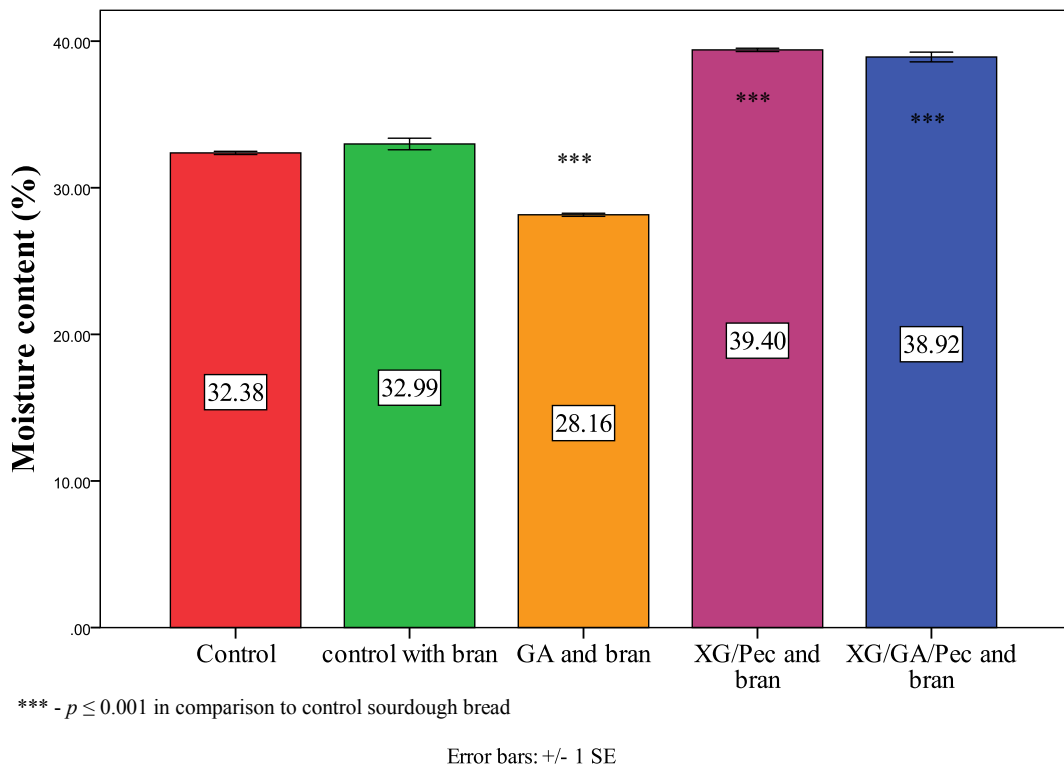
Figure 19. Moisture content of breads enriched with soluble and/or insoluble fibre; all values are means from three replications

3.3.7. Fibre determination

The content of fibre of the sourdough breads enriched with soluble fibres was shown in the summary Table 19. The graphic representation of these results is shown in Figure 20. ANOVA revealed that the addition of soluble fibre source (10% based on

flour) and insoluble fibre significantly ($p \leq 0.001$) increased the soluble, insoluble and overall fibre content of sourdough breads GA, XG/Pec and XG/GA/Pec when compared to the control sourdoughs. Addition of bran to control sourdough bread increased its content of insoluble and total fibre, although the increase was not statistically significant ($p = 0.061$ and $p = 0.071$, respectively).

Error bars: +/- 1 SE
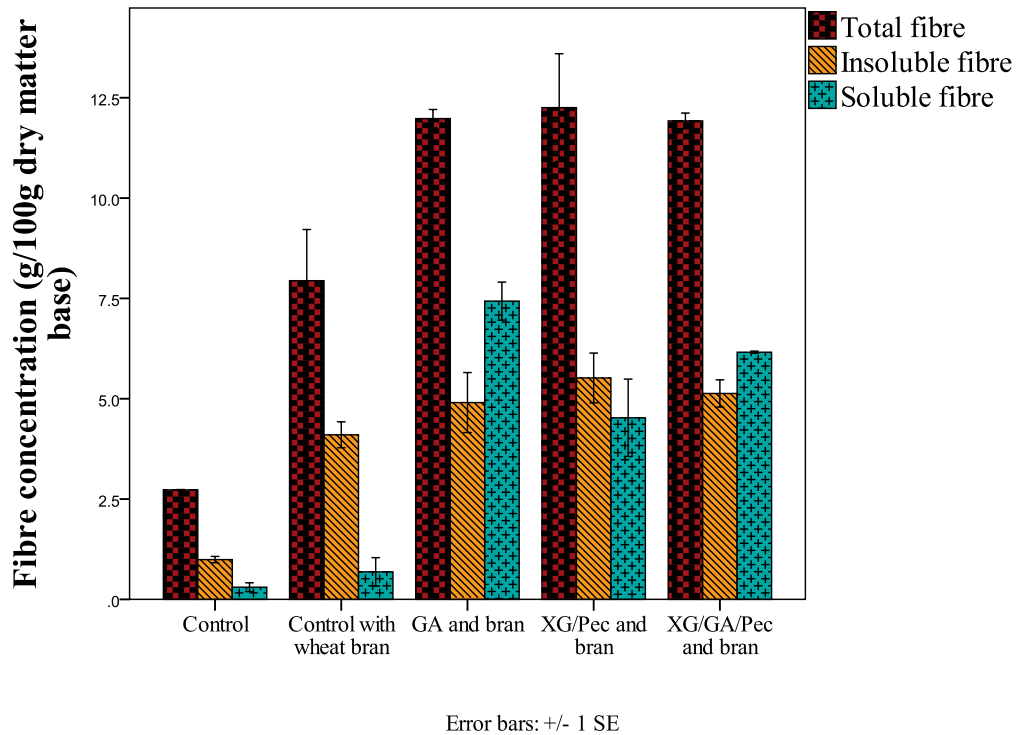
Figure 20. Content of total, soluble and insoluble fibre of sourdough breads; all values are means from two replications

3.3.8. Specific volume

Figure 21 shows that the mean values of specific volumes of breads enriched with soluble and/or insoluble fibre are lower than the mean value of specific volume of control sourdough. The difference of mean specific volume of sourdough control bread with added bran was significant statistically ($p \leq 0.05$). The differences for breads enriched with XG and Pec and XG, GA and Pec, were significant ($p \leq 0.001$). The result for bread enriched with GA and bran was not statistically significant. The bread with the lowest specific volume was sourdough bread obtained with combination of XG, GA and Pec.

93
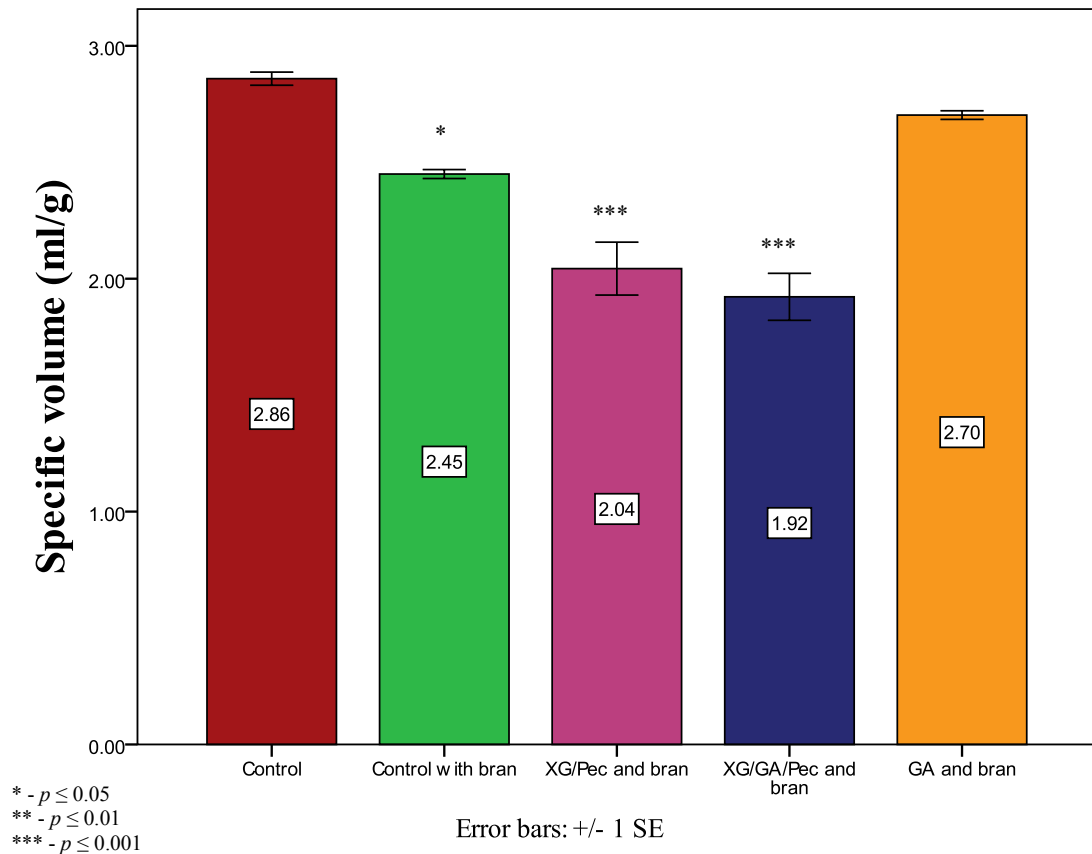
Figure 21. Specific volume of sourdough breads enriched with soluble fibres; all values are means from three replications

3.3.9. Water activity and shelf-life

The results of water activity (a_w) measurements are presented below. No significant differences were identified between the five types of sourdough breads enriched with soluble fibre. During storage the breads were observed and no visual signs of bacterial (ropiness) or fungal (mould growth) spoilage were noticed. The change of water activity of the crust and the crumb during the storage of the breads obtained in the course of this experiment is presented in Figures 22 and 23. Only for control sourdough breads, both without and with added wheat bran, were the changes of a_w within the crusts significant statistically ($p \leq 0.001$) and increased during storage. For sourdough breads enriched with mixtures of hydrocolloids and wheat bran these changes were insignificant ($p > 0.05$). The changes of water activity of the bread crumb during storage were insignificant. The smallest changes were observed in the crumb of breads containing XG, Pec and wheat bran, and XG, GA, Pec and wheat bran.

Error bars: +/- 1 SE
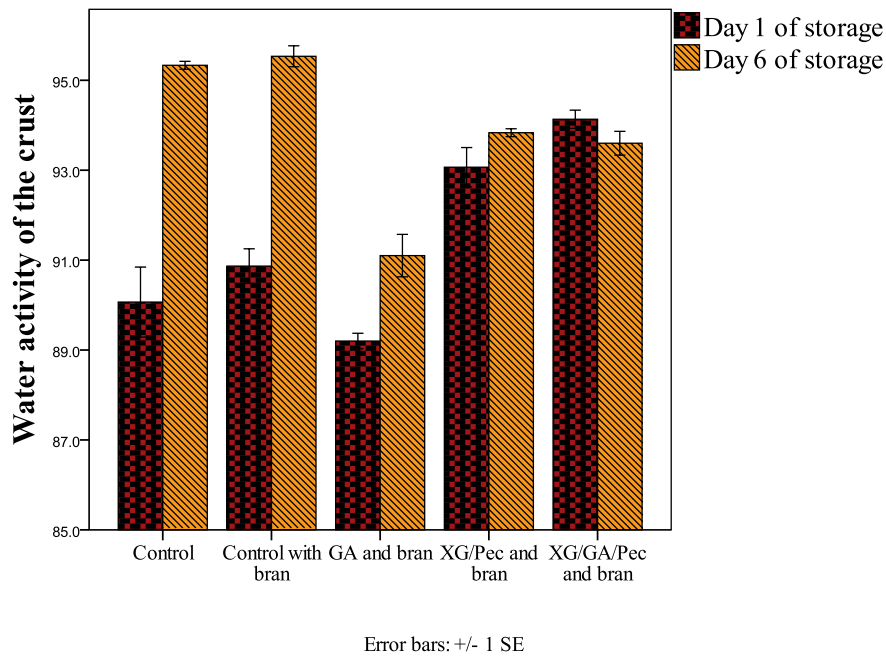
Figure 22. Water activity of crust of sourdough breads enriched with soluble fibres after 24 and 144 hours of storage. All values are means from three replications

Error bars: +/- 1 SE
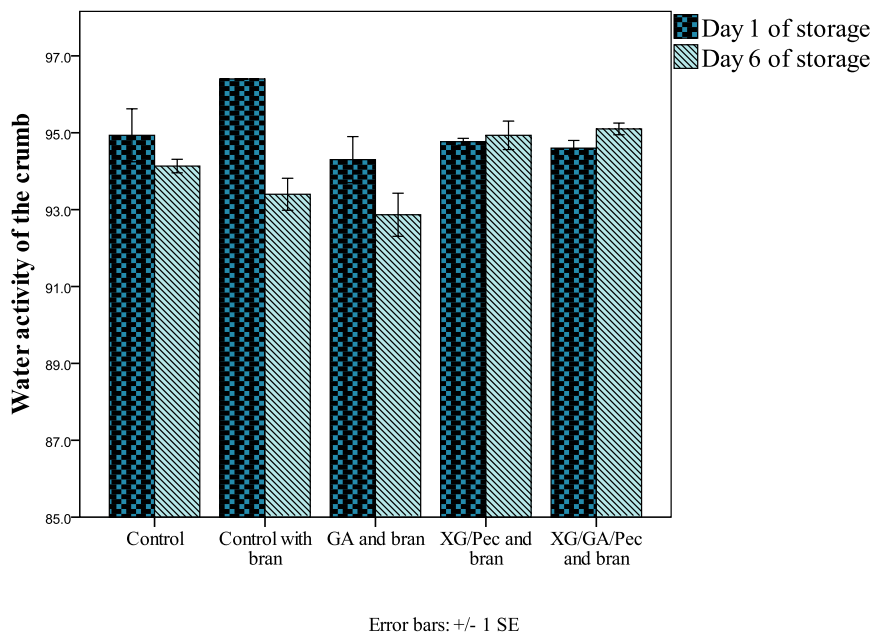
Figure 23. Water activity of the crumb of sourdough breads enriched with soluble fibres after 24h and 144h of storage. All values are means from three replications

3.3.10. Texture analysis and shelf-life: crumb firmness determination

The chart below summarises the crumb firmness of sourdough breads enriched with soluble fibres. Figure 24 shows that the only mean value of crumb firmness that was

significantly different ($p \leq 0.05$) from the mean crumb firmness of control sourdough bread, was the one of bread supplemented with xanthan gum, gum arabic, pectin SF 530 and wheat bran. However, Table 20 (p. 91) reveals more statistically significant differences.
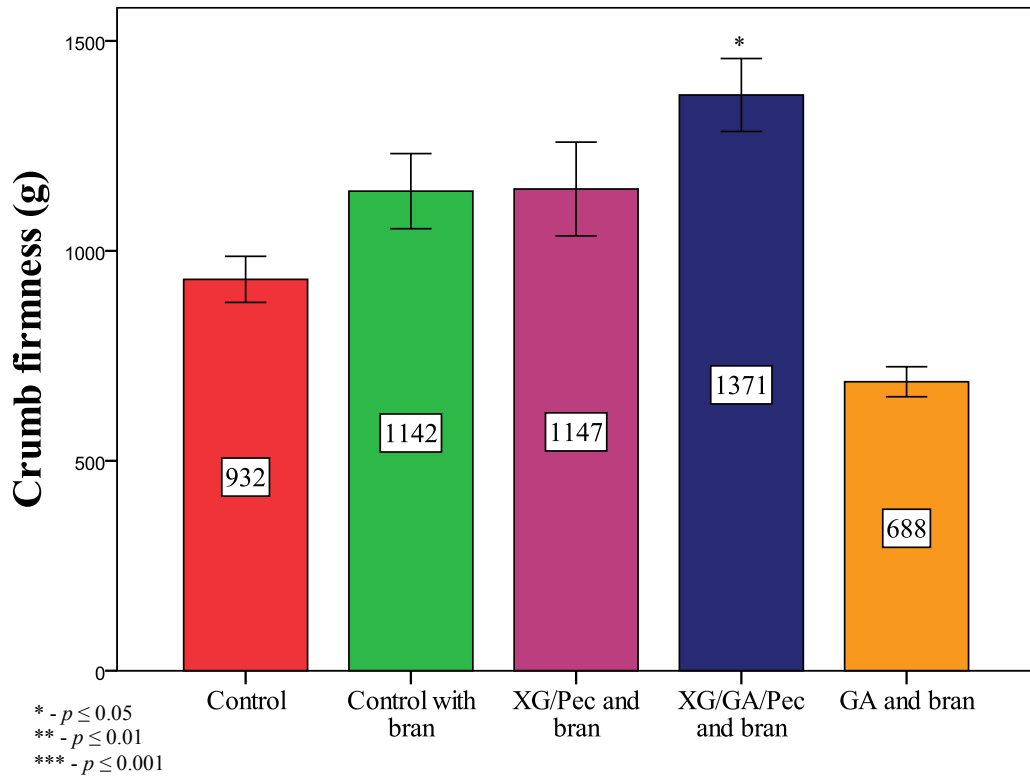
Figure 24. Crumb firmness (expressed as force) of sourdough breads enriched with soluble fibres; all values are means from three replications

ANOVA revealed that the mean value of crumb firmness of bread with gum arabic and wheat bran is significantly lower than the mean values of crumb firmness of control sourdough bread with added wheat bran ($p \leq 0.05$), sourdough bread with XG, Pec and wheat bran ($p \leq 0.05$), and sourdough bread with XG, GA, Pec and wheat bran ($p \leq 0.001$).

Despite the lack of significance of the difference between the mean values of crumb firmness of control sourdough bread and the bread with gum arabic and wheat bran, the latter had softer crumb than bread made with any other combination of soluble and/or insoluble fibre.

The profiles of staling of sourdough breads enriched with soluble fibres are presented in Figure 25. The results were obtained by the measurement of bread crumb firmness over the period of 6 days of storage of the bread in normal conditions, with the use of texture analyser (Stable Micro Systems, UK). The figure has been procured with the use of SPPS software.
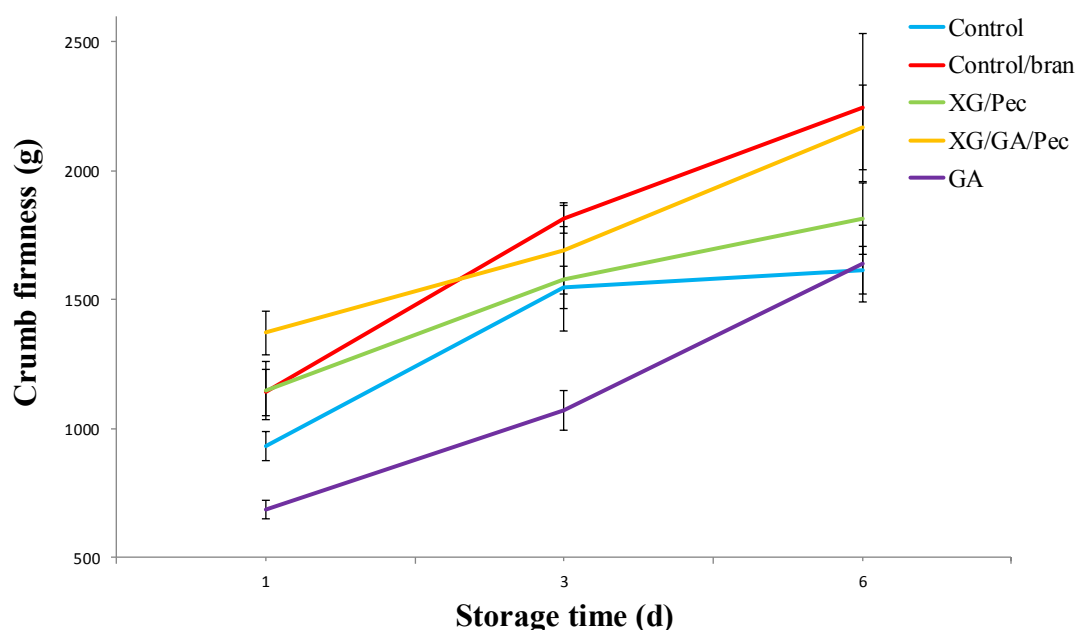
Figure 25. Change of crumb firmness of sourdough breads enriched with soluble fibres during 6 days of storage; all values are means from three determinations

ANOVA showed that after 3 day storage only crumb of the bread with gum arabic and bran was significantly softer than control with added bran, $p \leq 0.05$. Values of the same parameter for the rest of the breads were not significantly different from the control sourdough bread. The analysis of results showed also that after 6 days of storage, the differences in mean values of crumb firmness of sourdough breads enriched with soluble and/or insoluble fibre as compared to control sourdough bread bore no statistical significance.

3.3.11. C-cell analysis of the crumb porosity

The analysis of porosity of bread crumb was performed with the use of C-cell porosity analyser (Calibre Control International Ltd., UK). Only selected few parameters were considered summarised in Table 20 (p. 91) (slice area, crumb

brightness, cell number, % of slice area that cells constitute, cell diameter) are discussed below.
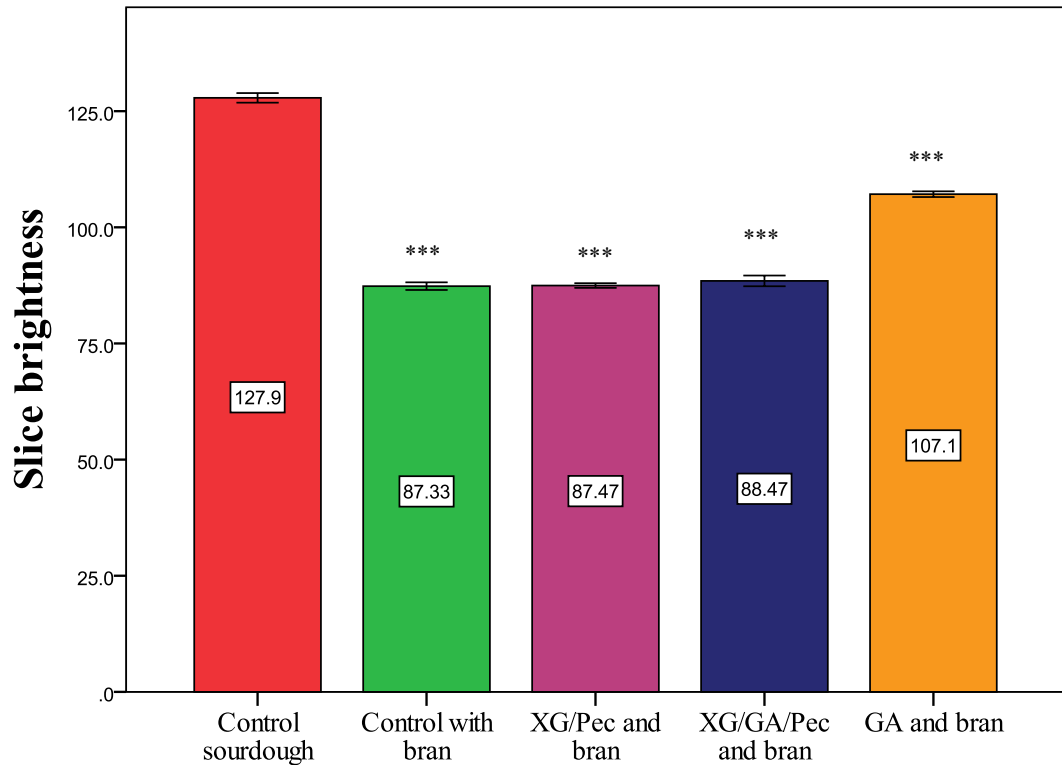
Error bars: +/- 1 SE

Figure 26. Brightness of the crumb of sourdough breads enriched with soluble fibres; all values are means from three determinations

Statistical analysis of C-cell results revealed that addition of fibre caused reduction of bread crumb brightness. The changes observed were statistically significant ($p \leq$ 0.001) for every combination of soluble and/or insoluble fibre added.

The number of gas cells, the area of the slice covered by gas cells and average cell diameter results provide more information on crumb structure. For bread with gum arabic and wheat bran the number of gas cells was significantly ($p \leq 0.001$) lower than for control sourdough bread. Because of the very soft bread crumb, the crumb was subject to damage during the slicing (Figure 27 e). Hence the C-cell results for bread containing GA and bran may be highly inaccurate.
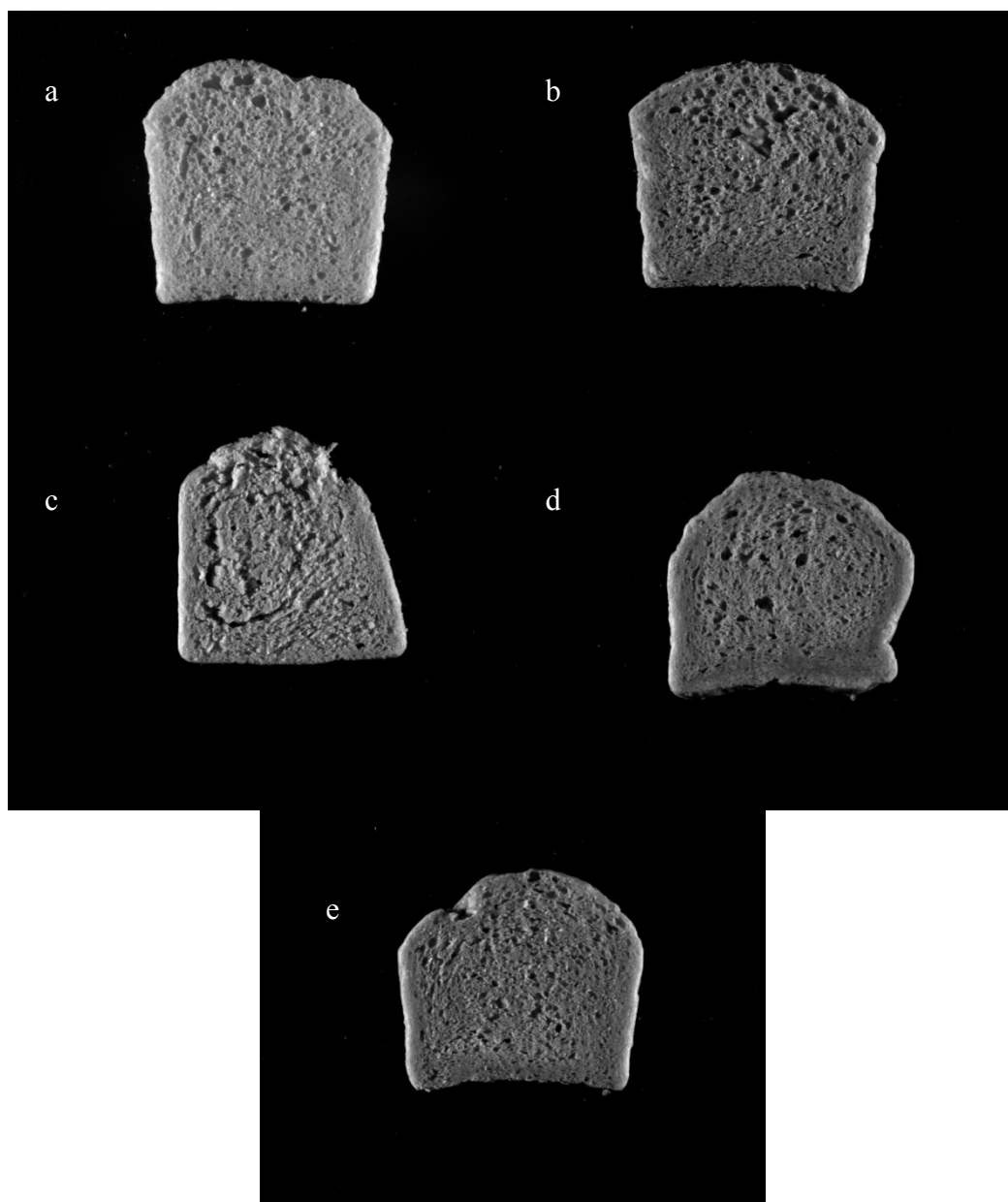
Figure 27. C-cell images sourdough breads. a - control sourdough bread, b - control with wheat bran, c - GA and bran, d - XG/Pec and bran, e - XG/GA/Pec

Mean slice area was influenced by all added mixtures of fibre. The differences between the average values for sourdough breads enriched with soluble and/or insoluble fibre were statistically significant in when compared to control sourdough bread ($p \leq 0.05$).

3.3.12. Fat, total carbohydrate and ash content

In this section the results of fat content, total carbohydrate and ash analysis are presented jointly. These assays were performed for both control breads and sourdough XG/GA/Pec selected for further analysis after the sensory panel of consumer acceptability. The results are presented in Table 21.

Table 21. Fat, total carbohydrate and ash content of sourdough breads

	Control sourdough bread		Control with bran		XG/GA/Pec and bran	
	Mean	SE	Mean	SE	Mean	SE
Fat content (g/100g dry matter)	0.39[b]	0.02	0.78[a,c]	0.04	0.46[b]	0.02
Fat content (g/100g wb)	0.57[b]	0.03	1.16[a,c]	0.06	0.75[b]	0.03
Total carbohydrate (g/100g)	55.19[c]	1.07	52.86[c]	0.90	46.83[a,b]	0.62
Ash (g/100g)	1.87[b]	0.02	2.07[a,c]	0.02	1.96[b]	0.03

Data presented in rows. Values with different superscripts are statistically different ($p < 0.05$). All values are means from three determinations

The sourdough bread enriched with wheat bran had statistically increased fat content in comparison to the two remaining breads ($p \leq 0.001$ for the results expressed as a percentage of dry matter and $p \leq 0.01$ for the results expressed as a percentage of wet matter).

The calibration curve equation used for calculation of total carbohydrates as glucose was:

$$y = 7.914x - 0.0129;$$

where y is the concentration of carbohydrates in the sample, x is the absorbance of the sample. Value of -0.0129 is the value of blank sample and 7.914 is the slope of the calibration curve. Sourdough bread enriched with soluble fibre mixture and bran had lower total carbohydrate content than both control sourdough breads. The results are statistically significant at $p \leq 0.01$ for sourdough bread with wheat bran and $p \leq 0.001$ for control sourdough bread.

The sourdough bread enriched with wheat bran only had significantly higher ash content than the controls. The results were statistically significant on level $p \leq 0.01$ for control sourdough bread and $p \leq 0.05$ for sourdough bread enriched with the mixture of soluble fibres and wheat bran.

3.3.13. Starch

The content of starch in sourdough breads obtained in the course of this project was determined with the use of Resistant Starch kit (Megazyme, Ireland) as described in

section 3.2.15. The RS kit allows the analysis of the content of total starch through determination of 'available' (i.e. digestible) starch and resistant (i.e. non-digestible) starch. The results of starch determination have been supplemented with the result of moisture content (Appendix 7) determined in parallel and enabling the presentation of the results of starch content as a percentage of dry matter and based on wet matter ('as eaten').

3.3.13.1.   *Available starch*

Figure 28 shows the content of available (potentially digestible) starch of sourdough breads enriched with soluble fibre. The content of available starch of the considered breads was as follows: 44.42 g/100g for control sourdough bread; 39.07 g/100g for control with wheat bran; 33.85 g/100g for bread XG/GA/Pec and bran; and 47.03 g/100g for WWB ('as eaten').

Analysis of variance of the results showed that sourdough bread with added soluble fibre had significantly ($p \leq 001$) lower content of digestible starch than both control breads. The mean difference was greater when bread high in soluble fibre was compared to control sourdough bread without added bran. All three sourdough breads had significantly ($p \leq 0.001$) lower content of available starch in comparison to white wheat bread. The mean difference was the greatest when compared to sourdough bread enriched with wheat bran and soluble fibre mixture.
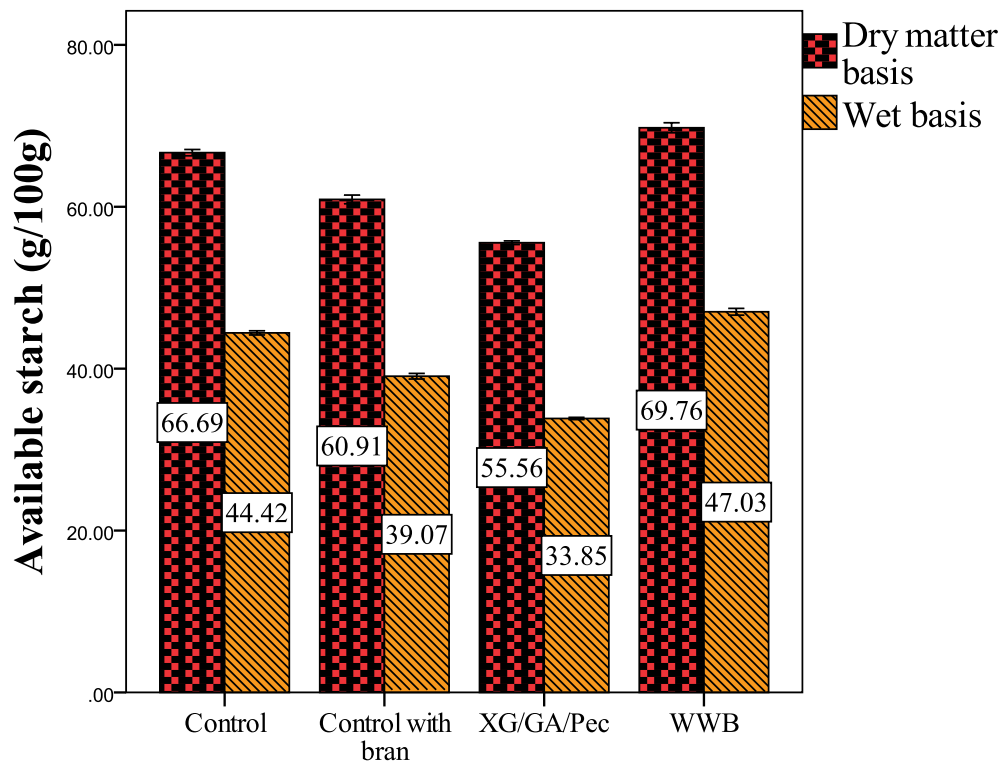
Error bars: +/- 1 SE

Figure 28. The content of 'available' starch in sourdough breads enriched with soluble fibre. All values are means from four determinations

3.3.13.2. *Resistant starch*

On Figure 29 the results of determination of resistant (non-digestible) starch are presented. The content of RS was 0.69 g/100g for control sourdough; 0.67 g/100g for control with bran; 0.47 g/100g for bread XG/GA/Pec/bran; and 0.58 g/100g for white wheat bread.

ANOVA procedure did not find a statistically significant difference between the resistant starch content of control bread and of control bread enriched with wheat bran. However, there was a statistically significant difference between both controls and sourdough bread enriched with wheat bran and soluble fibres as well as the white wheat bread. Sourdough bread enriched with soluble fibre and wheat bran contained less resistant starch ($p \leq 0.001$) than both control breads. Additionally, the same type of bread had significantly lower ($p \leq 0.01$) resistant starch content than commercially available white wheat bread. Resistant starch content of white wheat bread was significantly lower than that of both control breads ($p \leq 0.01$ for control bread and $p \leq 0.05$ for control with wheat bran). When expressed as a percentage of

the total starch content, the content of RS was a follows: 1.56% for control sourdough bread; 1.70% for control with wheat bran; 1.37% for bread XG/GA/Pec/bran; and 1.21% for WWB.
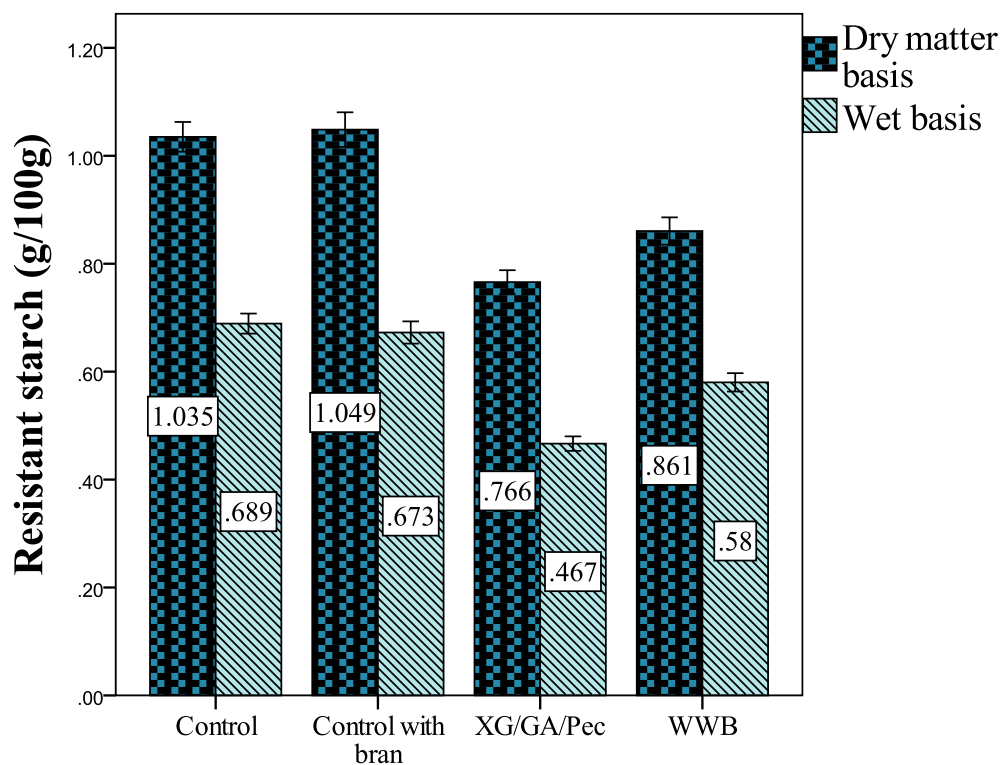
Error bars: +/- 1 SE

Figure 29. The content of resistant starch in sourdough breads enriched with soluble fibres; all values are means from four determinations

3.3.14. Lactic acid and ethanol by HPLC

Organic acids and ethanol were extracted according to modified procedure of Lefebvre et al. (2002) as described in section 3.2.11. The results are presented separately for lactic acid (section 3.3.14.1) and ethanol (3.3.14.2). Results obtained at each of the considered stages of the fermentation process were subject to ANOVA using SPSS software. Figure 30 shows a chromatogram of a mixture of acids (lactic, acetic, propionic, butyric acid) and ethanol All the results are presented in Figures 32 and 33. Table 22 gives the details of the concentrations obtained with the use of HPLC. On chromatograms, only peaks of lactic acid and ethanol were observed (Figure 31).
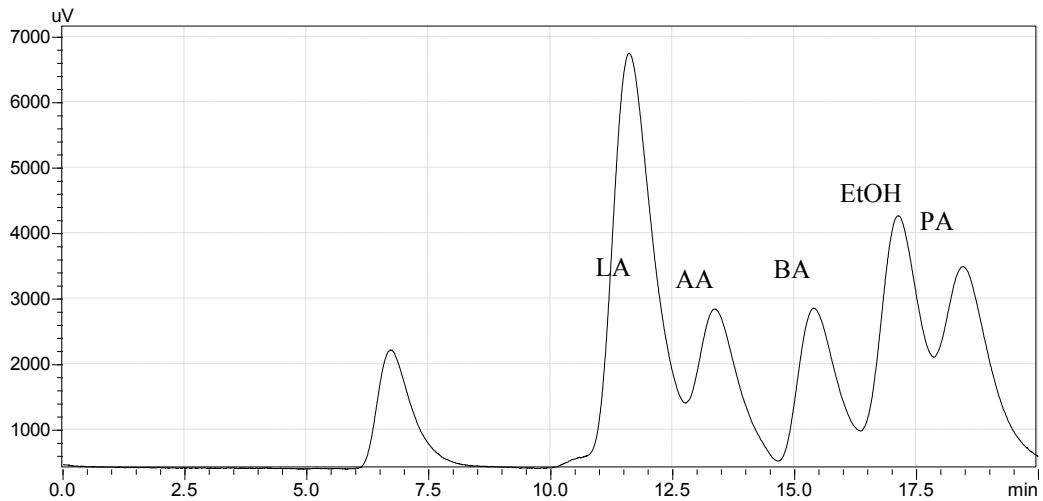
Figure 30. Chromatogram of a mixture of lactic acid (LA), acetic acid (AA), butyric acid (BA), ethanol (EtOH) and propionic acid (PA)
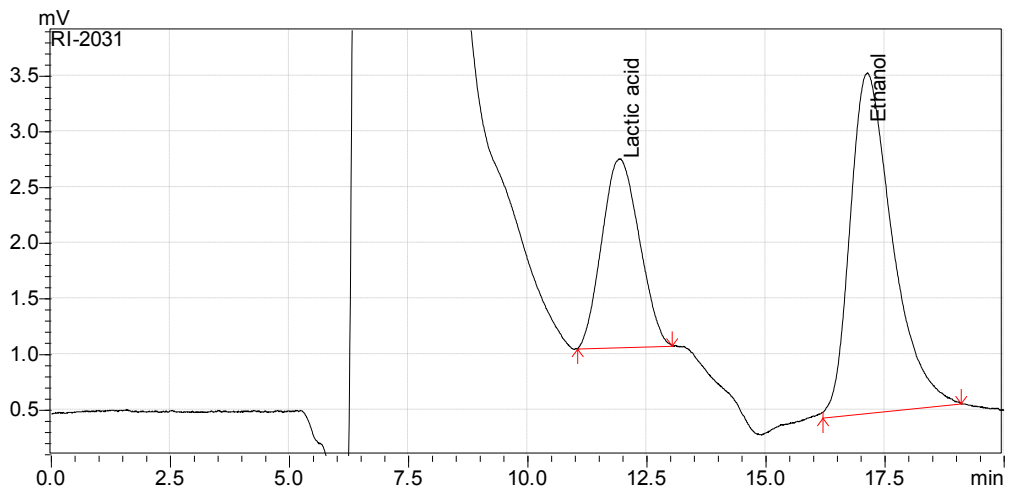
Figure 31. An example of a chromatogram of a sourdough extract (source: primary research)

3.3.14.1. *Lactic acid in sourdough during fermentation*

Figure 32 shows the concentrations of lactic acid in sourdoughs during the 4 hour fermentation of the sourdoughs. The increase in the concentration of lactic acid was observed in all the doughs. The initial content of lactic acid in sourdoughs was 0.0812 g/100g, for control sourdough, 0.1296 g/100g for control with wheat bran and 0.1126 g/100g for sourdough enriched with soluble fibres. Final lactic acid concentrations were 0.2487 g/100g for control sourdough, 0.2613 g/100g for control with wheat bran and 0.2581 g/100g for sourdough enriched with soluble fibres.
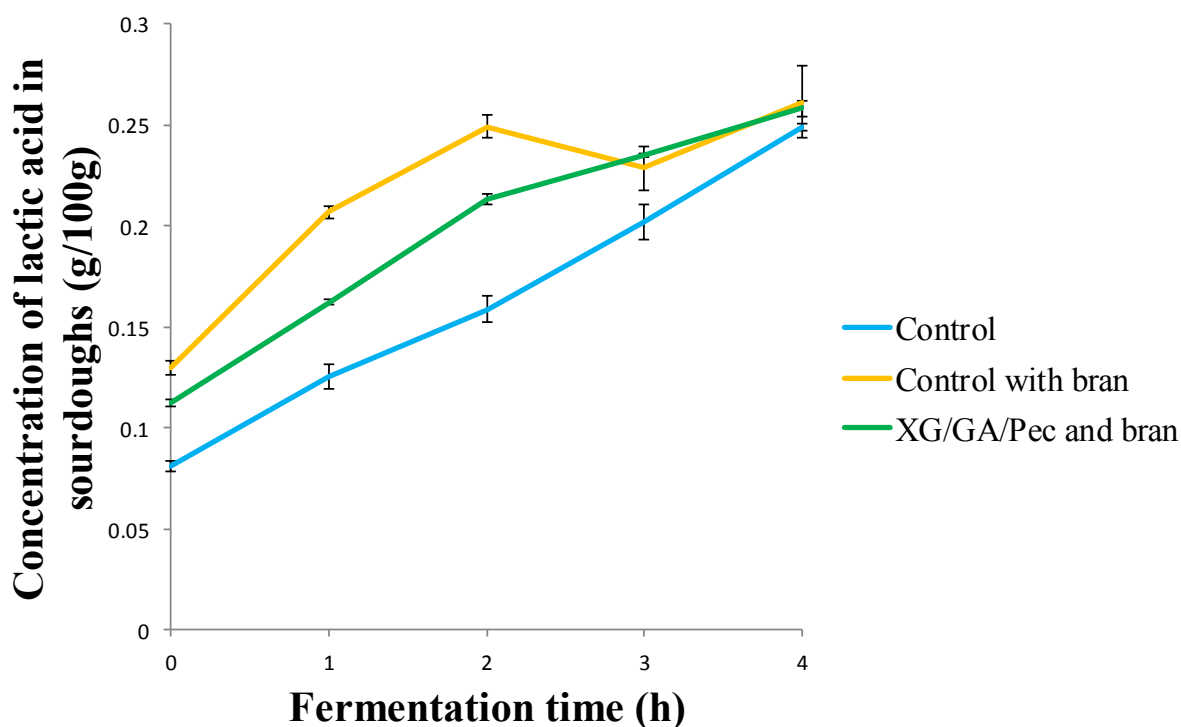
Figure 32. Concentration of lactic acid in sourdoughs during 4 hour fermentation; error bars +/- 1 SE; means from four determinations

Results of HPLC lactic acid determination were subject to ANOVA procedure. The increase of concentration of lactic acid was statistically significant ($p < 0.05$) at every stage of the fermentation in case of control sourdough and sourdough enriched with soluble fibre.

3.3.14.2. *Ethanol in sourdough during fermentation*

The changes of concentration of ethanol in sourdoughs during 4 hour fermentation are shown on Figure 33. The changes in ethanol concentration observed throughout the process were statistically significant ($p < 0.05$) at each stage of the process.

The initial concentrations of ethanol in sourdoughs were 0.0558 g/100g for control sourdough, 0.0539 g/100g for control with bran, and 0.0756 g/100g for the sourdough enriched with soluble fibres. The final ethanol concentrations were 0.4718 g/100g for control sourdough, 0.5745 g/100g for control with wheat bran, and 0.5418 g/100g for sourdough enriched with soluble fibres.
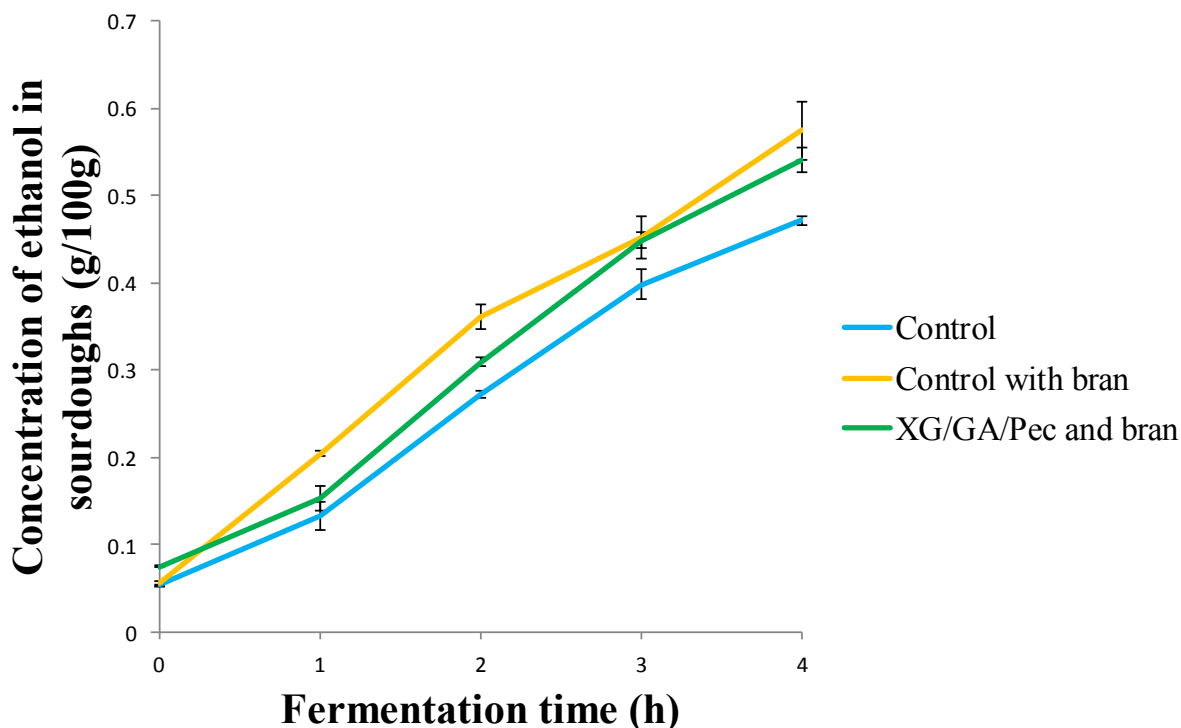
Figure 33. Concentration of ethanol in sourdoughs during 4 hour fermentation; error bars +/- 1 SE; means from *n* = 4 determinations

Table 22. Concentrations of lactic acid and ethanol during fermentation in sourdoughs

		Concentration of LA in sourdough (g/100g)					
		Control		**Control with bran**		**XG/GA/Pec and bran**	
		Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Fermentation time (hours)	**0**	0.0812	0.0025	0.1296	0.0032	0.1126	0.0018
	1	0.1253	0.0059	0.2068	0.0029	0.1622	0.0016
	2	0.1588	0.0064	0.2493	0.0054	0.2133	0.0026
	3	0.2018	0.0084	0.2285	0.0106	0.2349	0.0011
	4	0.2487	0.0018	0.2613	0.0177	0.2581	0.0042

		Concentration of EtOH in sourdoughs (g/100g)					
		Control		**Control with bran**		**XG/GA/Pec and bran**	
		Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Fermentation time (hours)	**0**	0.0539	0.0012	0.0558	0.0035	0.0756	0.0005
	1	0.1333	0.0165	0.2049	0.0029	0.1534	0.0141
	2	0.2718	0.0041	0.3619	0.0143	0.3097	0.0054
	3	0.3986	0.0170	0.4520	0.0249	0.4492	0.0090
	4	0.4718	0.0051	0.5745	0.0336	0.5418	0.0139

Changes of LA and EtOH concentrations presented in columns were at all points of fermentation statistically significant ($p < 0.05$). Pairwise comparisons between different types of doughs were not performed

3.3.14.3. *Correlation between the concentration of lactic acid and pH of sourdoughs*

The analysis of the results obtained through the use of HPLC in conjunction with the results of pH measured in the sourdoughs at the product development stage showed correlation between the lactic acid concentration in sourdough and its pH. The results of the Pearson's correlation analysis performed with the use of SPSS 19.0 (IBM) software, are presented in Table 23.

As showed in Table 23, for every kind of sourdough considered, a strong negative and statistically significant correlation between lactic acid concentration and pH was identified. The R^2 values were 0.808, 0.749 and 0.880 for control sourdough, control sourdough with wheat bran and sourdough enriched in soluble fibres.

Table 23. Pearson correlation between lactic acid concentration and pH of sourdoughs enriched with soluble and/or insoluble fibre

	R	*N*	*P*	R^2
Control	-0.899	15	≤ 0.001	0.808
Control with bran	-0.865	15	≤ 0.001	0.749
XG/GA/Pec	-0.938	15	≤ 0.001	0.880

3.3.15. Organic acids and ethanol in breads

The results of HPLC analysis of the content of lactic acid and ethanol in sourdough breads are presented in Figure 34. Two separate statistical analyses were performed. Mann-Whitney U Test was used to determine whether there was a difference in concentration of lactic acid and ethanol between the samples extracted in a single mode and double mode. The test was run separately for each type of bread considered. The second analysis was ANOVA to compare the concentrations of lactic acid in the sourdough breads.
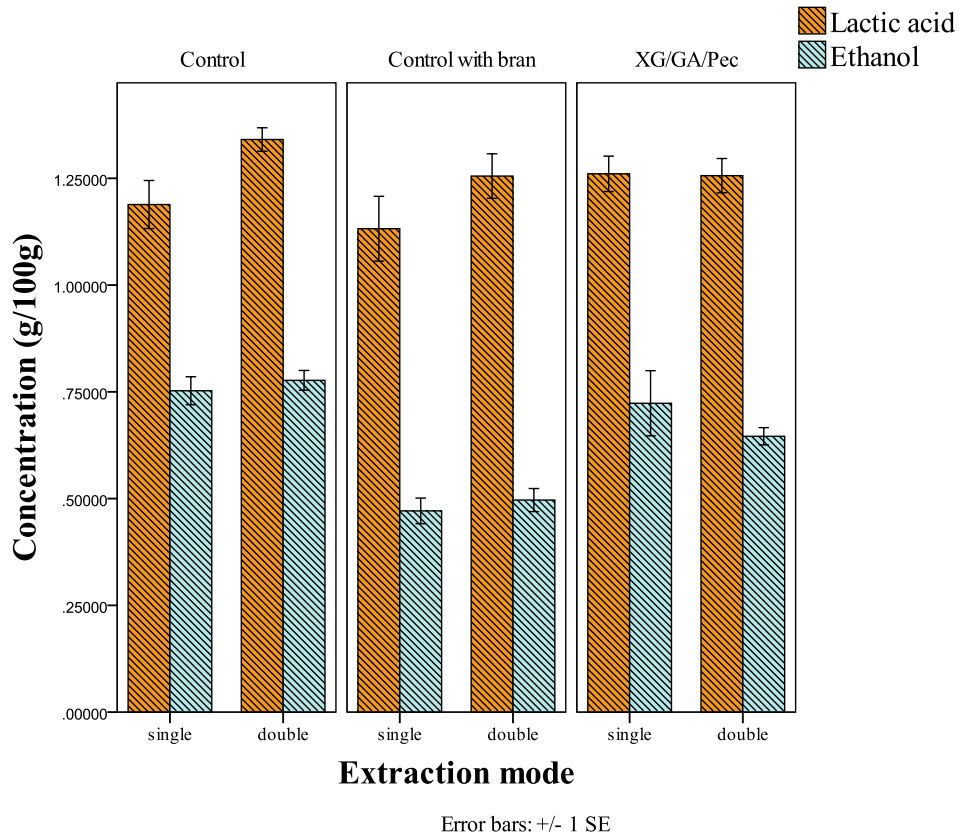
Figure 34. Concentrations of lactic acid and ethanol in sourdough breads; all values are means from two replications

Mann-Whitney U Test did not show statistically significant differences ($p > 0.05$) between the concentrations of lactic acid and ethanol obtained by single and double extraction. On this premise, it was concluded that there was no difference between the two methods of extraction therefore in future extraction could be performed in one stage process. Through ANOVA procedure, it was found that no statistical differences between the concentrations of lactic acid and ethanol in sourdough breads were present ($p > 0.05$). The mode of extraction did not influence this finding.

3.4. Calculation of GI test sample size

Table 24 shows the calculations of the size of bread samples containing 50 g of available carbohydrate in sourdough breads and commercially available white sliced bread. These values were obtained by deducting the concentrations of total dietary fibre and resistant starch from total carbohydrate content. The results shown in Table 24 were chosen as a source of information to be used for the purpose of GI trial.

Table 24. Calculation of available carbohydrate of sourdough breads based on deduction of total dietary fibre and resistant starch from total carbohydrate

Nutrients' content (g/100g 'as eaten')	Bread type		
	Control sourdough	Fibre mix (XG/GA/Pec)	White wheat bread
Total carbohydrate	55.19	46.83	48.60
Total fibre	1.80	7.22	3.20
Resistant starch	0.69	0.46	0.58
Available carbohydrate	52.70	39.15	44.82
Amount of bread containing 50g of available carbohydrate	**94.87**	**127.71**	**111.55**

3.5. Summary

In chapter 3 was presented a broad scope of methods used in data collection in this PhD study and results obtained using these methods. Five breads were developed and initially assessed for their physico-chemical properties. Following the product development stage the content of the key nutrients was analysed. Subsequently, the breads were analysed for their consumer acceptability in order to select one sourdough enriched with soluble fibres for the analysis of its nutritional aspects – glycaemic response and satiety, and other nutrients content. Additionally, in the selected sourdough fortified with fibre as well as in the control sourdoughs the content of lactic acid and ethanol was analysed using HPLC.

Sourdough fermentation resulted in the change of the pH of the doughs. This change was correlated to the increased stickiness of sourdoughs without added fibre. However, addition of soluble fibres reduced the stickiness of sourdoughs. Addition of hydrocolloids resulted in altered pasting properties of the flour-water mixtures. Addition of hydrocolloids' mixtures with bran to the dough enabled significant increase in total, insoluble and soluble dietary fibre content of sourdough breads. The negative influence of fibre on the bread properties of the breads was observed. The crumb firmness of all of the breads increased with storage. However, the water activity measurement pointed that hydrocolloids' presence retarded the migration of water from the breads' crumb to crust. Chapter 4 presents methodology and results of consumer acceptability test.

Chapter 4 Consumer acceptability evaluation

4.1. Sensory evaluation – an overview

Sensory evaluation and the description of modified or novel products is one of the key functions within the food industry (Stone and Sidel, 1993). Sensory analysis can provide an insight into the characteristics and acceptability of a new food product. By definition, sensory evaluation is:

> "a scientific method used to evoke, measure, analyse and interpret the responses to products as perceived through the senses of sight, smell, touch, taste, and hearing" (Lawless and Heymann, 1999, p.2).

Lawless and Heymann (1999) expanded on the above definition, in which the reference to "evoke, measure, analyse and interpret" are of crucial meaning. First, as a science, sensory evaluation gives and follows guidance for preparation and presentation of evaluated samples in the way which could minimise the panellists' bias ("to evoke"). Secondly, sensory evaluation aims to collect numerical data with the use of methods based on observation and quantification of human behaviour ("to measure"). The third aspect of sensory evaluation is the analysis of collected data with the use of appropriate statistical tests coupled with good quality of experimental design ("to analyse"). Good interpretation of the results contextualised in background knowledge and tested hypotheses, and supported with a critical discussion is the last process of the sensory evaluation ("to interpret") (Lawless and Heymann, 1999). These aspetcs of good practice were embedded within the present study as described in the following sub-sections.

4.1.1. Minimising panellists bias – "to evoke"

Lawless and Heymann (1999) repeat an axiom of perceptual psychology that:

"human beings are very poor absolute measuring instruments but are
very good at comparing things" (Lawless and Heymann, 1999, p.301).

According to the above statement, a question can be raised as to the nature of the reference material against which the selected sensory attribute is judged. Lawless and Heymann (1999) argue that based on their experience, humans establish reference for everyday items. Additionally, there are contextual (background) factors affecting perception. Bias, as defined by Lawless and Heymann (1999), is a process in which a change in response occurs. However, bias in sensory practices has a negative influence only if there is a way of judging known to be more accurate than others. Bias can be controlled for or eliminated if the conditions causing it are predicted and understood.

4.1.2. Line-marking scales – "to measure"

The data collection in sensory evaluation usually takes place through specifically constructed questionnaires. These questionnaires are based on scaling, i.e. an approach aiming to translate the sensory experience of a panellist into numbers (Lawless and Heymann, 1999). The fundament of scaling is the psychophysical model which assumes that a sensory sensation can be reported in a quantitative way by the person who perceives this sensation (Lawless and Heymann, 1999, Lawless, 2005). In case of the line-marking scales, the scale is expressed as a horizontal line, across which the panellists set a vertical mark as an expression of the level of perception of a tested attribute. The line scales have been successfully applied to quantitative description of sensory responses (intensity tests) and of product acceptance (hedonic tests) (Lawless and Heymann, 1999). Examples of the scales used in the current research are included in Appendix 1.

4.1.3. Sensory evaluation of fresh bread – "to analyse"

The quality of bread can be described by its volume, texture, flavour and aroma. However, it is the aroma of freshly baked bread that is sought after by bread consumers (Quílez et al., 2006), and determines the bread consumers' acceptability

of a bakery product (Heenan et al., 2008). Also, according to Corsetti and Settanni (2007) the flavour and aroma of bread are the main determinants of bread quality taken into account by the bread buyers. The perception of bread freshness is a complex issue, affected not only by the product olfactory, visual and textural characteristics. Heenan et al. (2008) noted that the consumers' social and demographic background, as well as their own experiences might influence their perception of bread freshness.

4.2. Methodology

The acceptability test was developed to test hypothesis 5 (H$_0$: there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads) presented in section 2.15. A taste panel was performed using Fizz Sensory Software in the sensory booths at the Hollings Faculty. The coded samples of breads (crust and crumb) were presented to 100 panellists in the sensory panel booth at the Hollings Faculty. The attributes evaluated by the panellists were: appearance, aroma (smell), taste, texture, aftertaste and overall acceptability. All of the attributes were judged on a nine-point hedonic scale constructed with the use of Fizz Sensory Software (Appendix 1). The anchor words used were "dislike extremely" and "like extremely". No midpoint on the scale was shown to avoid creating panellist bias. Panellists were asked to state their gender and age, and to state the usually consumed type of bread. Panellists were asked whether or not they had eaten sourdough bread prior to the sensory panel, and how often ("never", "rarely", "1-3 times a month", "1-3 times a week", 4-7 times a week").

4.3. Results

4.3.1. Panellists' profile

One hundred panellists completed sensory analysis. Out of 100 panellists (35 males, 65 females) participating in the test, 99 supplied the information about their age. With the use of SPSS software the age stated by the panellists was re-coded into four categories: 18-25 (59.6% of the panellists), 26-35 (13.13%), 36-45 (12.12%), and 46-60 (15.15%).

The usual consumption (% of the total answers) of different kind of bread by the panellists is presented in Figure 35. These data were gathered through question allowing multiple answers. The number of answers obtained through this question was 140, because 29 panellists supplied more than one answer.
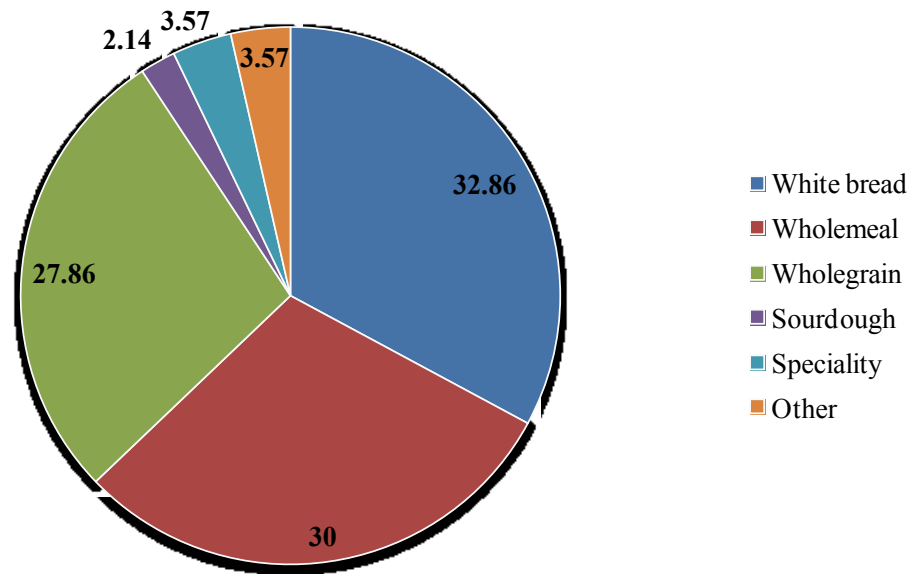
Figure 35. Types of breads consumed by the panellists participating in the consumer acceptability test (% of all the answers, *n* = 140)

As shown in Figure 35, "Sourdough" constituted only 2.14% of answers. The main answers were: white bread (32.86%), wholemeal bread (30%) and wholegrain bread (27.86%). In category "Other" the panellists had a possibility to state any additional kind of bread that they consumed.

The panellists were asked whether they had consumed sourdough bread in the past and how often they consumed it presently. 42% of the respondents declared that they consumed sourdough bread previously. However, the majority of the panellists (58%) had not previously tried sourdough bread. Only 9% of panellists consumed sourdough bread on a weekly or daily basis (Figure 36).
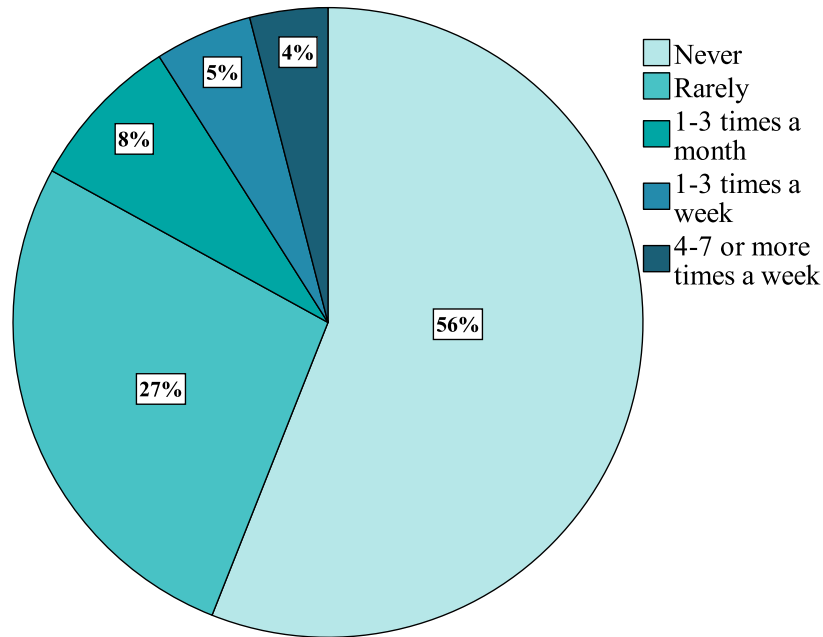
Figure 36. Frequency of sourdough consumption by panellists participating in the consumer acceptability test (*n* = 100)

4.3.2. Acceptability test – statistical analysis of the results

Table 25 shows the data collected in the consumer acceptability test. Additionally, for clarity, Figure 37 shows the data in the graphic form.

Table 25. Sensory scores from consumer acceptability test.

	Type of sourdough bread									
	Control sourdough bread (*n*=100)		Control with wheat bran (*n*=100)		GA/bran (*n*=100)		XG/Pec/bran (*n*=100)		(XG/GA/Pec/ bran (*n*=100)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Appearance score	5.12	0.19	5.23	0.20	5.25	0.18	4.54	0.18	5.14	0.18
Aroma score	4.48	0.18	4.84	0.18	4.91	0.19	4.37	0.17	4.77	0.19
Taste score	4.53	0.21	4.83	0.18	4.66	0.22	4.45	0.20	4.90	0.21
Aftertaste score	4.48	0.21	4.83	0.21	4.60	0.23	4.49	0.21	5.08	0.20
Texture score	4.53	0.19	4.38	0.21	4.25	0.20	4.24	0.20	4.57	0.21
Overall score	4.56	0.19	4.64	0.19	4.59	0.20	4.54	0.20	4.93	0.20

Mean values were not statistically significantly different ($p > 0.05$).

MANOVA procedure was performed for the results obtained in consumer acceptability test. The independent variable for this procedure was the type of bread (control sourdough, control sourdough with wheat bran, sourdough with wheat bran and gum arabic, sourdough with wheat bran, xanthan gum and pectin, and sourdough with wheat bran, gum arabic, xanthan gum and pectin).

To comply with the assumptions of MANOVA test, the data set was assessed for normality, linearity, univariate and multivariate outliers (total number of nine case rejected from further analysis), homogeneity of variance-covariance matrices, and multicollinearity. No violations of these assumptions were observed. Subsequently, Wilk's Lambda test for difference of variance returned a value of 0.933 with significance level of p = 0.083. On this basis it was assumed that there was no statistically significant difference between the scores of the five types of bread in the consumer acceptability test. Therefore, hypothesis 5 (H_0: there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads) was accepted.

Error bars: +/- 1 SE
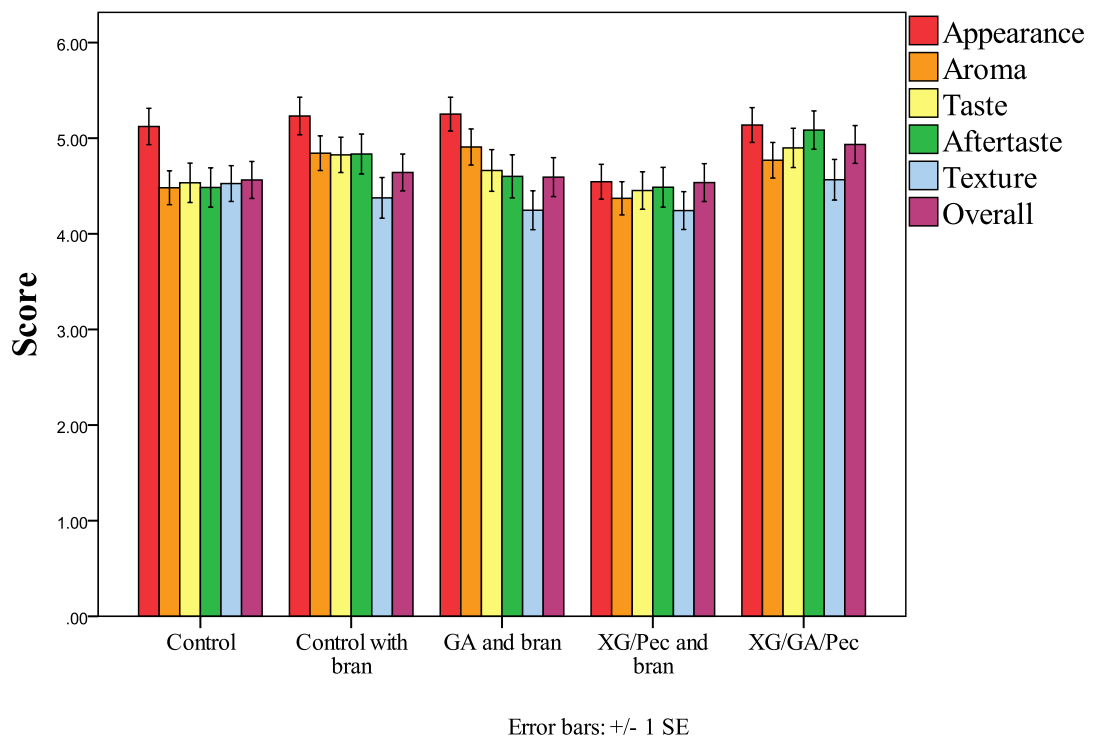
Figure 37. Summary of results obtained for five different sourdough breads in consumer acceptability test; all values are means from *n* = 100.

4.4. Summary

Chapter 4 showed the methodology and results of consumer acceptability tests. All of the breads had similar consumer acceptability. As a result, sourdough bread enriched with mixture XG/GA/Pec and bran was chosen for further analysis because of its properties and slightly higher overall consumer rating. In chapter 5 the methodological background and results of GI study will be presented. A summary of all the hypotheses tested using the experimental design shown in chapters 3, 4 and 5 will also be presented in chapter 5.

Chapter 5   Physiological responses

5.1. Introduction

The last stage of this PhD project was the measurement of glycemic index and satiety response following ingestion of the breads developed and characterised in previous stages of this study. This chapter introduces methodology and results obtained in the glycaemic index and satiety study.

5.2. Glycaemic index measurement methodology

5.2.1.   Measurement of glycaemic index of bread

The GI study was developed to test hypothesis 6, H_0: there is no difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread (section 2.15). The following subsections explain the methods used.

5.2.2.   Reference food

For the purpose of the primary research standard food consisted of 50g glucose dissolved in 250g of water. However, the measurement of the GI can be performed using white bread as a standard food Brouns (2005). The GI of the test foods will depend upon the reference food assumed for the study but can easily be converted between glucose and white bread (Atkinson et al., 2008).

5.2.3.   Blood sampling strategies

The site of the body from which the blood sample originates, has been disputed in the literature (Brouns et al., 2005, Hätönen et al., 2006). The article on methodological issues around the measurement of GI of foods by Brouns et al. (2005) constitutes a comprehensive base for the justification of methods used in the primary research. As noted by the authors, the ideal situation for GI measurement would be sampling of blood from an artery because arterial blood delivers glucose to the tissues. Arterial sampling, however, is an invasive method, so alternatives such as capillary blood (finger prick), vein blood (venopuncture or a cannula) or 'arterialised venous blood' are acceptable.

Brouns et al. (2005) discussed the differences between the capillary blood samples and venous blood samples. Brouns et al. (2005) pointed out that in the study by Granfeldt (1995) the results from blood obtained from the capillaries were higher than the results from the venous blood. Similarly, according to Jackson et al. (1983), greater values of the glycaemic response in capillary blood allow the detection of smaller differences in response to different foods (Wolever et al., 1991). Brouns et al. (2005) discussed also that in the study by Frayn et al. (1989) venous blood samples were characterised by glucose concentration 4mmol/L lower than the arterialised venous blood samples (Frayn et al., 1989, Brouns et al., 2005).

For the purpose of capillary blood sampling, fingertip is the preferable site. Aside from not being as problematic as sampling venous blood or arterialised venous blood, capillary blood gives sensitive measurements. It was noted also that although the repeated stabbing of the fingertip might not be liked by the subjects, the progress made in sampling of capillary blood has made it possible for this method to be almost painless (Brouns et al., 2005).

5.2.4. Blood sampling regime

In healthy subjects, blood sampling usually takes place at 0, 15, 30, 45, 60, 90 and 120 minutes after starting the ingestion of the test meal (Wolever et al., 1991). A FAO/WHO report (1998) recommends this regime of blood collection for GI measurement. In view of the results presented by Wolever (2004), this quarter-hourly regime of sampling of the blood for GI measurement is best suited for foods such as potatoes, bread, rice, pasta and barley. Although less frequent sampling could reduce the cost of the study and the distress inflicted on the participants, the frequency of sampling was shown to influence the mean and variation of the resulting AUC (Wolever, 2004, Brouns et al., 2005).

The result obtained with the use of fewer points of measurement may be less accurate and the precision of the GI determination may also be lower (Brouns et al., 2005, Wolever, 2004). Results obtained this way could lead to false claims of lower GI of tested foods while, in fact, it could be higher than calculated using four points. It could, of course, be argued that if the standard food and the test food are tested the same way, the ratio, which is glycaemic index, will be the same. However, various

foods show different peak values of glycaemic response and at different times after ingestion. The use of fewer points of measurement would disable the precise detection of the peak occurrence. As a result of these considerations, the seven-point protocol was adopted in this research.

5.2.5. Calculation of iAUC

The calculation of the GI is based on the incremental area under the curve (iAUC), ignoring AUC which falls below the baseline value (FAO, 1998, Brouns et al., 2005).
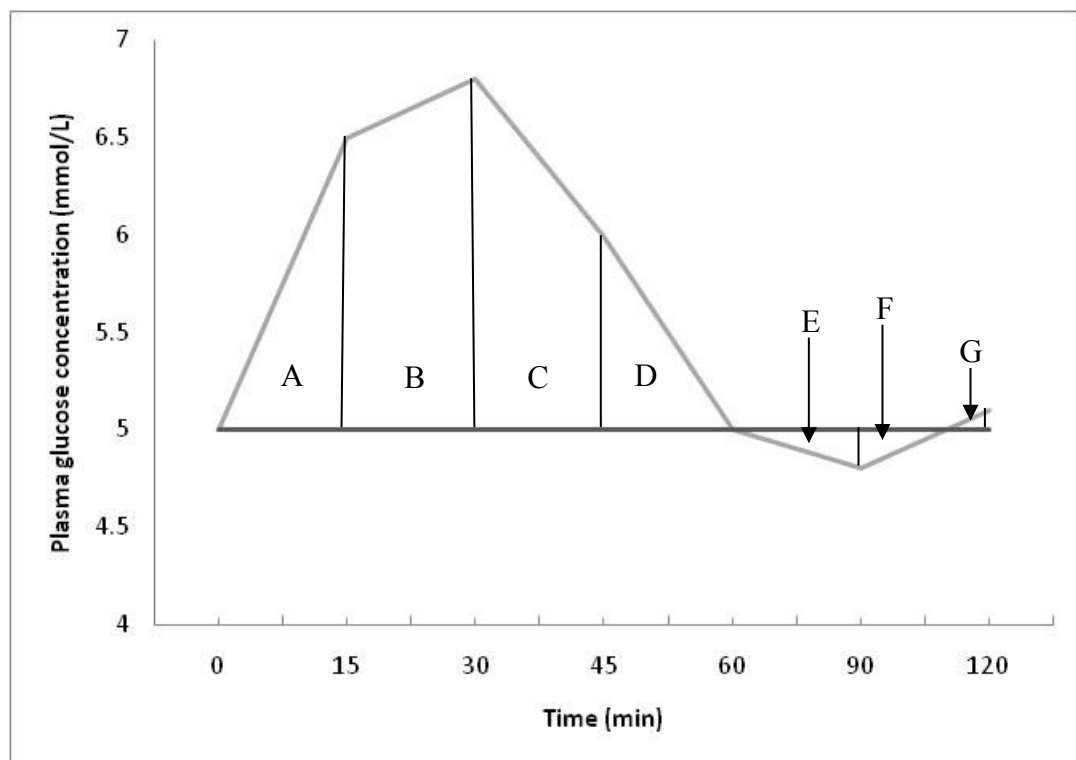
Figure 38. Typical curve of the glycaemic response

Figure 38 shows an example of typical glycaemic response curve obtained in the pilot run of the GI study. The incremental area under the curve refers to the area which does not fall below the baseline (plasma glucose concentration at the first measurement). In this case, measurement taken at 90 minutes gave a result lower than the baseline, so in the calculation of iAUC, the triangle between 60 and 110 minutes from the beginning of the test has to be discarded.

The iAUC is a sum of triangles and trapezoids fitting within the area under the curve (FAO, 1998, Brouns et al., 2005, Hätönen et al., 2006). In case of the glycaemic

119

response curve shown on Figure 38, the triangles E and F are not considered. So the iAUC is:

$$\frac{15 \times 1.5}{2} + \left(15 \times \frac{1.5 + 1.8}{2}\right) + \left(15 \times \frac{1.8 + 1}{2}\right) + \frac{15 \times 1}{2} + \frac{10 \times 0.1}{2}$$
$$= 11.25 + 20.25 + 21 + 7.5 + 0.5 = 60.5$$

5.2.6. GI calculation and validity

Glycaemic index is a ratio of iAUC of glycaemic response generated by standard food (glucose or white bread) and the iAUC of glycaemic response generated by test food. Brouns et al. (2005) put forward two methods of calculation of glycaemic index based on iAUC data gathered from a number of participants. The fundament of the first of the methods, called mean of ratios, is the calculation of the glycaemic index for every subject participating in the GI determination and subsequent determination of mathematical mean average of these values.

The second method is referred to as ratio of means. In this method, the mathematical mean of iAUCs for both, test food and standard food is determined and, based upon these mean values, the ratio is calculated. These two methods yield different GI values despite the use of the same data set (Wolever et al., 2003, Brouns et al., 2005). It is, therefore, recommended that for the reasons of statistical reliability and comparability of the results, mean of ratios should be used.

In this study, additionally, GL was calculated according to the formula:

$$GL = (GI \times dietary\ carbohydrate\ content)/100.$$

The size of a portion of bread (30g) was taken from international tables of GI and GL (Atkinson et al., 2008). The GL was calculated for every participant using GI obtained through measurement of their postprandial plasma glucose concentration, and the content of carbohydrate in 30g portion of relevant bread.

In terms of the validity of the methods of glucose determination used in GI research, Brouns et al. (2005) recommend that the coefficient of variance (CV) of the method used in scientific studies should not exceed 3%. Additionally, the accuracy of the

method (measured as glucose recovery) should not be lower than 98% (Brouns et al., 2005). Currently, glucose determination using enzymatic methods is often applied. For capillary blood glucose determination, Hätönen et al. (2006) used HemoCue Glucose 201 which is a glucose meter based on glucose dehydrogenase. In the same study, plasma glucose was determined using hexokinase method. De Angelis et al. (2009) used glucose oxidase method for determination of blood glucose concentration.

In the current study, the determinations of blood plasma glucose were performed with the use of GM7-Microstat (Analox, London, UK) which utilises an oxidase-catalysed reaction. In this reaction, the amount of oxygen proportionate to the amount of glucose in blood sample is used and measured with amperometric oxygen electrode. According to manufacturer's website (Analox, 2011), the method offers excellent linearity, precision and accuracy (Table 26). This fits within the recommendations made by Brouns et al. (2005) presented earlier in this section.

Table 26. Linearity, precision and accuracy of GM7-Microstat glucose oxidise method (Analox, 2011)

Linearity	0-30 mmol/L (10μl sample)
	0-15 mmol/L (5μl sample)
Precision	CV=1% for 10 mmol/L (n=20)
Accuracy	plasma vs. hexokinase
	y=0.96HK – 0.14 mmol/L; n=156, r=0.999

5.2.7. Subjects – recruitment and screening

Subjects were recruited from within the staff and students of the Hollings Faculty, Manchester Metropolitan University. The recruitment was performed by direct approach, e-mail, using social media, posters and word of mouth. The suitability of the volunteers for the GI trial was assessed using a general health questionnaire (Appendix 4). The initial screening assumption was that any of the volunteers declaring food allergy and/or gluten intolerance would be excluded from the trial. Additional exclusion criteria were: metabolic disorders (in particular type I and II diabetes), body mass index (BMI) > 25 kg/m^2, a serious surgical procedure within

previous six months, and recent inflammatory disease. The criterion of age exclusion was not applied. The subjects were asked also to state their smoking status.

The subjects were instructed to refrain from eating from 9p.m. in the evening preceding the trial. Participants were allowed to drink tea or coffee, with milk but no sugar. During the first visit, health questionnaires and consent were obtained, and the weight and height of the participants were measured. Each of the participants was presented with a sample of bread containing 50g of available carbohydrate and 250g of water. Bread samples were assigned to participants in systematic order. During the last of four visits in the physiology laboratory, the participants were given a reference food (50g of glucose dissolved in 250g of water). The participants were instructed to eat the breads within 10 minutes and to drink the glucose solution within 5 minutes.

5.2.8. Ethical considerations of the GI measurement

From the ethical point of view, fewer points of measurement would mean fewer finger pricks for the subjects participating. However, Brouns et al. (2005) stress that the progress made in blood sampling techniques for purposes of GI index enabled to make the sampling almost painless. Also, GI measured with the use of fewer points may be falsely lower than it would be in case of recommended protocol (Wolever et al., 2003, Brouns et al., 2005, Wolever et al., 2008).

The use of healthy subjects in GI measurement is justified. The study using the subjects with metabolic diseases such as diabetes would have to be conducted in a more clinical setting, accounting for the subjects using their hypoglycaemic medicines (insulin or oral hypoglycaemic agents). All the subjects were reassured of the confidentiality of the data collected through the use of the questionnaires. Written informed consent to participate in the trial was obtained from every subject (Appendix 5). Participants were required to provide a separate consent to obtain blood samples (Appendix 6). In adherence to The Declaration of Helsinki, participants were informed of their rights to withdraw from the study at any point without the need to provide the reason for doing so. The study was granted the approval from Hollings Faculty Research Ethics Committee (Appendix 9).

5.3. Methodology of the measurement of satiety

As discussed in chapter 2, satiety induced by foods has been successfully measured with the use of various objective and subjective methods. Due to time constraints dictated by the time span of this PhD project, a subjective method was chosen for assessment of the effects that consumption of various types of bread had on the perception of hunger/satiety of participants of the GI study. This experiment was used to test hypothesis 7, H_0: there is no difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread (Table 7, section 2.15).

A bidirectional, 10cm satiety labelled intensity magnitude (SLIM) scale was chosen and designed exactly in accordance with Cardello et al. (2005). An example of the used scale can be seen in Appendix 2. No magnitude values were given next to the descriptor anchors. The participants were asked to rate the degree of their fullness/hunger at the same time as the blood sample was obtained for plasma glucose analysis (0, 15, 30, 45, 60, 90 and 120 minutes).

5.4. Results – glycaemic index

5.4.1. Baseline characteristics of the participants

Thirteen healthy volunteers were recruited to participate in the study. All the participants started the study but after the first trial two of the participants dropped out of the study. The anthropometric details of the remaining eleven volunteers who completed the study (eight females, three males) are summarised in Table 27.

Table 27. The baseline characteristics of the eleven volunteers who completed the GI study

Subjects	Age (years)		Weight (kg)		Height (m)		BMI (kg/m^2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
11(8F;3M)	35	10.4	69.1	12.7	1.70	0.09	23.7	2.86

The age of the participants ranged from 21 to 51 years. The BMIs of two participants were higher than 25 kg/m^2. However, this was attributed to the high mass of skeletal

muscles and was treated as normal. The baseline (fasting) values of blood glucose concentrations of the eleven participants on four occasions are presented in Table 28.

Table 28. The fasting glucose concentrations in GI tests; the values are means of the results from $n = 11$ participants

Control sourdough bread		Sourdough bread with XG/GA/Pec		White wheat bread		Glucose	
Plasma glucose (mmol/L)	SE	Plasma glucose (mmol/L)	SE	Plasma glucose (mmol/L)	SE	Plasma glucose (mmol/L)	SE
4.19	0.06	4.30	0.08	4.34	0.12	4.20	0.04

5.4.2. Glycaemic index

The incremental postprandial changes of glucose plasma concentrations in capillary blood of the eleven participants are shown on Figure 39. The values of the incremental concentrations are included in Appendix 8. Two factor repeated measures ANOVA was performed for these results (intervention type, time and intervention type*time) and demonstrated the mean effect of type of meal ($p < 0.001$), time of blood sampling ($p < 0.001$) and interaction of the two ($p < 0.001$). *Post-hoc* repeated measures ANOVA showed that all of the test foods generated statistically significantly different concentration of plasma glucose.

Post-hoc one-way ANOVA of the results revealed that at all measurement points the differences of glycaemic responses generated by the three types of bread were not statistically significant. However, at 15 minutes, incremental glucose plasma concentrations generated by breads were significantly lower ($p \leq 0.001$ for all three bread samples) than this generated by 50g of glucose. Also at 30 minutes, plasma glucose concentrations generated by breads were significantly lower than this generated by the glucose standard ($p \leq 0.01$ for control sourdough bread; $p \leq 0.001$ for XG/GA/Pec; and $p \leq 0.001$ for WWB).

Table 29. Peak values of the glycaemic curves generated by test foods in GI study

	Test food type			
	Control sourdough	XG/GA/Pec	WWB	Glucose
Peak time (min)	30	45	45	30
Peak plasma glucose increment (mmol/L)	1.82	1.61	1.82	3.03
SE	0.20	0.23	0.17	0.14

As shown on Figure 39 and in Table 29, peak glucose concentrations occurred at 30 minutes after the consumption of glucose (increment of 3.03 mmol/L) and control sourdough bread (increment of 1.82 mmol/L), and at 45 minutes after the consumption of white wheat bread (increment of 1.82 mmol/L) and sourdough bread enriched with XG/GA/Pec (increment of 1.61 mmol/L). Additionally, it can be seen on Figure 39 that glycaemic response curve generated by 50g of glucose is characterised by a steep peak, followed by a steep slope whilst glycaemic curves generated by the breads take on flatter shape.
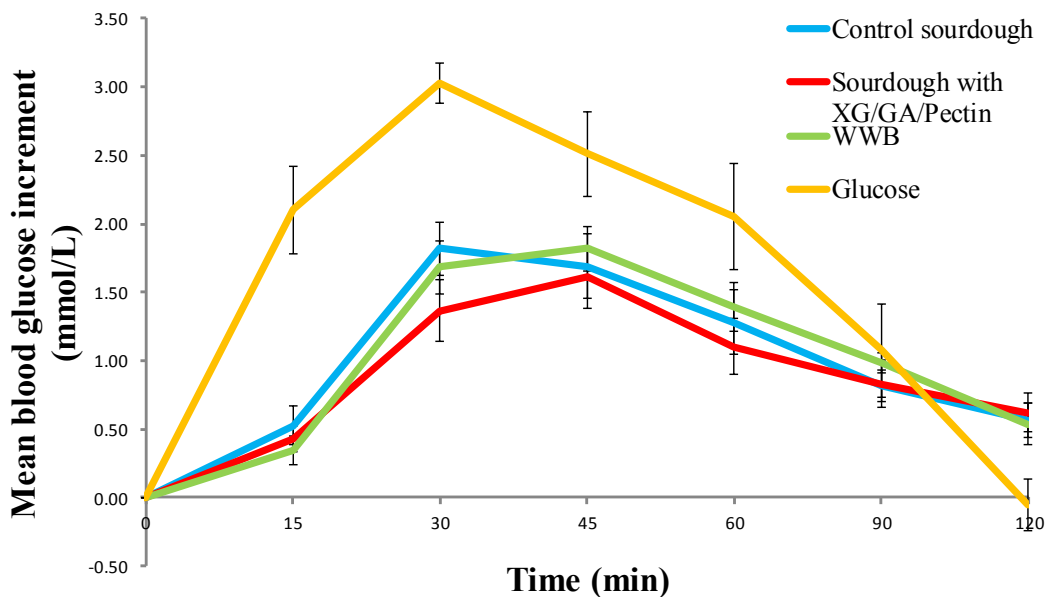
Figure 39. Change in blood plasma glucose concentration over 120 minutes following the consumption of test foods; error bars +/- 1 SE; *n* = 11

5.4.3. Incremental area under the curves of glycaemic responses

The iAUCs of glycaemic response were calculated for every participant and are presented in Table 30.

Table 30. Values of iAUC and calculation of the GI of test foods as ratio of means

Participant no	iAUC			
	Sourdough	XG/GA/Pec	WWB	Glucose
1	231.75	161.25	87.75	202.5
2	75.75	138	127.5	162
3	129.75	105	136.5	183.75
4	104.25	123.75	128.3	362.25
5	142.5	176.25	125.25	313.5
6	180	172.5	182.36	148.5
7	113.25	52.5	137.25	154.91
8	141	102.75	110.25	154.5
9	61.5	95.25	115.5	129.39
10	116.25	34.61	117.75	219.54
11	54	52.89	124.88	176.34
Mean	122.73[d]	110.43[d]	126.66[d]	200.65[a,b,c]
SE	15.69	14.91	6.95	22.07
GI	**61.16**	**55.04**	**63.13**	**100**

The differences of the values with different superscripts are statistically significant ($p < 0.05$).

High values of standard error of mean obtained for these results reflect the between-subjects variability of glucose plasma concentration under standardised measurement conditions. Table 30 shows that the values of iAUCs obtained for all three types of bread against 50g of glucose were statistically significant at $p \leq 0.05$. However, ANOVA showed no statistically significant difference in values of the iAUCs between the three types of bread. The greatest of the differences was observed in the pair: glucose – XG/GA/Pec ($p = 0.018$). Although there were differences between the mean values of iAUCs generated by the remaining two breads and glucose, these were found not to be statistically significant ($p > 0.05$ for both control bread and WWB). The GIs calculated as ratio of means were 61 for control sourdough (medium-GI), 55 for sourdough enriched with soluble fibre (low GI) and 63 for white wheat bread(medium GI).

Another method of calculating the GI of test foods is shown in Table 31. This method is referred to as mean of ratios. Using this method, the GI is calculated for every participant using their iAUC of glycaemic response to 50g of glucose as a

reference. Table 31 shows that the GI values obtained as mean of ratios were statistically significantly different from the GI of glucose standard for all three breads $p < 0.05$).

Table 31. GI if test foods calculated as mean of the ratios

| Participant no | Glycaemic index | | | |
	Control sourdough	XG/GA/Pec	WWB	Glucose
1	114.44	79.63	43.33	100
2	46.76	85.19	78.70	100
3	70.61	57.14	74.29	100
4	28.78	34.16	35.42	100
5	45.46	56.22	39.95	100
6	121.21	116.16	122.80	100
7	73.11	33.89	88.60	100
8	91.26	66.50	71.36	100
9	47.53	73.61	89.26	100
10	52.95	15.76	53.64	100
11	30.62	29.99	70.81	100
Average	**65.70[d]**	**58.93[d]**	**69.83[d]**	**100[a,b,c]**
SE	9.56	8.85	7.79	2.34E-15

The differences of the values with different superscripts are statistically significant ($p < 0.05$).

Tables 30 and 31 show that values of glycaemic indices obtained in two different methods using the same dataset are not the same. For clarity, the comparison of these results is presented in Table 32.

Table 32. Comparison of glycaemic indices calculated as ratio of means and as mean of ratios

| | Glycaemic index | | | |
	Control sourdough bread	Sourdough with XG/GA/Pec	White wheat bread	Glucose
Ratio of means	61.16	55.04	63.13	100
Mean of ratios	65.70	58.93	69.83	100
Absolute difference	4.54	3.89	6.7	-

The values of GI obtained as mean of ratios were 66 for control sourdough bread ($p = 0.03$), 59 for XG/GA/Pec ($p = 0.006$) and 70 for white sliced bread ($p = 0.019$) with glucose as reference food (GI = 100).

5.4.4. Glycaemic load

The calculation of GL values of the three breads subject to GI testing in this study is shown in Table 33. The calculation was based on GI values calculated for every participant presented previously in Table 31.

Table 33. Calculation of GL of breads used in GI trial

| Participant no | Glycaemic load | | |
	Control sourdough bread	Sourdough XG/GA/Pec and bran	WWB
1	17.66	8.16	5.79
2	7.21	8.73	10.52
3	10.89	5.85	9.93
4	4.44	3.50	4.73
5	7.01	5.76	5.34
6	18.70	11.90	16.41
7	11.28	3.47	11.84
8	14.08	6.81	9.54
9	7.33	7.54	11.93
10	8.17	1.61	7.17
11	4.72	3.07	9.46
Mean	**10.14[b]**	**6.04[a,c]**	**9.33[b]**
SE	1.47	0.91	1.04

The differences of values with different superscripts are statistically significant ($p < 0.05$).

Repeated measures ANOVA of these results revealed that the GL of sourdough bread enriched with the mixture of soluble fibres was significantly lower than GL of both control sourdough bread ($p = 0.014$) and white sliced bread ($p = 0.017$). There was no statistically significant difference between the GL of control sourdough bread and white bread. As a result of the data analysis, the null hypothesis 6 (H_0: there is no difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread) was rejected and the alternative hypothesis accepted.

5.5. Results – satiety response

Changes of perceived satiety over 120 minutes after the ingestion of test foods are shown in Figures 40 and 41 (incremental changes). The negative values represent hunger and the values above the X axis represent fullness. Whereas solution of 50g of glucose in 250g of water did not produce the feeling of fullness, all of the bread

samples containing 50g of available carbohydrates did produce the feeling of fullness which was most pronounced by 15 minutes after the start of consumption. Fullness declined at different rates with hunger reported at 60 minutes after consumption of WWB, 90 minutes after consumption control sourdough bread, and towards the end of the experiment (120 minutes) after XG/GA/Pec.
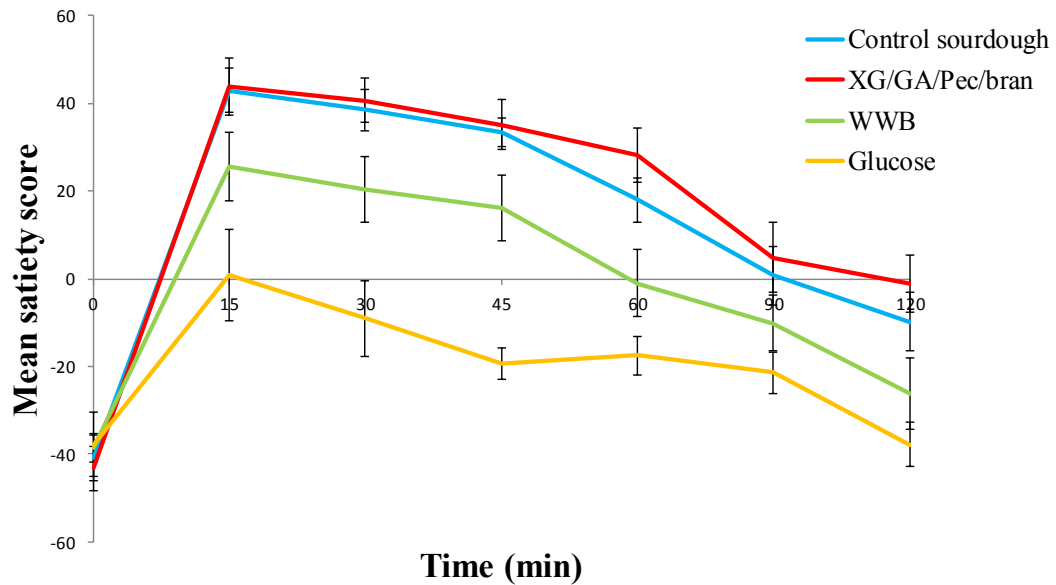
Figure 40. The changes of satiety response over 120 minutes following the ingestion of the test foods; error bars +/- 1 SE; *n* = 11
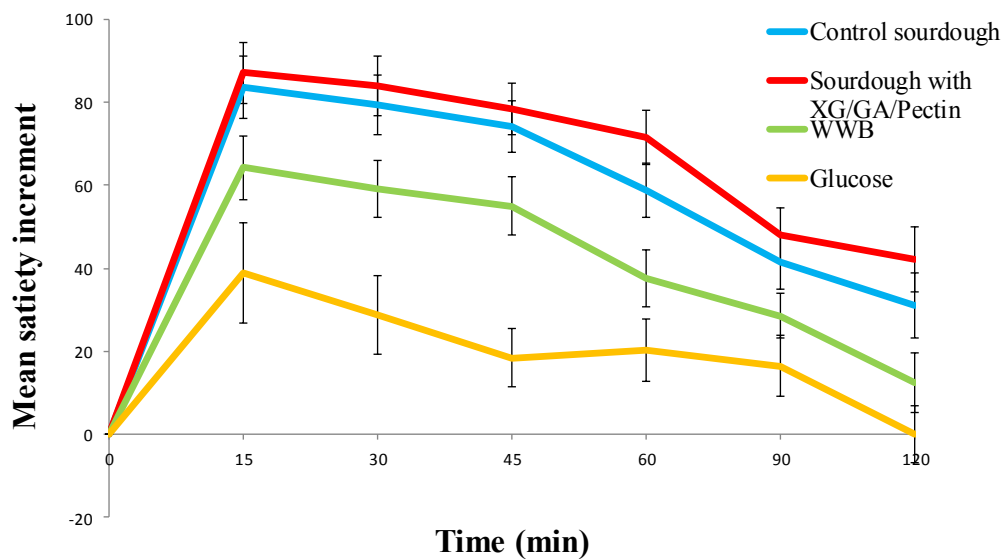
Figure 41. Incremental changes of satiety ratings over 120 following the ingestion of test foods; error bars +/- 1 SE; *n* = 11

129

Two way repeated measures ANOVA of the incremental satiety perception scores was performed (type, time, and type*time) and demonstrated main effect of type of intervention type ($p < 0.001$), time ($p < 0.001$) and interaction ($p <0.001$). *Post-hoc* repeated measures ANOVA showed statistically significant changes in satiety response for all the breads. Glucose, however, did not produce statistically significant changes to postprandial satiety scores. *Post-hoc* ANOVA showed that after 15 minutes from ingestion, control sourdough bread and sourdough bread with XG/GA/Pec generated higher satiety response than glucose or white sliced bread. The difference was statistically significant for pairs, glucose-control ($p = 0.011$) and glucose-XG/GA/Pec ($p = 0.005$). After 30 minutes from ingestion all breads generated higher satiety than glucose ($p < 0.001$ for control and XG/GA/Pec and $p = 0.009$ for white wheat bread). After 60 minutes control sourdough generated higher satiety than glucose ($p = 0.004$). At the same time, the subjects felt more satiated after the consumption of sourdough bread with XG/GA/Pec than after WWB ($p = 0.013$) or glucose ($p < 0.001$). After 90 minutes the only statistically significant difference in satiety was observed between sourdough bread with XG/GA/Pec and glucose ($p = 0.14$).

Table 34. Incremental AUC of satiety generated by the test foods

	iAUC			
Participant no	**Control sourdough**	**XG/GA/Pec**	**WWB**	**Glucose**
1	5827.5	7050	4695	103.8
2	7980	2175	3555	68.6
3	7260	9105	4335	154.3
4	5280	3922.5	2400	3842.7
5	5610	6375	2835	1710
6	6285	9765	3862.9	4995
7	5415	6270	4770	2460
8	9960	9315	8445	937.5
9	4695	7215	5820	1372.5
10	8940	9195	4155	4590
11	3235	4410	4185	6180
Average	**6407.96**[c,d]	**6799.77**[c,d]	**4459.81**[a,b]	**2401.31**[a,b]
SE	591.41	752.24	487.34	655.92

The differences of values with different superscripts are statistically significant.

After 120 minutes from ingestion, subjects reported higher satiety after control sourdough bread ($p = 0.027$) and sourdough with XG/GA/Pec ($p = 0.001$) than after

glucose. Additionally, the bread with XG/GA/Pec was perceived by the subjects to be more satiating after 120 minutes than WWB ($p = 0.036$).

Statistical analysis of the results presented in Table 34 using repeated measures ANOVA revealed significant differences between the mean values of iAUCs of satiety response. The iAUC for control sourdough bread and sourdough bread with XG/GA/Pec was higher than those of glucose ($p = 0.018$ and $p = 0.007$ respectively) and WWB ($p = 0.045$ and $p = 0.036$ respectively). The iAUC of satiety after consumption of WWB was higher than that of glucose although this difference was not statistically significant. Also the value of iAUC of XG/GA/Pec was higher than that of control sourdough bread. This result did not reach statistical significance in statistical analysis.

As a result of the analysis of the results gathered through the satiety questionnaire, the null hypothesis 7 (H_0: there is no difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread) was rejected, and the alternative hypothesis accepted.

5.6. Summary

The glycaemic responses of control sourdough and bread XG/GA/Pec were similar. However, fibre addition resulted in delayed and reduced glucose peak value. The GIs calculated in the study were: 100 for glucose, 70 for WWB, 66 for control sourdough and 59 for XG/GA/Pec. Greater differences were observed in GL of the three breads. It was observed that consumption of bread with XG/GA/Pec resulted in increased satiety perception.

These key findings point that there is an influence of soluble fibre addition upon the properties of sourdoughs and the resultant bread. In the next chapter, these findings will be discussed and critically analysed in the context of the literature reviewed previously in chapter 2 in order to substantiate the conclusions of this study. Table 35 summarises hypotheses rejected as a result of experiments presented in chapters 3, 4 and 5.

Table 35. The outcomes of the hypotheses' testing in this PhD

	Null hypothesis	Alternative hypothesis	Statistical test	Rejected hypothesis
1)	H_0: inclusion of soluble fibre mixtures does not influence the stickiness of sourdoughs during fermentation	H_1: inclusion of soluble fibre mixtures influences the stickiness of sourdoughs during fermentation	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one-way ANOVA *post-hoc*	H_0
2)	H_0: there is no correlation between the pH of sourdoughs and their stickiness	H_1: there is correlation between pH of sourdoughs and their stickiness	Pearson's correlation	H_0 for control; H_1 for sourdoughs enriched with soluble fibres
3)	H_0: addition of different soluble fibres and bran does not alter the gelatinisation parameters of flour pastes	H_1: addition of different soluble fibres and bran alters the gelatinisation parameters of flour pastes	ANOVA	H_0
4)	H_0: inclusion of soluble fibres and bran does not alter physico-chemical properties of sourdough breads	H_1: inclusion of soluble fibre and bran alters the physico-chemical characteristics of soluble fibres	ANOVA	H_0
5)	H_0: there is no difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads	H_1: there is difference in appearance, aroma, texture, taste, aftertaste and overall scores in consumer acceptability test between sourdough breads enriched with soluble fibres and control sourdough breads	MANOVA	H_1
6)	H_0: there is no difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread	H_1: there is difference between the glycaemic response generated by sourdough bread enriched with soluble fibres and the glycaemic response of control sourdough bread and white sliced bread	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one way ANOVA *post-hoc*	H_0
7)	H_0: there is no difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread	H_1: there is difference between the perceived satiety generated by sourdough bread enriched with soluble fibres and satiety generated by control sourdough bread and white sliced bread	two-factor repeated measures ANOVA; one-way repeated measures ANOVA *post-hoc*; one-way ANOVA *post-hoc*	H_0

Chapter 6   Discussion

6.1. Introduction

In chapter 2 it was demonstrated that sourdough bread and soluble fibres are characterised by nutritional properties that allow them to be categorised as functional food ingredients. The findings of this PhD demonstrated the influence of sourdough technology and soluble fibre addition upon the properties of sourdoughs and resultant breads in chapter 3. The observations emerging from the findings of this study suggest that the changes in the properties of doughs and breads may have influenced the nutritional aspects of the sourdough breads enriched with soluble fibre. The discussion presented within this chapter allows the elucidation of the mechanisms underlying the enhanced nutritional properties of sourdough breads enriched with soluble fibre. First, the observations made during the development of sourdough breads enriched with soluble fibre are discussed. Consumer acceptability and nutrient analysis results are described subsequently. Next, the focus of this chapter is placed on the glycaemic and satietogenic properties of the breads considered in this study. The sum of the findings and their analysis will allow for the development of a model describing the influence of soluble fibre upon the glycaemic and satietogenic properties of sourdough breads, and discuss it with reference to existing knowledge (aim 4). Following this, the conclusions of this study are drawn and recommendations for future research are made.

6.2. Product development

The results presented within this thesis showed the physico-chemical properties of sourdough breads enriched with soluble fibre. The sources of soluble fibre used in the study were EU approved food additives (xanthan gum, gum arabic and Grinsted Pectin SF 530) which were combined with a source of mainly insoluble fibre (wheat bran). As demonstrated in sections 3.2.1 and 3.3.2, the gums used as soluble fibres in this research altered the dough water absorption and strengthen the dough.

A statistically significant correlation between the pH and dough stickiness was identified for control sourdough bread ($R^2 = 0.618$) and for control sourdough bread with added wheat bran ($R^2 = 0.532$). The lack of a correlation between the pH and

dough stickiness, and no statistically significant change in the stickiness during fermentation of the doughs with added soluble fibre was also demonstrated (see sections 3.3.2 and 3.3.3). In other words, the presence of hydrocolloids in sourdough prevented the increase of stickiness during the fermentation. A plausible explanation of this phenomenon is the water-holding capacity of soluble fibres used (Chaplin, 2010). The greatest net increase of stickiness was observed for control sourdough. In the control enriched with wheat bran, this effect was slightly attenuated, possibly due to the presence of wheat bran, and its water holding capacity. However, for sourdoughs enriched with hydrocolloids the net change of stickiness was close to zero (section 3.3.2).

In this study, the experimentally determined water absorption increased from 62.5% for control sourdough bread to 90% for sourdoughs containing 10% soluble fibre (mixture of XG and Pec, and mixture of XG, GA and Pec). This result is in agreement with findings of Rosell et al. (2001), who reported xanthan gum as the fibre having the most pronounced effect on water absorption of bread dough. The increase in water absorption was observed in previous studies concerning the application of dietary fibre sources in breadmaking (Pomeranz et al., 1977, Huebner and Wal, 1979, Rosell et al., 2001, Wang et al., 2002) and the production of biscuits (Sudha et al., 2007).

Dough stickiness can be affected by water content, flour extraction, the presence of soluble pentosans, protein composition, the activity of alpha-amylase and proteolytic enzymes (Chen and Hoseney, 1995). Thus, it is to be expected that the addition of soluble fibre which has a high water holding capacity will influence the stickiness of a sourdough. This study reflected this expectation; the weakening of dough (increase of stickiness) was observed with a decrease of pH. Weakening of dough, increased water absorption and decrease of mixing time were reported as a result of organic acid addition by several authors (Tanaka et al., 1967, Maher Galal et al., 1978, Wehrle et al., 1997, Arendt et al., 2007), and might be responsible for diminished quality of a sourdough product. The findings of the current study agree with the previous research in this aspect.

The gluten network was shown to be liable to extensive proteolysis and depolymerisation by sourdough LAB (Thiele et al., 2004, Clarke et al., 2004). This

mechanism might explain the weakening of dough and its increased stickiness. The technological implication of the increased stickiness of dough is the detriment to its handling properties. On the other hand, the current study shows that the addition of soluble fibre seems to have a strenghtening effect on dough (lack of stickiness increase). This finding supports the research of Rosell et al. (2001) in which the strengthening of dough and the improvement of gas retaining properties of the dough by the addition of hydrocolloids were reported alongside the improvement of dough handling properties. This outcome was due to the interaction between the hydrocolloids and the proteins of the flour (Jones and Erlander, 1967, Huebner and Wal, 1979).

The dough strengthening effect was most pronounced for the doughs containing XG. Rosell et al. (2001) concluded that the use of hydrocolloids improved the stability of doughs during fermentation. In case of sourdough, it is possible that the water released from gluten network depolymerised during the fermentation is bound by added hydrocolloids. This is a plausible mechanism of the decreased stickiness of sourdoughs with hydrocolloids as a source of soluble fibre.

6.2.1. Starch gelatinisation in the presence of soluble fibres

Rapid Viscosity Analysis showed that the addition of 10% of soluble fibres had an effect on the pasting properties of wheat flour. Therefore, the addition of soluble fibre significantly influenced not only the rheological properties of dough but also the gelatinisation properties of starch (section 3.3.4).

The results presented in this thesis show that the addition of 10% of hydrocolloids to wheat flour or flour with wheat bran (9:1) changed the temperature of gelatinisation of starch. The pasting temperature was significantly lower ($p < 0.05$) for the mixtures containing XG. The reduction of pasting temperature to 50-52 °C was observed for mixtures FXG and FBrXG. The mixtures containing gum arabic (FGA and FbrGA) showed the opposite trend and increased the pasting temperature from 78 °C to 88-89 °C. Additionally, by the addition of hydrocolloids, the viscosity of flour pastes was altered. A similar trend was observed by Rojas et al. (1999) in mixtures containing XG. However, in their research, Rojas et al. (1999) tested pastes of flour and hydrocolloids at concentrations of 0.5 and 1% and using different equipment

(Brabender Viscograph). This methodological difference may account for contrasting findings.

Through the analysis of the pasting properties of starch, it is possible to determine not only the swelling characteristics of starch granules in flour pastes but also the retrogradation of starch granules (Rojas et al., 1999). Retrogradation of starch granules is one of the processes occurring during the staling of fresh bread. In RVA analysis, the setback value describes the retrogradation of the starch in flour pastes on cooling of the paste. Lower values of setback point towards slower firming of the crumb (Rojas et al., 1999). In this study, it was found that the setback value was influenced by the presence of wheat bran in the flour paste (section 3.3.4). The value of setback was increased by the presence of pectin. Nevertheless, XG had the greatest effect upon the setback value. Hence, it could be expected that bread made with 10% XG would be characterised with a quick firming of the crumb. The finding that XG promotes the setback (increases the starch retrogradation) might be due to the fact that XG has a high affinity to water ('water holding capacity'). On this premise, XG should also influence the gelatinisation of starch in flour paste. It was seen earlier in this section and in the section 3.3.4 that the flour pastes obtained with the addition of XG had the highest peak viscosity, trough viscosity, final viscosity, setback and lowest pasting temperature. However, it needs to be emphasised that the finding that XG promotes setback rather than reduces it remains in disagreement with the study by Rojas et al. (1999), possibly due to the difference in the concentration of XG used.

The mixture of hydrocolloids chosen for production of the sourdough bread enriched with soluble fibres (XG/GA/Pec) produced the lowest setback value ($p < 0.05$); therefore, it could be expected that the increase of crumb firmness due to starch retrogradation in bread made with this hydrocolloid mixture would be slower.

6.2.2. Physico-chemical analysis of the properties of breads

The detrimental effect of the addition of high amounts of fibre to the dough upon the properties of bread was also observed. The resultant breads (except for the bread with GA and bran) were characterised by increased crumb firmness and diminished specific loaf volume. However, after six days of storage in the same conditions, the

crumb of breads enriched with soluble fibre was softer than that of sourdough bread with added wheat bran. The negative influence of fibre addition upon the properties of bread was seen in the secondary research. Poor gas retention was a reason for the diminished loaf volume as reported by Pomeranz et al. (1977). The degradation of the texture, darker crumb colour and increased crumb firmness were also reported. In a more recent study on the application of hydrocolloids in breadmaking, the crumb firmness was increased by XG (Rosell et al., 2001). The increased crumb firmness of the breads containing XG was confirmed by the findings of the current study. On the other hand, the softer crumb of sourdough bread can be explained by the acidification of dough with lactic acid which was linked to improved softness of bread crumb (Katina et al., 2006a). Katina et al (2006a) showed an improvement of specific loaf volume and justified the increase of crumb softness with the higher loaf volume.

In the case of sourdough breads developed in the course of the current research, increased crumb firmness for sourdough breads enriched with soluble fibre was paired with the reduced specific loaf volume and crumb brightness (with the exception of bread made with GA and bran). However, it needs to be noted that Armero and Collar (1998) reported sourdough to generally produce breads with an increased firmness in comparison to the straight (baker's yeast-leavened) bread. It can be expected, though, that the addition of fibre to sourdough bread will have a complex effect on the physico-chemical properties of bread. Furthermore, the properties of bread can be translated onto consumers' perceptions of its quality – mouthfeel, texture, chewiness (Brady and Mayer, 1985).

In this study, the crumb firmness of all of the breads with soluble fibre (except for bread containing GA and wheat bran) was increased in the initial stage of the shelf-life experiment. However, sourdough bread with wheat bran had the hardest crumb after 144 hours of storage, which suggests, that the addition of soluble fibre slows down the crumb firming during storage.

An increase in TDF and SDF fraction of sourdough breads was achieved with the use of GA, XG and pectin. The results of fibre determination presented within this thesis show that the addition of 10% soluble and/or insoluble (wheat bran) fibre to sourdough bread increases the TDF from below 3% to ~12% (dry matter basis). The

SDF content of breads containing pectin was lower than that of bread containing only gum arabic and wheat bran. An explanation for this result was found in the study by Kondo (1998), who showed low pectin recovery rate with the use of the enzymatic-gravimetric method. It was concluded that the pectin is stable at low pH (3-5) but disintegrates into smaller molecular weight compounds on heating at neutral pH (5-7).

6.2.2.1. *Fibre content*

The Food Standards Agency requires a food product to contain 25% RDI dietary fibre in order to have a health claim. Level of DF incorporated with the use of XG, GA, Pec and bran enabled the manufacture of bread containing more than 25% RDI of DF (more than 6.25g) in 100g of the product. Recent UK dietary surveys (Nelson et al., 2007, Bates et al., 2011) clearly point out that the population of the UK, independent of the socio-economic background, fails to achieve high levels of dietary fibre in its diet and. Therefore, the bread obtained in the course of this study could contribute towards adequate fibre intake and provide the nutritional goodness of soluble dietary fibre. It is worth pointing out that increased consumption of fibre has been shown to reduced the risk of CVD, diabetes and, through colonic production of SCFAs, certain types of cancer (not only bowel cancer). Moreover, gums are classed as viscous soluble fibre (Slavin et al., 2009) and this class of dietary fibre was recently demonstrated to elicit a lowering effect upon the blood cholesterol concentration (Vuksan et al., 2011). However, the effects of the breads developed in the current study on the metabolism of lipoproteins in human body are, as of now, unknown. Therefore, it is recommended that this branch of viscous fibre in bread technology research is pursued in the future.

6.2.3. Water activity and shelf-life

The water activity (a_w) measurements suggest that the water migration between the crust and crumb is slower in the sourdough breads enriched with mixture of soluble fibres and wheat bran than in control sourdough breads. This, in turn, could possibly prolong the staling period probably due to water binding capacity of hydrocolloids. The fact that hydrocolloids in general bind water, and prevent its diffusion (Chaplin,

2010) can explain the lack of significant changes in a_w of crust and crumb of sourdough breads with hydrocolloid content.

The lack of apparent signs of bacterial (ropiness) or fungal (mould growth) spoilage contributed to the prolongation of the shelf life of the products developed in the course of the experiment. The preserving properties of sourdough bread are well documented (Corsetti et al., 1998b, Gobbetti et al., 2005). It was previously observed by Corsetti et al. (1998b) that spoilage was delayed in breads made with LAB and occurred sooner in breads made with bakers' yeast. It is possible that *L. brevis* (obligate heterofermentative LAB) present in the sourdough starter used in this study prevents spoilage and thus prolongs the shelf-life of the breads. Sourdough has been renowned for its antifungal activity; the mixture of acetic, caproic, formic, propionic, butyric and *n*-valeric acids produced by sourdough lactic acid bacteria was shown to have an inhibitory effect on the growth of spoilage moulds such as *Fusarium*, *Aspergillus* and *Penicillium* (Gobbetti et al., 2005). Antifungal activity is normally associated with the metabolism of heterofermentative lactobacilli (Messens and De Vuyst, 2002). However, the HPLC analysis of organic acids present in the sourdoughs in this experiment yielded only lactic acid. Therefore, an explanation offered is that the content of lactic acid might be high enough to suppress the bacterial and fungal spoilage of sourdough bread based only on the pH of the bread crumb. Alternatively, the presence of other antibacterial compounds needs further research to clearly explain the mechanism of lack of the spoilage during prolonged storage of the sourdough breads.

The reduced amount of protein in sourdough breads enriched with gum arabic, xanthan gum, pectin or mixtures of these fibres was demonstrated in the current study by analyzing the content of protein in the baked bread. This result is in agreement with dilution of gluten proteins by the addition of fibre observed by Pomeranz et al. (1977). However, the current study shows the strengthening effect of hydrocolloids which seems to be partially counteracting the effect of dilution of the gluten proteins. The current study shows the potential for the use of hydrocolloids gum arabic, xanthan gum and pectin as a source of SDF in sourdough bread. Additionally, the application of gum arabic, xanthan gum and pectin not as bread improvers but as nutrients adds to the novel use of these hydrocolloids. The

bakery applications of hydrocolloids used as SDF in this study were subject to research in the past. The addition of 0.5% of hydrocolloids was used by Rosell et al. (2001), 0.3% by Mettler and Seibel (1995) and 0.1% by Guarda et al. (2004). In comparison to the current study (use of 10% hydrocolloid based on flour weight) the levels considered by researchers in the past, were small and probably could not contribute towards increased DF consumption.

6.3. Consumer acceptability of breads enriched with soluble fibre

The findings of this research demonstrated that the addition of soluble fibres and wheat bran to sourdough bread had no significant effects on its consumer ratings of the appearance, aroma, flavour, texture, aftertaste and overall acceptability. Gómez et al. (2003) applied 2% and 5% of various fibre sources (cellulose, coffee, cocoa, orange, pea, long and short wheat fibre) in wheat bread making. Gómez et al (2003). found that as a general rule, the acceptability of breads containing fibre was decreased to a varying degree, depending on the type and amount of fibre used in breadmaking). In this regard, the findings of the current research disagree with findings of Gómez et al. (2003). The demographic results gathered in this study indicate, however, that sourdough bread was not the usual kind of bread of choice for the panellists selected for this study. The fact that on a nine-point hedonic scale the scores given to the breads all fit in the range 4.48-5.25 might be pointing at a 'central tendency' caused by panellists' unfamiliarity with the tested product.

It was previously demonstrated in studies concerning sourdough bread technology and bread enriched with fibre, that the loss of consumer acceptability is not always the case when developing a healthy product. For example, de Angelis et al. (2009), who developed sourdough bread enriched with oat and rye fibre, demonstrated that this sourdough bread scored higher in sensory tests than wheat sourdough bread and wheat bread fermented only using baker's yeast. Dewettinck et al. (2008) demonstrated that consumers with a strong positive attitude towards health and sensory attributes of bread, formed the largest group of bread consumers. It is known that sourdough can improve the palatability of wholegrain, gluten-free or high-fibre breads (Katina et al., 2005, Poutanen et al., 2009). The results of this PhD study

show that the addition of fibre did not influence the acceptability of the sourdough breads.

6.4. Available and resistant starch

Results of the resistant starch assay in the breads considered for the GI trial revealed statistically significant differences in their RS content. It was found that the RS content of WWB was significantly lower than that of control sourdough breads (both with and without the addition of wheat bran). Moreover, the total starch values were found to be different for all of the considered breads (control sourdough 68%, control with added wheat bran 62%, fibre enriched sourdough 56%, WWB 71%). The addition of soluble fibre did not appear to have an effect on the resistant starch content of the breads. The values of resistant starch (1.2-1.7% based on total starch) were consistent with the findings of previous studies. Liljeberg and Björck (1994) found that resistant starch content of various barley breads and breads enriched with intact barley kernels was 0.8-1.7%. A high amount of resistant starch was found in pumpernickel bread (8%) (Liljeberg and Björck, 1994). In another study, Liljeberg et al. (1995) found that resistant starch content of bread made with addition of sourdough or salts of organic acids was in the range of 1.3-2.1% (based on total starch).

Previously, RS content was reported to increase from 3% (based on total starch) to 6.6% in wholemeal rye breads baked with addition of lactic acid and to 5.7% in breads baked with added acetic acid (Liljeberg et al., 1996). In another study by Scazzina et al (2009), the content of resistant starch in bread was noted to have increased due to sourdough fermentation in products made from both refined (from 6.1% in *S.cerevisiae* fermented bread to 7.7 %) and wholemeal wheat flour (from 3.3 % in *S. cerevisiae* to 4.7%). Conversely, in the current study, sourdough bread made with a mixture of flour and wheat bran (9:1) had slightly higher content of RS than sourdough without bran. This finding, however, was not statistically significant. The amount of the resistant starch also remained below the levels previously reported by Liljeberg et al. (1996) and Scazzina et al (2009).

Another factor contributing towards the resistant starch formation in bakery products is the amylose content of flour. Previously, Åkerberg et al. (1998) found that a

greater amount of RS is formed in breads baked from flours with high amylose content and under slow-baking ('pumpernickel') conditions (20h, 120 °C). As an unlikely explanation of the RS content in this experiment, it requires more research to determine the influence of amylose on RS formation in sourdough bread under various baking conditions.

The addition of high amounts of soluble fibres to sourdough in this PhD explains the lower starch content (both, RS and 'available' starch) through the replacement of the contents of wheat flour. In De Angelis et al. (2009) no resistant starch content was offered. In turn, the hydrolytic index (HI) of starch was presented. It was found that the starch in bread fermented by bakers' yeast alone was more prone to enzymic hydrolysis than the starch in breads fermented by LAB. This could explain the low GI of the sourdough bread enriched with oat fibre in the study by de Angelis et al. (2009). Slower digestion of the breads considered in the current study is a possible explanation of the reduced GI. However, as a limitation of this research, starch hydrolysis *in vitro* was not performed.

6.5. Glycaemic index and satiety

6.5.1. Glycaemic index and glycaemic load

GI in this study was reported as two sets of values: ratio of means and mean of ratios. Brouns et al. (2005) recommended that the mean of ratio values of GI should be used. The GIs of the breads in this study calculated as ratio of means were as follows: control sourdough: 66 (medium GI), sourdough bread with bran and XG/GA/Pec: 59 (medium GI), white sliced bread: 70 (high GI). The GL obtained for these breads were: control sourdough bread: 10 (low GL), XG/GA/Pec: 6 (low GL), WWB: 9 (low GL). The GL of the fibre-enriched sourdough bread was significantly lower than this of remaining two breads.

The peak increment of plasma glucose concentration occurred at 30 minutes after consumption of glucose (2.9 mmol/L) and control sourdough bread (1.82 mmol/L), and at 45 minutes after consumption of sourdough bread with bran and XG/GA/Pec (1.61 mmol/L) and white sliced bread (1.82 mmol/L). The delay in the peak of plasma glucose could be explained by the fact that the digestion of starch contained within the bread, and subsequent absorption of the released glucose, needs to take

place prior to the peak occurrence. Therefore, it can be concluded that a difference exists in the rate at which the bread enriched with soluble fibres releases glucose from the starch. However, the GI of bread containing high amounts of soluble fibre found in this study was higher than the value obtained from previous research in the field (Lu et al., 2000, De Angelis et al., 2007, De Angelis et al., 2009). It was reported that sourdough technology can yield bread characterised by a low GI. De Angelis et al. (2009) obtained a bread with a GI of ca. 41 (low-GI) by subjecting the dough enriched with oat fibre to two stage fermentation. Prior to that, De Angelis et al. (2007) obtained a similar to the study from 2009, bread with a GI of ca. 54 (low-GI). Nevertheless, this PhD study's results replicate the trend observed previously by De Angelis et al (2007) and De Angelis et al. (2009).

6.5.1.1. *The role of organic acids in the regulation of postprandial glycaemic response*

It has been known for a quarter of a century that white bread containing gluten produces lower postprandial glycaemic response than gluten free breads. This has been attributed to the interaction of gluten proteins network with starch granules, possibly slowing down starch digestibility in the intestine (Jenkins et al., 1987a). The finding of Jenkins et al. (1987a) can provide an explanation for the reduced GI of sourdough breads, in conjunction with the effects produced by the organic acids present in sourdoughs.

Some evidence exists relating to the influence of organic acids on the GI of starchy foods. It was demonstrated that acetic, propionic and lactic acid have the potential to lower the postprandial glycaemic response (Östman et al., 2002). Several groups of scientists elucidated the acids' mechanism for lowering postprandial glucose response. Liljeberg and Björck (1998) observed gastric emptying rate using paracetamol as a marker, and found that presence of acetic acid slowed gastric emptying and resulted in reduced GI of bread. No such effect was observed for bread with added lactic acid (Liljeberg and Björck, 1996). In a previous study it was suggested that the potential of weak acid to slow gastric emptying is inversely related to its molecular weight (Hunt and Knox, 1972). According to this line of thinking, acetic acid ($M_w = 60$) is more potent in slowing gastric emptying than lactic acid ($M_w = 90$) (Östman et al., 2005).

Conversely, the presence of lactic acid in a food product during heat treatment was shown to play a role in reducing the predicted glycaemic index of food (Östman et al., 2002). It was demonstrated that lactic acid lowers the hydrolytic index of starch only when gluten is present in the mixture. Additionally, no lowering effect on postprandial glycaemia was observed for barley gruels fermented with lactic acid bacteria to pH 3.4 - 3.5 after the heat treatment. On this basis, Östman et al. (2002) concluded that lactic acid influences interactions between gluten and starch, which, in turn, are responsible for the reduced digestibility of starchy foods. However, two conditions must be fulfilled: the presence of gluten and lactic acid before the thermal processing of the food product. Both of these conditions were satisfied in the research presented within this thesis. Lactic acid in sourdoughs was produced by LAB starter during the four hour fermentation period, and all of the sourdoughs contained wheat flour, and therefore, gluten. The interactions between gluten and starch brought about by the presence of lactic acid in the sourdough during baking, might have influenced the behaviour of starch in the gastrointestinal tract. Several facts support this conclusion: 1) no acetic acid was detected in the sourdoughs, therefore it was unlikely to have reduced the rate of gastric emptying; 2) the concentration of resistant starch in sourdough breads was too low to produce significant effects upon the GI values; and 3) lactic acid was previously demonstrated to reduce postprandial glycaemic response on the basis of promoting interactions between dough proteins and starch (Östman et al., 2005).

In the study by Najjar et al. (2009) it was shown that sourdough bread consumption resulted in lower glycaemia. Other metabolic effects of different breads' consumption were explored in the same study. No differences in insulinaemic response were indentified by Najjar et al. (2009), but it was shown that insulin sensitivity was acutely higher and glucagon-like peptide-1 (GLP-1) plasma concentration was lower than after the consumption of white, wholewheat and barley bread. The importance of GLP-1 in carbohydrate homeostasis and satiety regulation is recognised (Williams et al., 2009). Additionally, reduction of insulin resistance (increased insulin sensitivity) is a protective factor in prevention of development of type 2 diabetes and other diseases (Salmerón et al., 1997a, Salmerón et al., 1997b, Barclay et al., 2008, De Angelis et al., 2009). The metabolic responses after the consumption of breads developed in the course of this PhD remain unknown.

Therefore, it is recommended that further research should be performed in order to more firmly establish the influence of soluble fibre and sourdough on postprandial hormonal response of the human body.

6.5.1.2. *Resistant starch and soluble fibres' content and postprandial glycaemia*

The content of RS in breads in this study seems an unlikely mechanism for the lower GI of sourdough breads. Additionally, the GI of sourdough bread enriched with XG/GA/Pec was lower than that of the control sourdough bread with no fibre added. Therefore, it seems logical to make an assumption that the mixture of soluble fibres applied in breadmaking does exert some effect on either the rate of digestion of the bread or the rate of absorption of glucose from the small intestine. However, the lower GI of fibre enriched sourdough bread was not statistically significantly lower; therefore, this conclusion is drawn cautiously.

The effects of viscous soluble fibres (gums, pectins, β-glucans) on glycaemic response are well documented (Frati Munari et al., 1998, Lu et al., 2000, Dikeman and Fahey Jr, 2006, Atkinson et al., 2008). In the past two mechanisms were suggested for the decreased transport of glucose through the mucous membrane of the intestine: 1) increased resistance of the mucous membrane caused by the viscosity of the intestinal fluid in the presence of gum, and 2) obstruction of glucose molecules' mobility caused by the gel-forming macromolecules of gums (Johnson and Gee, 1981). More recently, a suggestion was put forward that the increased viscosity of intestinal fluid due to the presence of soluble fibre could influence the mobility of α-amylase (Brennan et al., 2012). In this PhD study, the reduced mobility of α-amylase is a plausible explanation for the reduced GI of sourdough bread after eliminating the presence of acetic acid in the dough (and, subsequently, its effect upon the gastric emptying rate), and the improbable influence of the RS on the GI of the breads in this experiment. However, it is recommended to further investigate the mechanisms behind the reduction of GI in the presence of high amount of soluble fibres.

As presented in section 3.3.8, the specific volume of the sourdough bread enriched with soluble fibres (XG/GA/Pec) was significantly lower than that of control

sourdough bread ($p \leq 0.001$). Burton and Lightowler (2006) demonstrated that the volume of bread can influence the postprandial glycaemic response. Burton and Lightowler (2006) found that bread obtained with the shortest proving time and characterised by the lowest volume generated the lowest glycaemic response. Additionally it was found that a lower core temperature of bread during baking could affect the swelling and gelatinisation of the starch, and the stability of protein-starch interactions could lead to a reduction in GI (Burton and Lightowler, 2006).

The results of this PhD showed that the presence of hydrocolloids in the flour pastes affected the processes of gelatinisation and retrogradation of starch. Perhaps through the modification of the amount of free water available during the thermal processing of starch, it is possible to affect the nutritional properties of starch. This would seem a plausible explanation for the reduced GI of bread enriched with soluble fibre. However, in this study these effects were elucidated in systems containing lactic acid, which exerts its own influence on the nutritional properties of starch. Therefore future research could be undertaken to clarify the influence of high amounts of soluble fibres on the behaviour of gluten-starch systems during digestion and the subsequent absorption of the nutrients.

6.5.1.3.   *Criticism of the glycaemic index concept*

Brouns et al. (2005) acknowledged that glycaemic index has its "pros" and "cons". Dewettinck et al. (2008) labelled the usefulness of GI as debatable because the GI of bread (which is rarely consumed on its own) may be lowered by food commodities served as an accompaniment. Arguably, this is true for most starchy foods, perhaps with the exception of ready-to-eat snacks. However, it was demonstrated in the section 2.11 that GI is a useful research tool in epidemiologic studies. Some evidence exists to suggest that high-GI diets lead to metabolic syndrome, type 2 diabetes and cardiovascular disease. Certain types of cancer may stem from high-GI diets (Jenkins et al., 1987b, Jenkins et al., 1987c, Salmerón et al., 1997a, Salmerón et al., 1997b, Jenkins et al., 2000, Liu et al., 2000, Ford and Liu, 2001, Liu et al., 2001, Brand-Miller, 2003, McMillan-Price and Brand-Miller, 2006, Barclay et al., 2008, Lin et al., 2012).

The glycaemic index represents the effects of standard amount of available carbohydrate which is normally 25g or 50g. The size of the sample containing this amount of carbohydrate may not be representative of what is usually consumed (Kirpitch and Maryniuk, 2011). However, glycaemic load (GL) value can be easily calculated if the GI, portion size and carbohydrate content of a food is known, therefore tying the glycaemic effect of a food product to its portion (see section 2.11).

On the other hand, it was observed in this study that the GI value does not provide much of an explanation for the physiological properties of dietary carbohydrates when presented on its own. Previously it was remarked that several factors may influence the GI value of food products and these factors may not have the same health implications (Englyst and Englyst, 2005). Moreover, it is entirely possible that two meals might generate differently shaped GR curves which have the same iAUC, and therefore have the same glycaemic index. Therefore, the information about the shape of the glycaemic response curve, or at least the peak glucose concentration and the time at which the peak concentration of glucose after ingestion occurred should be considered together with the value of glycaemic index. In other words, with GI, peak concentration and the time of the peak, it is possible to discuss the rate of release of glucose from a food samples during digestion. In the view of above, the testing of the glycaemic properties of dietary CHO should not be limited only to GI. Food products should be thoroughly evaluated in terms of the content of any food components, which may alter the release of glucose from food samples.

The between- and within-subject variation in glycaemic response to foods is an inevitable problem associated with GI testing (Venn and Green, 2007). The precision of the measurements could be improved by repeated testing of test and reference foods but with the disadvantage of increasing the cost and duration of the trial as well as the burden to the participants (Brouns et al., 2005). This issue poses a limitation of this PhD study. To reduce the burden on participants, and to perform the research activities within the timeframe prescribed by MMU, all the samples in this study were tested only once.

6.5.2. Satiety response

Satiety response to a meal is a very complex cascade of events (Blundell, 1999). It was not the purpose of this thesis to research in-depth the metabolic implications of the consumption of sourdough bread enriched with soluble fibre. However, without an attempt to explain the phenomena reported in the findings chapter, this thesis would be incomplete.

The results gathered using the SLIM scale show that control sourdough bread and sourdough bread enriched with the mixture of soluble fibres (XG/GA/Pec) produced prolonged satiety sensation in subjects who ingested samples of bread equal to 50g of available carbohydrate. As demonstrated in section 5.5 of this thesis, the iAUC of the satiety responses generated by both of these sourdough breads were significantly higher than those of commercially available white sliced wheat bread or 50g of glucose dissolved in 250g of water. Unlike the bread samples, the glucose solution does not require digestion, which could explain the limited amount of time that this test food spent in the upper gastro-intestinal tract of the participants. The larger size of the sample of sourdough bread enriched with soluble fibres is an effect of high moisture (section 3.3.6) and dietary fibre content (section 3.3.7), which added to the bulk of the sample. This might explain partially the prolonged satiety after ingestion of bread XG/GA/Pec.

The feeling of hunger was present and increasing in participants who ingested WWB 60 minutes from ingestion (section 5.5). After the consumption of sourdough bread the hunger-free period was extended by 30 minutes and, after the consumption of sourdough bread with XG/GA/Pec, the feeling of hunger appeared only towards the end of the experiment (120 min). Östman et al. (2005) observed that the consumption of breads supplemented with 18, 23 and 28g of vinegar produced significantly higher satiety response in comparison to white bread at 30, 90 and 120 minutes after ingestion. In this PhD study no acetic acid was detected in sourdough samples during fermentation, hence the acetic acid mechanism seems an unlikely explanation of increased satiety of sourdough breads enriched with viscous soluble fibre. However, lactic acid in sourdough bread might have slowed down the rate of digestion of the sourdough bread, perhaps prolonging the passage of the bread through the upper intestinal tract. Soluble fibre content in XG/GA/Pec-enriched

bread could explain the even more pronounced satiety responses reported by the subjects.

Viscous dietary fibre is associated with a reduced rate of gastric emptying and gastrointestinal transit (Dikeman and Fahey Jr, 2006). Pectin was demonstrated to promote satiety through a modification of gastric emptying (Di Lorenzo et al., 1988). A similar trend was observed for other sources of soluble fibre (psyllium, guar gum) (Slavin and Green, 2007). It was discussed in chapter 2 that GA forms highly viscous solutions in concentrations of 50% and over (Verbeken et al., 2003, Belitz et al., 2004). However, XG and pectins create viscous solutions in lower concentrations, and for this reason both of the substances are used as thickeners in food and beverage industry. It is possible that these two viscous fibres modified the transit time of the sourdough bread from stomach to duodenum in the present study, thus promoting the satiety perception as effected through nerve vagus stimulation.

Increased consumption of two blends of gum arabic also has been reported to promote satiety and reduce the calorific intake during the second *ad libitum* meal (Calame et al., 2011). Because of the previously mentioned low viscosity in diluted solutions, the mechanism of this action of gum arabic is presently unknown and requires further research. Nonetheless, together with the application of viscous fibres, such as xanthan gum and pectin, gum arabic might offer a promising perspective in weight loss programmes based on reduced calorie intake. Additionally, if future research confirms the satiatogenic properties of this type of bread, these might be of interest to the hospitality industry, offering a way to reduce the size of the portion of the main meal, if a starter containing satiating bread was served first.

6.6. Model describing the influence of soluble fibre upon the glycaemic and satietogenic properties of sourdough breads

This section presents a model describing the influence of soluble fibre upon the nutritional characteristics of sourdough breads (Figure 42). Discussion presented in section 6.5 allowed the elucidation of mechanisms which may be involved in eliciting lower glycaemic responses by the consumption of sourdough bread enriched with soluble fibres. It is concluded that sourdough and fibre work synergistically;

however, their effects stem from separate mechanisms. For sourdough, it was shown that lactic acid's promotion of gluten-starch interactions was the likely mode of action, since no acetic acid was detected in the doughs. Soluble fibres have the potential to delay gastric emptying and perhaps to reduce the mobility of α-amylase by increasing the viscosity of the digests. The increased compactness of bread crumb (reduced loaf volume) is a possible factor influencing the glycaemic index (Burton and Lightowler, 2006); therefore, it is included in the model as a means of reducing of the rate of starch digestion.
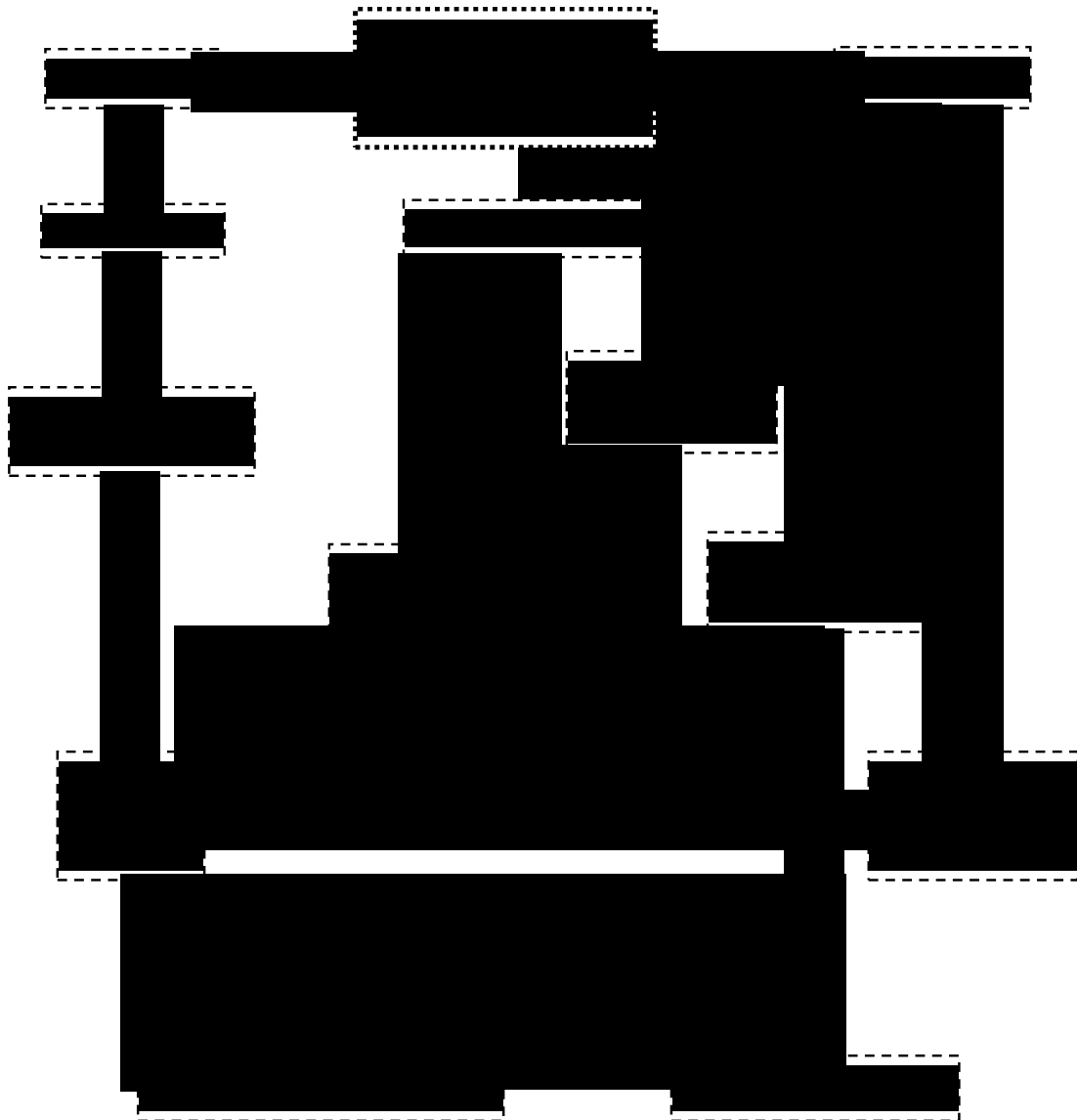
Figure 42. Model describing the influence of soluble fibre upon the glycaemic and satietogenic properties of sourdough breads

Due to the content of sourdough and viscous soluble fibres, it was assumed that their combined effects − strengthening the protein-starch interactions, delayed gastric

emptying, influence on gelatinisation and retrogradation of starch, and reduced mobility of α-amylase − all lead to the slow release of glucose from starch granules. Additionally, the reduced rate of digestion and slow gastric emptying may both contribute towards the increased perception of satiety generated by sourdough bread enriched with soluble fibres.

6.7. Sourdough bread enriched with soluble fibre as a functional food

Sourdough bread enriched with XG/GA/XG fulfils the definition of a functional food product. To reiterate the definition presented in section 1.1,

1. functional food should offer a health benefit, *e.g.* to maintain health;
2. functional food should be produced by either fortification with a functional ingredient or removal of undesired or unhealthy ingredients (*e.g.* salt);
3. the functionality of food should be enhanced beyond the basic function of food as a vehicle for nutrients;
4. the pattern of consumption – functional food should be a part of a daily diet (Doyon and Labrecque, 2008).

The findings of this study fit within the current view that, through the use of sourdough and its lowering of GI of foods, it is possible to achieve the control of glycaemia in healthy subjects (De Angelis et al., 2009). Additionally it was previously shown that sourdough allowed for glycaemic control in subjects with impaired glucose tolerance (Maioli et al., 2008). Moreover, the addition of viscous soluble fibre in this study allowed for further reduction of the glycaemic index. Reduced glycaemia and increased TDF content may contribute to other health benefits, the research of which was not an aim of this study.

The bread XG/GA/Pec was prepared using sourdough technology, which has a proven record of health benefits (section 2.6), and with additional fortification with sources of soluble fibre. The functionality of the bread extends beyond the basic function of bread as a nutrient source. Reduced glycaemia and increased satiety shown in sections 5.4 and 5.5 support this statement, although this study may give a rise to a number of studies pursuing the effects of short and longer-term consumption of this kind of bread. Lastly, bread forms a staple part of daily diet of cultures around

the world, therefore an intervention into the nutritional properties of sourdough breads may yield a functional product. Despite the fact that functionality of the breads developed in this study has been demonstrated, a verification of a health claim by manufacturers interested in the production of sourdough bread enriched with soluble fibres should take place according to EFSA requirements.

6.8. Conclusions

A range of sourdough breads enriched with soluble fibres was developed during this study. One type of bread was chosen from this range of products to evaluate its nutritional properties. This thesis contains the guidelines for the production and processing conditions of this new functional bread. The content of TDF for this bread is 7.28g/100g of product "as eaten". The bread with XG/GA/Pec fits within the definition of functional foods.

Soluble fibre content influenced the behaviour of the dough during the fermentation by counteracting the negative handling properties (stickiness) brought about by sourdough fermentation. It is postulated that the mechanism of this phenomenon is an effect of water binding capacity of the soluble fibres, which results in apparent dough strengthening and reduction of dough stickiness. Hydrocolloids used as a soluble fibre source in this study were demonstrated to influence the pasting properties of flour and flour-bran (9:1) pastes. It was also shown that the addition of soluble fibres to wheat flour can improve retrograding properties of the starch, and therefore delay staling. Soluble fibre had an effect on the shelf-life of the sourdough breads by reducing the water migration from the crumb to crust. This process is one of the mechanisms of the complex process of bread staling. The process of staling was shown to be delayed by the application of high amounts of soluble fibres. Sourdough breads subject to the experiments described within this thesis did not show any visible signs of microbial spoilage, even if stored for a prolonged period of time (several weeks). Sourdough is renowned for being a natural biotechnological method of preserving the food.

The addition of soluble and insoluble fibre to sourdoughs changed the physico-chemical properties of sourdough breads. Crumb firmness, loaf volume and breadcrumb brightness were negatively influenced by the presence of hydrocolloids.

In particular, the bread chosen for the GI test (enriched with XG/GA/Pec) was characterised by low volume and dark crumb which suggests a dense structure of the bread. Low resistant starch concentrations have been recorded for the breads subjected to RS analysis. The concentrations were below 2% based on total starch. Therefore it is not likely that the reduction of GI of sourdough bread enriched with soluble fibres is a result of resistant starch formation.

The glycaemic indices of breads tested in the GI study were: 70 for white sliced bread, 66 for sourdough bread and 59 for sourdough bread enriched with soluble fibre and wheat bran. No acetic acid was detected in sourdoughs considered in this study, and the presence of acetic acid was shown in previous studies to influence gastric emptying and, subsequently, the glycaemic index. An alternative explanation for the reduced GI of the sourdough bread in this experiment is the content of lactic acid and its influence upon protein-starch interactions resulting in slower digestion of the sourdough bread.

Further reduction of GI was achieved by the application of soluble dietary fibres in sourdough bread making – xanthan gum, gum arabic and pectin. The plausible explanation based on previous research into the potential of these hydrocolloids to reduce the postprandial glycaemia, is the increased viscosity of the digests containing these soluble fibres which results in delayed gastric emptying. Furthermore, it is suggested that the viscosity of hydrocolloids-containing digests affects the mobility of α-amylase, delaying the digestion of starch in the bread samples.

Sourdough bread and sourdough bread enriched with the mixture of soluble fibres were characterised by increased perceived satiety by subjects participating in the study. The increased content of viscous soluble fibres and delayed gastric emptying is a plausible explanation of the satietogenic properties of sourdough breads enriched with XG/GA/Pec.

6.9. Recommendations for further work

Based upon the discussion presented within this section, the model developed previously (section 6.6), was updated to include the possible future research (Figure

43). Sourdough breadmaking with flours with varying content of amylose and under varying baking conditions could help to explore the determinants of resistant starch formation. Furthermore, the influence of lactic acid on viscosity of flour-soluble fibre pastes could enable the exploration of the full mechanisms of action of the breads obtained in this study.

This thesis makes a contribution to the field of human nutrition. The pasting properties of hydrocolloid-containing flour pastes might influence the subsequent digestibility of bread, which most probably was the reason for reduced GI of sourdough bread enriched with soluble fibre in this study. However, a further gap in knowledge has been identified as a result of this research: the effects of organic acid addition upon the pasting properties of hydrocolloids-containing flour pastes are unknown and are yet to be elucidated. Furthermore, it has been known for some time that gluten-free breads are characterised by higher GI than wheat and gluten containing breads (Jenkins et al., 1987a). As a result of this study, it is clear that the application of sourdough in conjunction with high amounts of viscous soluble dietary fibre can reduce the GI of bread. However, a new research question can be raised: can these two functional ingredients work in the same manner for gluten-free breads? Both, sourdough and hydrocolloids have been used in gluten-free technology, to improve the texture and palatability of gluten-free breads (Katina et al., 2005, Poutanen et al., 2009). It remains to be investigated whether it is possible to produce low- to medium-GI gluten-free breads using the same or similar technology to the one presented within this thesis.

A potential of sourdough bread containing xanthan gum, gum arabic and pectin in weight management programmes has been revealed through this study. That is not to say, that the consumption of this particular bread would be beneficial to anyone who is willing to lose weight. Based on this research, however, further studies will be required to confirm/disprove the satietogenic potential of sourdough breads enriched with soluble fibres. In particular, the second meal effect should be taken into account and in-depth metabolic studies involving the measurement of blood insulin and incretins should follow to explore the full potential of functional food offered through this research.
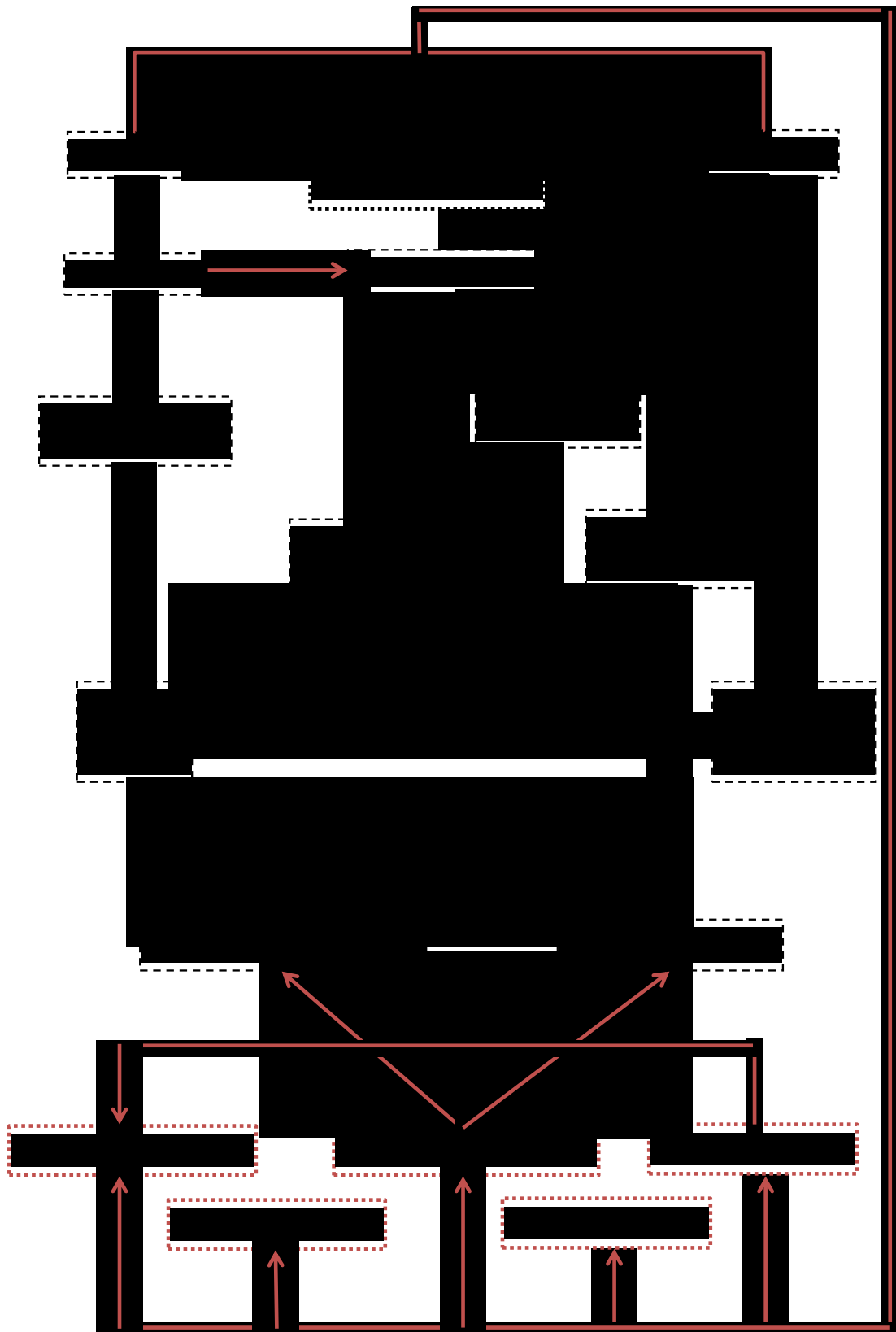
Figure 43. Model showing possible future research based on this PhD study

The mechanism behind satiety is very complex and it is impractical to generalise that reduced gastric emptying and lower starch digestibility were fully responsible for the

increased satiety perception in this study. The metabolic effects of the breads considered in this thesis, other than postprandial glycaemic effect, remain undetermined; therefore, a longer study of a larger group of subjects should be considered. Metabolic effects including insulin and incretin (GIP, GLP-1) response could be performed based on the research described within this thesis. The effect of both types of functional ingredients – organic acids and soluble fibres – in various types of starchy foods might be worth investigating.

Second-meal effects (the calorific intake of *ad libitum* second meal) of sourdough breads enriched with soluble fibre should be investigated alongside the metabolic effects of the consumption of sourdough bread enriched with soluble fibre. By doing so, a greater clarity will be provided with regards to the possibility of reducing food intake during the second meal after the consumption of sourdough bread with high soluble fibre content.

The *in vitro* modelling of large intestinal fermentation of the breads' samples could provide a description of fermentability of such food products. It is expected that a physiologically significant amount of SCFAs is produced during the passage of the breads enriched with soluble fibres through the large intestine. If this was the case, the influence of a sub-acute consumption of this kind of bread on blood pressure, fasting concentration of glucose, and blood concentrations of triglycerides, free fatty acids, HDL and LDL should be elucidated, perhaps in conjunction with the faecal content of SCFAs.

Finally, the in-depth investigation of the influence of the addition of soluble fibres upon the dough and breadcrumb structure of sourdoughs could deliver valuable information which might help to explain reduced GI and increased satiety observed after the consumption of sourdough breads into which soluble fibres are incorporated.

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Appendices

Appendix 1. Example of screenshots used in consumer acceptability test of sourdough breads enriched with soluble fibres

Welcome to this

Sensory Analysis session...

You will be testing five samples of sour dough bread today. Please provide feedback on the sensory properties of the samples by rating the selected attributes.

All your responses will be anonymous and kept confidential.

The samples contain wheat (gluten), and salt. Kindly **participate only if you are not allergic** to any of these ingredients.

Please click on the 'Next Screen' icon below to begin.

Please answer the following.

Your age in years

25 | K

Your gender

☑ **Male**
☐ **Female**

Next screen

What type of bread do you normally use? If you tick other, please specify in the line below.

☑ **White bread**   ☑ **Wholemeal**
☑ **Wholegrain**   ☑ **Sour dough**
☑ **Speciality**   ☐ **Other**

| K

How often do you consume sour dough bread?

☐ **Never**
☐ **Rarely**
☑ **1-3 times/month**
☐ **1-3 times/week**
☐ **4-7 or more times/week**

Have you eaten sour bread before?

☑ **Yes**   ☐ **No**   Next screen

Look at the first sample on the left. The number on the plate should correspond with the **3 digit** number on the screen.

Indicate how much you like the appearance of the sample using the scale below.

Appearance |————————————|————————| 565

Dislike extremely Like Extremely

Next screen

Smell the sample. Indicate how much you like the aroma using the scale below.

Smell |————————————|————————| 565

Dislike extremely Like Extremely
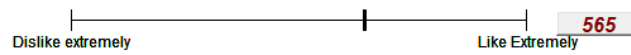
Next screen

.

Taste the sample. Indicate how much you like the flavour of the sample using the scale below.

Flavour

Dislike extremely   Like Extremely   *565*

Next screen

Please indicate how much you like the texture of the sample (how the sample feels in the mouth) using the scale below.

Texture

Dislike extremely   Like Extremely   *565*

Next screen

Please indicate how much you like the aftertaste of the sample using the scale below.

Aftertaste is the taste lingering in the mouth after the sample has been consumed.

Aftertaste

Dislike extremely   Like Extremely   *565*
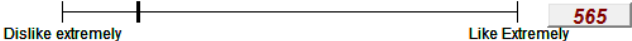
Next screen

Indicate how much you like the sample overall.

Please comment on the product using the box below the scale.

Overall

Dislike extremely   Like Extremely   *565*

565

K
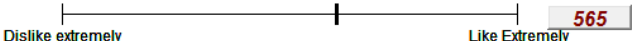
Next screen

Please cleanse your palate by drinking some water.

When the timer shows 00:00, please press the 'Next screen' button.

00:18

Next screen

Appendix 2. Satiety labelled magnitude intensity (SLIM) scale

Please rate the degree of fullness/hunger you feel at this time.

Please put a slash (/) mark somewhere on the line below.

Greatest imaginable fullness

Extremely full

Very full

Moderately full

Slightly full

Neither hungry nor full

Slightly hungry

Moderately hungry

Very hungry

Extremely hungry

Greatest imaginable hunger
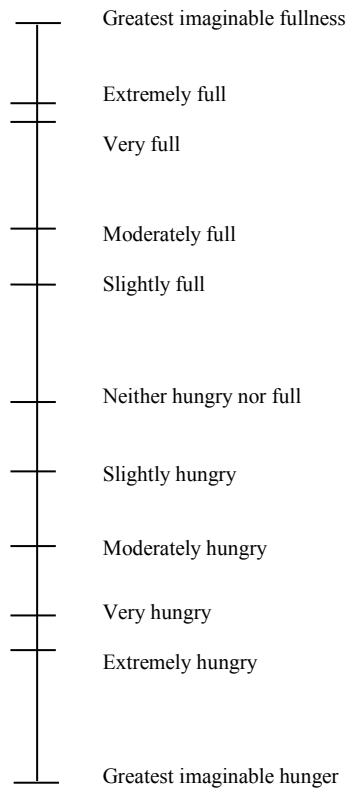
Thank you

Appendix 3. Research information sheet for participants in the GI study

A comparison of different breads' ability to raise blood sugar levels (part of a PhD project, Bartosz Buczkowski)

NB: The samples provided in this trial contain gluten. Anyone suffering gluten/wheat intolerance (Coeliac disease) or food allergies should not participate.

It is recommended that our daily diet should be high in dietary fibre. There is some evidence that regular consumption of high-fibre foods may help to regulate body weight, improve cholesterol profile, blood sugar levels and reduce diabetes risk. Some studies also show that high-fibre diets can reduce the risk of certain types of cancer.

The Food Standards Agency (FSA) advises that adults should aim to consume about 25g of fibre a day. However, findings from UK dietary surveys show that these recommendations are generally not being met.

This study aims to investigate whether eating sourdough bread rich in soluble fibre can help to lower the rate at which sugars from the bread are released into the bloodstream (a measure of glycaemic index) which may, in turn, help to reduce the risk of chronic diseases such as diabetes.

If willing, we would like you to help us test whether the amount of sugar released into human blood stream within two hours after eating is significantly lower for sourdough bread rich in soluble fibre as opposed to control bread. This way you would help us to make a determination whether one kind of bread is healthier than the other kinds of bread.

What would you need to do to take part?

1. **Pre-Study Questionnaire -** If you would like to take part in this study you will need complete a short questionnaire to make sure that you are healthy.
2. **Informed Consent** - After completing the questionnaire, you will be asked to read and sign a consent form before taking part in the main study.
3. **Main study** – In total you will be asked to visit our physiology laboratory four times. On first visit your weight and height will be measured which will enable us to calculate your body mass index (BMI). On each occasion you will be given a sample of bread (approximately 100g) and on one occasion, a sample of 50g of glucose.
4. **Blood samples -** During each visit we will collect 7 blood samples from you. Using finger prick tests we will collect small sample after 0, 15, 30, 45, 60, 90 and 120 minutes. Using these samples your blood sugar levels will be measured.

Each visit will last 2 hours, take place in the morning and you will need to have not eaten anything since 9pm on the night before (only fluids may be consumed without sugar).

Points for consideration:

The trial - Your participation is fully voluntary and appreciated. However, you have a right to withdraw from the trial at any point without giving the reason for your decision.

Your data - We will handle confidentially any information relating to you, according to the Data Protection Act.

The results - The results of the trials may be published in scientific journals after the study is completed. Any work published will not contain names of the participants or any other data related to the participants. However, should you wish to see the results of your trials, please contact Bartosz Buczkowski. He will be happy to answer any questions you might have regarding the study.

Symptoms - It is possible that the increased consumption of dietary fibre during the trials may cause bloating and/or abdominal discomfort. Please report any symptoms to the researcher.

How to contact us

Should you need to get in touch, or have any questions that you might want us to answer for you, please contact:

Mr. Bartosz Buczkowski (researcher), e-mail: b.buczkowski@mmu.ac.uk, tel: 0161 247 2641 or

Dr Emma Derbyshire (supervisor): e-mail: e.derbyshire@mmu.ac.uk, tel: 0161 247 2483.

Thank you.

Appendix 4. Health questionnaire used in the screening of subjects

GENERAL HEALTH QUESTIONNAIRE

A comparison of different breads' ability to raise blood sugar levels (part of a PhD project, Bartosz Buczkowski)

This questionnaire will only take a few minutes to fill. All the information will be kept confidential.

The samples contain gluten. Anyone suffering from gluten intolerance (Coeliac disease) or food allergies should not participate in the trial.

1. Do you have any special dietary requirements?   YES /   NO

If yes, please state: ....

2. Have you been diagnosed with a medical condition, i.e. diabetes, coeliac disease etc.?   YES /   NO

If yes, please state: ....

3. Do you currently take any medicines?   YES /   NO

If yes, please state: ....

4. How many times a week do you exercise?

less than once a week   1-2 times a week   3-4 times a week   more than 4 times a week

5. Are you a regular smoker?   YES /   NO

6. What is your weight?

....

7. What is your height?

....

8. What's your date of birth?

....

9. What's your occupation?

....

Appendix 5. Informed consent form used in the GI study

INFORMED CONSENT FORM

A comparison of different breads' ability to raise blood sugar levels
(part of a PhD project, Bartosz Buczkowski)

	Please initial
I have read and understood the information sheet	....
I understand that the research involves consumption of approximately 100g of bread	....
I understand that I will have to visit Hollings Faculty 4 times	....
I understand that on every occasion 7 blood samples will be taken	....
I understand that I can withdraw from the research at any time without providing a reason	....
I understand that any information about me is confidential and will be treated carefully according to Data Protection Act	....
I have had the opportunity to ask questions	....

I consent to take part in the stated study

Name of participant	Signature	Date
Name of researcher	Signature	Date
Bartosz Buczkowski		

Please contact Bartosz Buczkowski (b.buczkowski@mmu.ac.uk, 0161 247 2641) or Dr Emma Derbyshire (e.derbyshire@mmu.ac.uk, 0161 247 2483) should you require any further information or have any questions.

Appendix 6. Phlebotomy consent form used in the GI study

Participant's ID:.........................

PHLEBOTOMY CONSENT FORM

A comparison of different breads' ability to raise blood sugar levels
(part of a PhD project, Bartosz Buczkowski)

Mr Mark Kelly, technician and a trained phlebotomist, will obtain blood samples using a finger prick. We have to check your medical history and obtain your consent. Please, take your time to answer the following questions:

	PLEASE CIRCLE
Have you had recently or do you currently have any infection?	YES/NO
Have you had recently any surgical procedure?	YES/NO
Have you ever had a stroke or a heart attack?	YES/NO
Do you take any medicines which can influence your blood clotting time (*e.g.* warfarin, acenocoumarol, clopidogrel, low-dose aspirin)?	YES/NO
Do you faint when a blood sample is taken from you?	YES/NO
Do you feel anxious or suffer panic attacks because of needles and/or blood?	YES/NO

I consent to have my blood samples taken for the purpose of the named study

Name of participant	Signature	Date
Name of researcher	Signature	Date
Bartosz Buczkowski		

Should you have any questions or concerns, please speak to the phlebotomist or the researcher, Bartosz Buczkowski (b.buczkowski@mmu.ac.uk, 0161 247 2641).

Alternatively, please contact Dr Emma Derbyshire (e.derbyshire@mmu.ac.uk, 0161 247 2483) should you require any further information or have any questions.

Appendix 7. The content of resistant and available starch of sourdough breads enriched with soluble and insoluble fibre

	Bread type							
	Control		Control with bran		Fibre mix (XG/GA/Pec)		White sliced bread	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Moisture content	33.39 (*n*=3)	0.15	35.86 (*n*=3)	0.13	39.08 (*n*=3)	0.06	32.59 (*n*=3)	0.22
Available starch (% dry matter)	66.69[b,c,d] (*n*=6)	0.39	60.91[a,c,d] (*n*=6)	0.54	55.56[a,b,d] (*n*=6)	0.23	69.76[a,b,c] (*n*=6)	0.62
Available starch (% wb)	44.42[b,c,d] (*n*=6)	0.26	39.07[a,c,d] (*n*=6)	0.35	33.85[a,b,d] (*n*=6)	0.14	47.03[a,b,c] (*n*=6)	0.42
Resistant starch (% dry matter)	1.04[c,d] (*n*=6)	0.03	1.05[c,d] (*n*=8)	0.03	0.77[a,b] (*n*=8)	0.02	0.86[a,b] (*n*=4)	0.03
Resistant starch (% wb)	0.69[c,d] (*n*=6)	0.02	0.67[c,d] (*n*=8)	0.02	0.47[a,b,d] (*n*=8)	0.01	0.58[a,b,c] (*n*=4)	0.02
Total starch (available + resistant)	67.73	-	61.96	-	56.33	-	70.62	-
Resistant starch (% TS)	1.56	-	1.70	-	1.37	-	1.21	-

Differences of values with different superscripts are statistically significant ($p < 0.05$).

Appendix 8. Data obtained from participants: incremental glucose concentrations and satiety response

participant no	time	control sourdough		XG/GA/Pec		WWB		glucose	
		glucose increment (mmol/L)	satiety increment	glucose increment (mmol/L)	satiety increment	glucose increment (mmol/L)	satiety increment	glucose increment (mmol/L)	satiety increment
1	0	0	0	0	0	0	0	0	0
	15	0.7	84	0.6	102	0.3	65	3	4
	30	3.2	76	1.5	89	1.4	54	3.3	-12
	45	2.4	75	2	73	0.9	48	1.9	-13
	60	2.3	62	1.7	64	1.1	38	0.6	-20
	90	2.1	18	1.4	36	0.3	31	1.4	8
	120	1.5	31	1.3	38	1	27	1.6	-22
2	0	0	0	0	0	0	0	0	0
	15	0.1	100	0.4	26	0.3	18	1.7	-2
	30	1	92	2.1	18	1.6	30	2.8	-6
	45	0.5	82	2.6	18	2.2	34	3	-10
	60	0.7	84	1.2	18	1.6	34	2.2	0
	90	0.8	48	1	18	0.7	38	0	-8
	120	0.8	36	0.3	20	0.6	28	-0.4	6
3	0	0	0	0	0	0	0	0	0
	15	0.3	76	0	102	0.2	52	0.6	-2
	30	1.4	70	0.6	102	0.7	38	2.4	12
	45	1.6	68	1.4	87	1.8	46	2.6	-2
	60	1.1	68	1.6	72	1.6	38	2.3	-1
	90	1.4	54	1.2	72	1.4	38	1.4	-6
	120	0.9	60	0.2	64	1.2	20	0.4	-22
4	0	0	0	0	0	0	0	0	0
	15	-0.3	44	0.9	20	-0.1	38	1.2	48
	30	0.6	44	1.3	36	1.4	38	3.3	44
	45	1.9	54	1.9	20	2	31	4.6	26
	60	2.1	38	1.3	49	2	14	5.3	26
	90	0.6	50	0.6	49	0.8	20	3.3	20
	120	0.2	53	1	14	0.6	6	0.5	-2
5	0	0	0	0	0	0	0	0	0
	15	0.9	94	0.9	94	0.4	82	4.5	66
	30	2.1	84	2.3	84	1.6	69	3.8	48
	45	1.7	72	2.7	84	2.1	38	2.4	0
	60	1.2	56	1.9	82	1.5	0	2.7	0
	90	1	20	1.1	20	0.9	-8	2	-12
	120	1	0	0.6	0	0.2	-36	-0.5	-38
6	0	0	0	0	0	0	0	0	0
	15	0.9	74	0.2	120	0.2	78	2.7	24

197

	30	2.7	70	2.5	120	2.5	44	2.9	28
	45	3	70	1.8	118	3.2	38	0.4	40
	60	2.6	50	1.2	78	2.5	24	1	58
	90	0.7	46	1.9	62	1.3	34	1.2	64
	120	0.1	38	1.4	52	-0.1	-8	0	26
7	0	0	0	0	0	0	0	0	0
	15	0.8	116	0.4	130	1.2	102	1.4	114
	30	1.3	118	0.4	114	1.6	102	2.6	44
	45	1.4	112	0.5	118	1.4	102	2.4	36
	60	1.1	56	0	114	0.7	52	2	26
	90	0.9	26	0.7	26	1.4	20	0.6	28
	120	0.6	18	0.8	36	1.1	16	-0.5	8
8	0	0	0	0	0	0	0	0	0
	15	0.5	116	0.5	110	0.3	96	1.7	16
	30	1.7	112	1.5	98	1	92	2.5	16
	45	2.5	98	1.9	98	1.6	88	2.4	14
	60	1.8	92	1.3	98	1.3	86	1.2	12
	90	0.6	74	0.3	62	1.2	62	0.6	6
	120	0.8	52	0.4	44	0.1	34	0.7	-6
9	0	0	0	0	0	0	0	0	0
	15	0.1	50	0.1	78	0.3	54	1.7	22
	30	1.8	48	0.8	82	1.8	61	3.4	18
	45	1	40	1.4	70	1.3	58	2.5	16
	60	0.4	42	1.5	74	1.2	58	1	22
	90	0.1	50	0.7	78	1	39	-0.9	2
	120	0.4	12	0.4	68	0.5	50	-0.9	-6
10	0	0	0	0	0	0	0	0	0
	15	1.2	102	0.4	92	0.2	48	2.4	34
	30	2.5	94	0.7	96	2.1	48	3.5	22
	45	1.9	88	0.8	98	2	48	3.1	40
	60	0.5	80	0.5	100	0.5	42	2.9	44
	90	0.7	66	-0.2	96	0.9	30	0.9	50
	120	0	60	-0.3	90	1	10	-1.2	44
11	0	0	0	0	0	0	0	0	0
	15	0.6	64	0.3	84	0.5	74	2.2	102
	30	1.7	64	1.3	84	2.8	74	2.8	102
	45	0.7	56	0.7	78	1.5	74	2.3	56
	60	0.3	18	-0.1	38	1.3	28	1.4	56
	90	0.1	4	0.4	10	0.9	10	1.3	28
	120	-0.1	-20	0.7	38	-0.3	-10	-0.3	12

Appendix 8. Baseline characteristics of participants in the GI study

participant no	age	gender	weight (kg)	height (m)	BMI (kg/m^2)
1	37	F	55	1.65	20.20
2	36	M	88	1.87	25.17
3	24	F	90	1.73	30.07
4	51	F	62	1.62	23.62
5	31	M	80	1.75	26.12
6	46	F	68	1.64	25.28
7	50	F	57	1.63	21.45
8	24	F	67	1.68	23.74
9	21	M	77	1.83	22.99
10	31	F	58	1.63	21.83
11	34	F	58.5	1.685	20.60

Appendix 9. Hollings Faculty Research Ethics Approval Form

Date.........16 January 2012...................

Manchester
Metropolitan
University

MANCHESTER METROPOLITAN UNIVERSITY
HOLLINGS FACULTY

APPLICATION FOR ETHICAL APPROVAL

Introduction

All university activity must be reviewed for ethical approval. In particular, all undergraduate, postgraduate and staff research work, projects and taught programmes must obtain approval from their Faculty Academic Ethics committee (or delegated Departmental Ethics Committee).

APPLICATION PROCEDURE

The form should be completed legibly (preferably typed) and, so far as possible, in a way which would enable a layperson to understand the aims and methods of the research. Every relevant section should be completed. Applicants should also include a copy of any proposed advert, information sheet, consent form and, if relevant, any questionnaire being used. The Principal Investigator should sign the application form. Supporting documents, together with one copy of the full protocol should be sent to the Administrator of the appropriate Faculty Academic Ethics Committee.

Your application will require external ethical approval by an NHS Research Ethics Committee if your research involves staff, patients or premises of the NHS (see guidance notes)

Work with children and vulnerable adults
You will be required to have a Criminal Disclosure, if your work involves children or vulnerable adults.

The Faculty Academic Ethics Committee meets every ......Teem...... and will respond as soon as possible, and where appropriate, will operate a process of expedited review. Applications that require approval by an NHS Research Ethics Committee or a Criminal Disclosure will take longer - perhaps 3 months.

1. DETAILS OF APPLICANT (S)
1.1 Principal Investigator: (Member of staff or student responsible for work)
Name, qualifications, post held, tel. no, e-mail

Bartosz Buczkowski, PhD student, Ex 2641, b.buczkowski@mmu.ac.uk

1.2 Co-Workers and their role in the project: (e.g. students, external collaborators, etc)
Dr Emma Derbyshire, supervisor
Mr Mark Kelly, technician, trained to obtain blood samples

1.3 University Department/Research Institute/Other Unit:

Department of Food and Tourism Management

2. DETAILS OF THE PROJECT
2.1 Title:
Physico-chemical, sensory and nutritional properties ofsourdough breads enriched with soluble fibres.

2.2 Description of Project: (please **outline** the background and the purpose of the research project, 250 words max.).

The application of sourdough in bread production has a long tradition and in recent times the health benefits of sourdough breads were proven by various food scientists. On the other hand, the low intakes of dietary fibre in developed societies remain a problem. This PhD study was developed to address the novel use of hydrocolloids as a source of soluble dietary fibre in sourdough breads. The initial aim of this research was to provide the process enabling the production of sourdough bread rich in soluble fibre. Secondary aims were the description of the breads' physico-chemical properties and the assessment of their sensory properties by taste panel.
At the current stage, however, the nutritional properties of breads obtained previously should be addressed. It is proposed that the Glycaemic Index (postprandial glycaemic response generated by a food product in relation to glycaemic response generated by glucose) of the breads be tested.

Describe what type of study this is (e.g. qualitative or quantitative; also indicate how the data will be collected and analysed). Additional sheets may be attached.

The GI measurement is a quantitative study. The data will be collected through blood glucose level measurement in healthy volunteers. The measurement will be performed according to standard protocol at 0, 15, 30, 45, 60, 90 and 120 minutes from food product consumption. This standard protocol is recommended by FAO and WHO (1998) and its use has been appraised in numerous reviews of GI methodology (e.g. Wolever et al., 2003, Brouns et al., 2005). Each volunteer will be asked to visit the

physiology laboratory four times. Each visit will be preceded by an overnight fasting. On three occasions, bread sample containing 50 g of available carbohydrates (approximately 100 g or two slices of bread) and on one occasion, 50 g of pure glucose will be given to the volunteers.

The breads tested will be:

- soft white bread,
- white sourdough bread,
- sourdough bread enriched with soluble fibre.

with each of the samples, 250 g of water will be provided. Glucose will be dissolved in 250g of water.

The blood samples will be obtained through a finger prick (using a single-use lancing device). The amount of blood required for one determination is 10 μl.

The data will be analysed by repeated measures ANOVA with the use of SPSS v. 19.

2.3 Are you going to use a questionnaire?   YES
(Please attach a copy)

A screening questionnaires will be used to include/exclude the volunteers.

2.4 Start Date / Duration of project:

January – February 2012.

2.5 Location of where the project and data collection will take place:

Hollings Faculty – Physiology Laboratory (Room 2)

2.6 Nature/Source of funding

Part of a PhD, tuition fees paid by RIHSC

2.7 Are there any regulatory requirements? NO
If yes, please give details, e.g., from relevant professional bodies

3. DETAILS OF PARTICIPANTS
3.1 How many?

Up to 20 participants.

3.2 Age:

Adults (18 – 60)

3.3 Sex:
both sexes

3.4 How will they be recruited?
(Attach a copy of any proposed advertisement)

word of mouth, a message in ManMetLife, All staff/students e-mail

3.5 Status of participants: (e.g. students, public, colleagues, children, hospital patients, prisoners, including young offenders, participants with mental illness or learning difficulties.)

public, possibly students and colleagues.

3.6 Inclusion and exclusion from the project: (indicate the criteria to be applied).

Inclusion: healthy adults can participate. All the samples are vegetarian.

Exclusion: diabetes, glucose intolerance and/or other metabolic disorders declared by the volunteers on the General Health Questionnaire; celiac disease, food allergies.

3.7 Payment to volunteers: (indicate any sums to be paid to volunteers).

No incentive will be offered to the volunteers

3.8 Study information:
Have you provided a study information sheet for the participants? YES
Please attach a copy of the information sheet, where appropriate

3.9 Consent:
(A written consent form for the study participants MUST be provided in all cases, unless the research is a questionnaire.)

Have you produced a written consent form for the participants to sign for your records? YES

Please attach as appropriate.

4. RISKS AND HAZARDS
Please respond to the following questions if applicable

4.1 Are there any risks to the researcher and/or participants?
(Give details of the procedures and processes to be undertaken, e.g., if the researcher is a lone-worker.)

Small blood samples will be obtained. The risk of an accidental needlestick injury using a single-use lancing device is very small. The technician obtaining blood samples underwent an appropriate training. The technician is to wear latex gloves and use sterile antibacterial swabs.

Every effort has been taken to avoid creating risks to the participants. The greatest risk to the participants in this study might be a food allergy. All the volunteering participants will be initially screened for food allergies and special dietary

requirements (see questionnaire attached) and any participant declaring a food allergy of any nature or wheat/gluten intolerance will be rejected.

There is a small probability, that the consumption of dietary fibre on one occasion as a part of this research may cause bloating and/or stomach discomfort. This is an unlikely occurrence as some dietary fibre is consumed on a daily basis. However, the participants will be warned of the possibility of experiencing this side-effect.

4.2 State precautions to minimise the risks and possible adverse events:

The person sampling blood has to avoid direct contact with blood by wearing protective gloves (latex or vinyl) when working with blood.

Participants will be screened using a questionnaire (attached) and any participant declaring a food allergy of any nature, will be excluded from the study.

4.3 What discomfort (physical or psychological) danger or interference with normal activities might be suffered by the researcher and/or participant(s)? State precautions which will be taken to minimise them:

There is no danger to the participants. No danger to the researcher.

5. PLEASE DESCRIBE ANY ETHICAL ISSUES RAISED AND HOW YOU INTEND TO ADDRESS THESE:

1. Blood samples are required in order to measure postprandial blood glucose concentration (glycaemic index, GI). 7 samples are going to be obtained from participants on 4 occasions (total of 28 blood samples). The blood is going to be taken by a finger prick with the use of a disposable lancing device at 0, 15, 30, 45, 60, 90 and 120 minutes of experiment. This is the current accepted practice for GI measurement and is required for adequate sensitivity and accuracy of the results (Brouns et al., 2005, FAO/WHO, 1998). Additionally, the amount of blood required is small (10 µl) and no tissue is going to be stored or used in any other way except for the immediate blood glucose level assessment.

2. Food and substances used in the experiment and administered to subjects are wheat sourdough bread, high-fibre sourdough bread (containing soluble fibre

and wheat bran), white sliced wheat bread and pure glucose (50 g in 250 ml of water). The subjects are going to be given a sample of bread equal to 50g of available carbohydrates (approximately 100g of bread) with butter or dairy-free spread if preferred and with 250 ml of water. The samples are going to be allocated randomly, to avoid creating bias. Similar kind of bread was used in a trial by De Angelis et al. (2009).

3. Repetitive and prolonged testing: 7 blood samples on 4 occasions. This is justified as in point 1 (see Brouns et al., 2005).

4. It is anticipated that up to 20 participants will take part in the proposed study. The statistical strength of studies based on 10 subjects was shown to be sufficient (Wolever et al., 2003, Brouns et al., 2005). However, if smaller differences in GI are sought by researchers, or greater precision is needed, the size of the sample may be increased (Brouns et al., 2005). Twenty participants have been used in a recent study of postprandial glycaemic response to sourdough bread with elevated level of dietary fibre (De Angelis et al., 2009).

6. SAFEGUARDS /PROCEDURAL COMPLIANCE
6.1 Confidentiality:

(a) Indicate what steps will be taken to safeguard the confidentiality of participant records. If the data is to be computerised, it will be necessary to ensure compliance with the requirements of the Data Protection Act.

All participants are going to have a random code (ID) assigned for the purpose of the study. The only person to know the codes is the principal researcher (Bartosz Buczkowski).

(b) If you are intending to make any kind of audio or visual recordings of the participants, please answer the following questions:

a. How long will the recordings be retained and how will they be stored?

b. How will they be destroyed at the end of the project?

c. What further use, if any, do you intend to make of the recordings?

6.2 Human Tissue Act:

The Human Tissue Act came into force in November 2004, and requires appropriate consent for, and regulates the removal, storage and use of all human tissue.

    a. Does your project involve taking tissue samples, e.g., blood, urine, hair,   etc., from human subjects?

YES

    b. Will this be discarded when the project is terminated?

     If NO – Explain how the samples will be placed into a tissue bank under the Human Tissue Act regulations:

    The required blood volume (10 µl) will be obtained and immediately used for determination of blood glucose level. No samples will be stored for any period of time.

6.3 Insurance:

The University holds insurance policies that will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their courses. This does **not** extend to clinical negligence. There are no arrangements to provide indemnity and/or compensation in the event of claims for non-negligent harm.

Will the proposed project result in you undertaking any activity that would not be considered as normal University business? If so, please detail below:

The study discussed within this form is a part of a PhD project, and similar kind of research has been conducted within MMU.

6.4 Notification of Adverse Events (e.g., negative reaction, counsellor, etc):
(Indicate precautions taken to avoid adverse reactions.)

Please state the processes/procedures in place to respond to possible adverse reactions.

In the case of clinical research, you will need to abide by specific guidance. This may include notification to GP and ethics committee. **Please seek guidance for up to date advice**, e.g., see the NRES website at http://www.nres.npsa.nhs.uk/
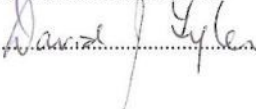
SIGNATURE OF PRINCIPAL INVESTIGATOR   DATE:

B.Buczkowski   28-11-2011

....

SIGNATURE OF FACULTY ACADEMIC ETHICS   DATE:
COMMITTEE CHAIRPERSON:

..............David J Tyler...................................   ......16 January 2012......

APPENDIX

Checklist of attachments needed:
1. Participant consent form
2. Participant information sheet
3. Full protocol
4. Advertising details
5. Insurance notification forms
6. NHS forms (where appropriate)
7. Other evidence of ethical approval (e.g., another University Ethics Committee approval)

REFERENCES

BROUNS, F., BJORCK, I., FRAYN, K. N., GIBBS, A. L., LANG, V., SLAMA, G. & WOLEVER, T. M. S. 2005. Glycaemic index methodology. *Nutrition Research Reviews,* 18, 145-171.

DE ANGELIS, M., DAMIANO, N., RIZELLO, C. G., CASSONE, A., DI CAGNO, R. & GOBBETTI, M. 2009. Sourdough fermentation as a tool for the manufacture of low-glycaemic index white bread enriched in dietary fibre. *European Food Research and Technology,* 229, 593-601.

FAO/WHO 1998. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. *World Health Organization technical report series,* 868, i-viii, 1-69.

WOLEVER, T. M. S., VORSTER, H. H., BJÖRCK, I., BRAND-MILLER, J., BRIGHENTI, F., MANN, J. I., RAMDATH, D. D., GRANFELDT, Y., HOLT, S., PERRY, T. L., VENTER, C. & WU, X. 2003. Determination of the glycaemic index of foods: Interlaboratory study. *European Journal of Clinical Nutrition,* 57, 475-482.

Appendix 10. Chromatogram of the calibration solutions for lactic acid, acetic acid, butyric acid, ethanol and propionic acid.

211
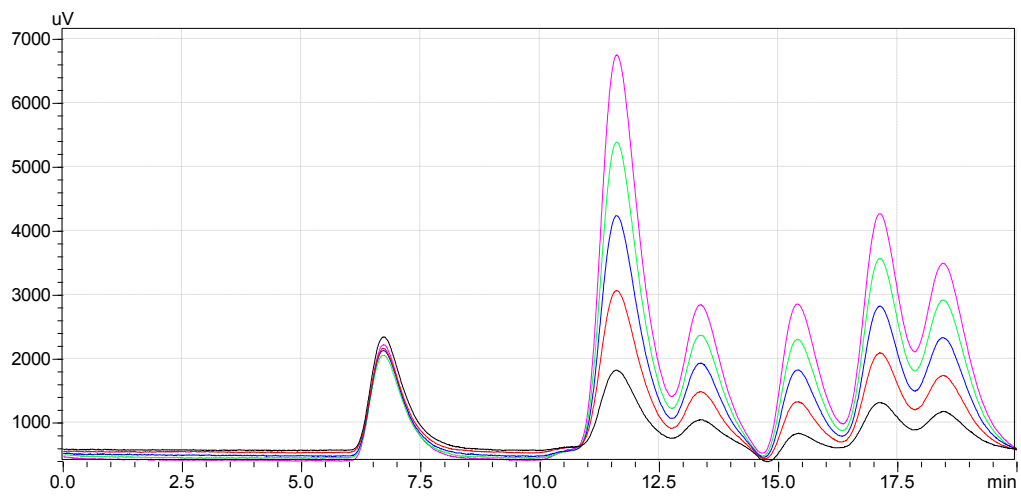
Appendix 11. Scatter plots and correlations between the pH and stickiness of a) control sourdough; b) control sourdough with added wheat bran; c) sourdough with GA and wheat bran; d) sourdough with XG/Pec and wheat bran; and e) sourdough with XG/GA/Pec and wheat bran

c)

R^2 Linear = 0.015

d)

R^2 Linear = 0.083
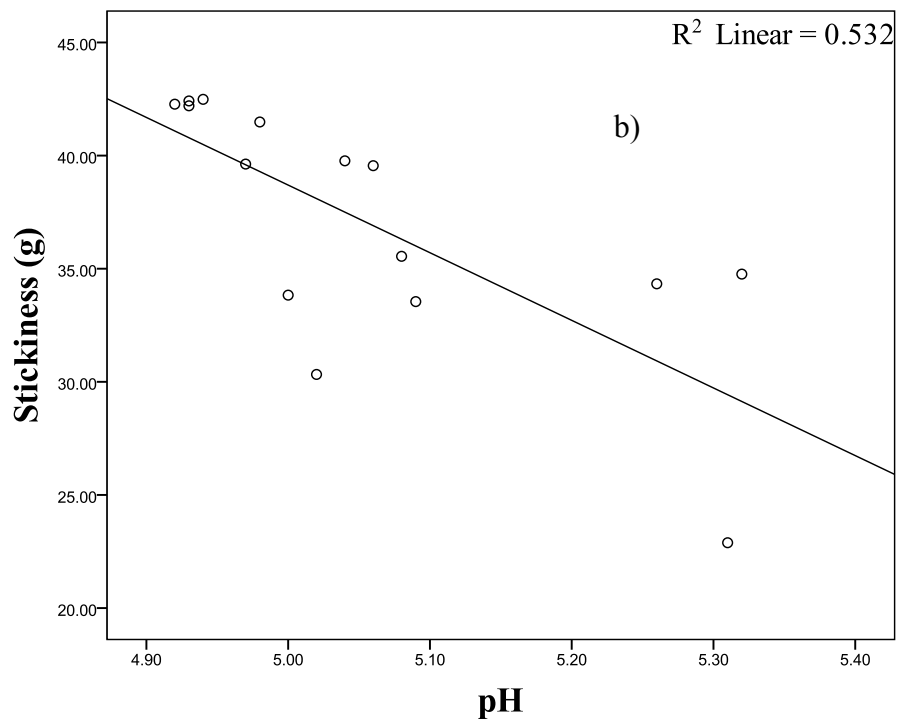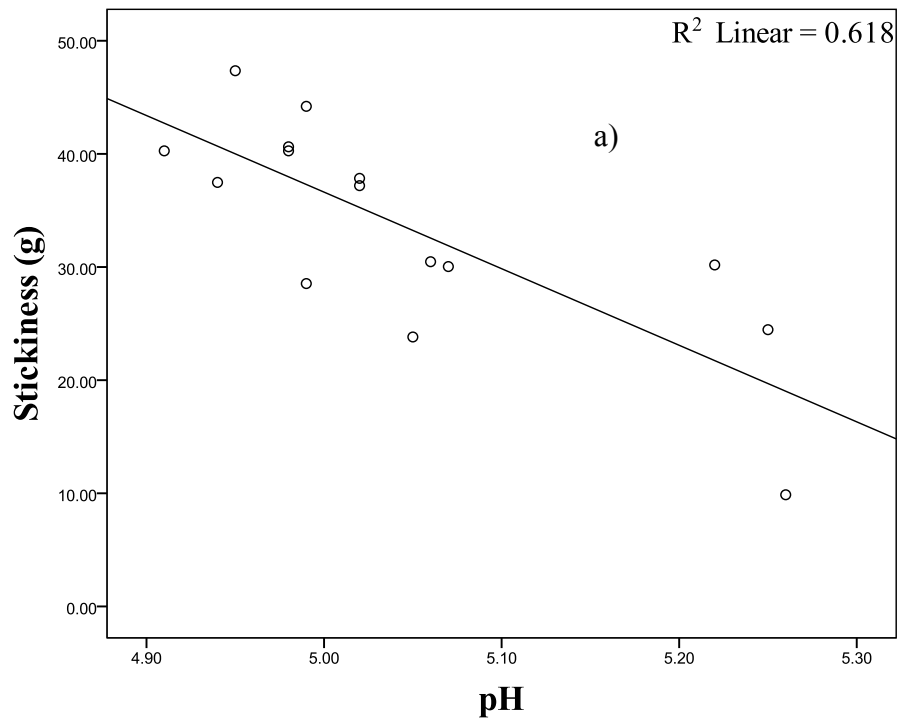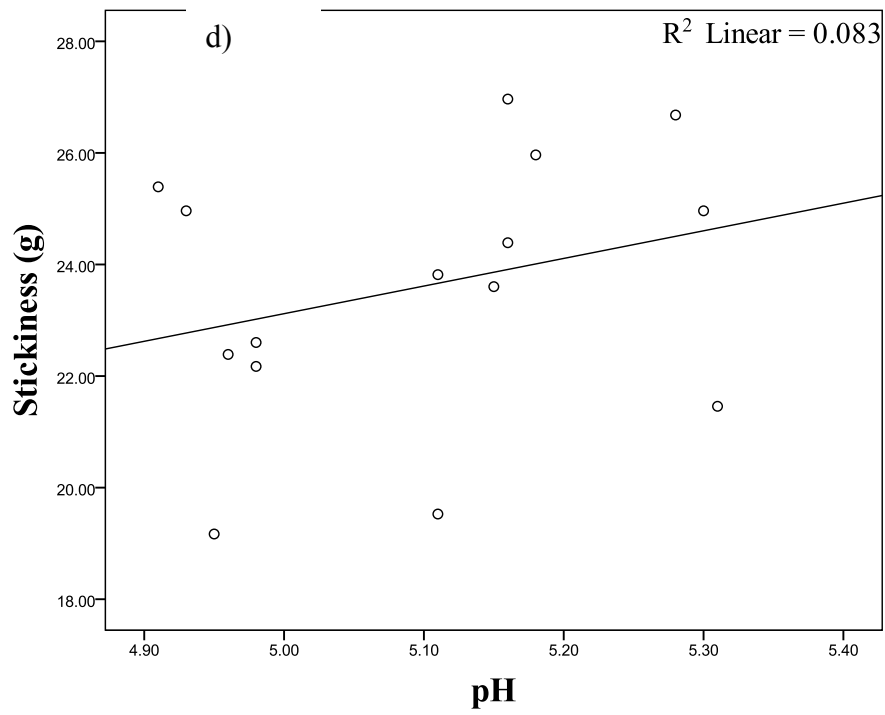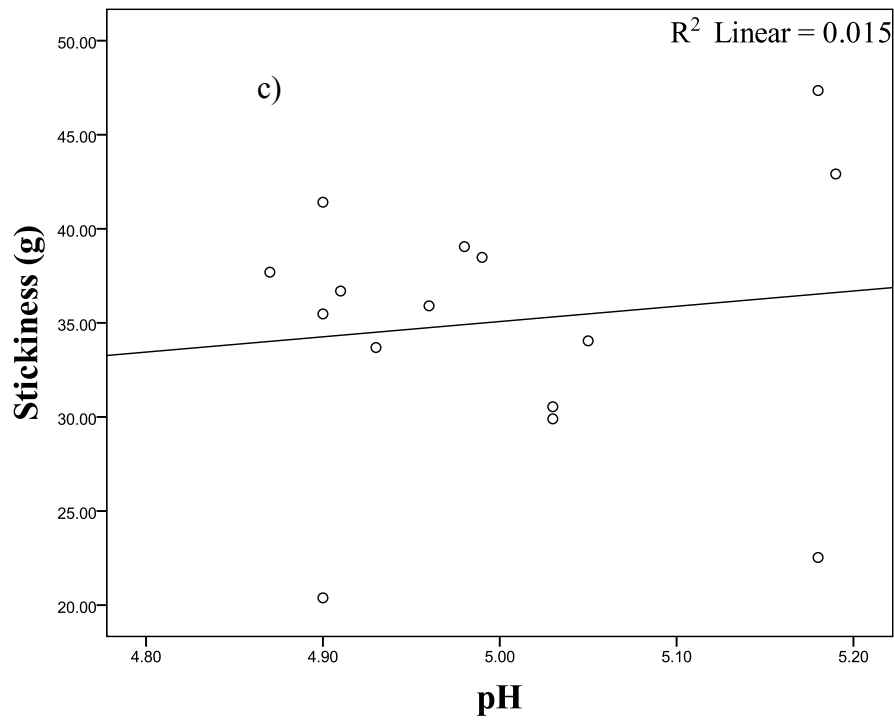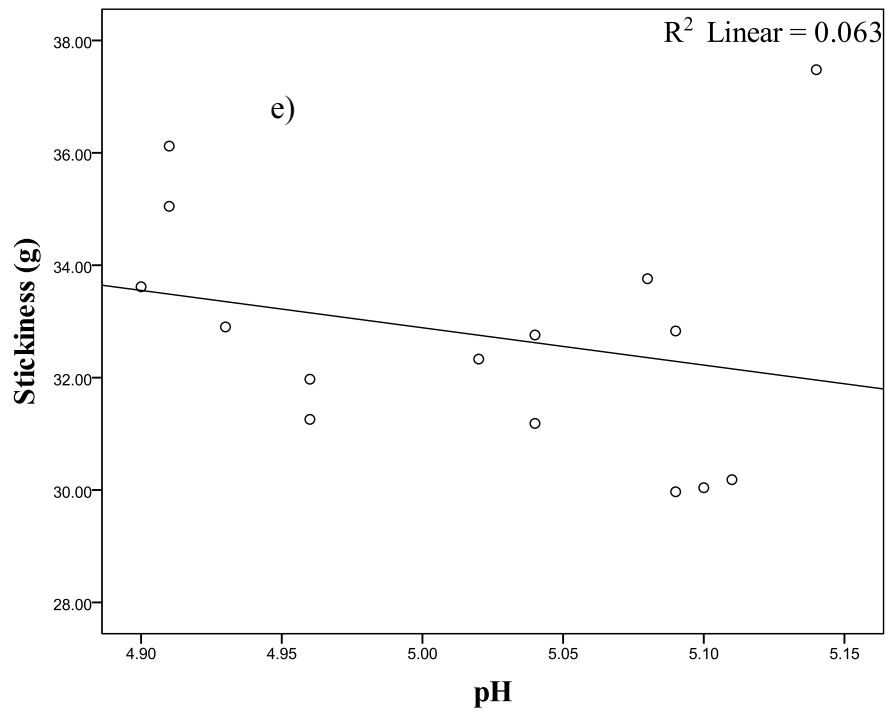
Appendix 12. Scatter plots and correlations between lactic acid concentration and pH of sourdoughs; a) control sourdough, b) control with added bran, and c) sourdough with XG/GA/Pec and bran