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QUALITY AND SHELF LIFE OF PITA AND TANDOORI BREADS SUPPLEMENTED WITH THREE NOVEL FUNCTIONAL INGREDIENTS

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QUALITY AND SHELF LIFE OF PITA AND TANDOORI BREADS
SUPPLEMENTED WITH THREE NOVEL FUNCTIONAL INGREDIENTS

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A thesis submitted to the Plymouth University
in partial fulfilment for the degree of

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Abstract:

The interest for functional foods rich in dietary fibre (DF) and low in glycaemic response (GR) is steadily increasing. This is because DF could reduce GR of food products and protect and restrict diet-associated diseases which have become common in society. But DF rich products are not always high in quality and palatability. Therefore, more fibre-rich functional products need to be designed and developed with good quality, palatability and longer shelf life.

A response surface methodology (RSM) was used to develop both pita and Tandoori breads rich in DF with good quality, palatability and longer shelf life. So, a wide range of three different functional ingredients (FI) (inulin (IN) up to 8%, Guar gum (GG) up to 2% and waxy wheat flour (WWF) up to 15%) alone and in combination was selected, to investigate their influences on the cooking properties, bread quality, sensory characteristics, shelf life and GR. The results showed that the FI affected cooking properties differently. IN and WWF reduced almost all the viscosity parameters. In contrast, GG increased them. The data from dough assessment showed that none of the additives changed the quality and processability. Furthermore, the outcomes from the physical and instrumental assessments of the breads indicated that although there were some modifications in the bread quality, the FI did not change the quality of the breads drastically. The sensory evaluation results showed that the FI addition resulted in acceptable sensory attributes and palatability. The results also showed that some of the sensory attributes were significantly improved through adding the FI. The scanning electron microscopy (SEM) images clearly demonstrated the influence of the FI on the starch granules gelatinisation. It is very evident that the granules are less swollen when DFs are added.

The in vivo trial results indicate that DFs incorporated into bread could change the digestibility of starch and lowered the GR in comparison to the controls. This could be attributed to a number of factors. Gelatinisation may influence the granule structure and degree of amylase and amylopectin readily solubilised. The gels formed by DFs could restrict the accessibility of α-amylase to the starch granules. A mechanism would be linked to viscous layers of soluble DFs becoming physical barriers which could limit the uptake of digested carbohydrate in the small intestine.

Keywords: Glycaemic response, sensory evaluation, shelf-life, IN, GG, WWF
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Author's Declaration:

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Relevant scientific seminars and conferences were attended at which work was presented.

Paper presented at conference-oral presentation


Paper presented at conference-Poster presentation


Word count: 31,790
List of abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AUC</td>
<td>Area under the blood glucose response curve</td>
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<tr>
<td>BD</td>
<td>Breakdown</td>
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<tr>
<td>BSG</td>
<td>Brewer’s spent grain</td>
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<tr>
<td>CHD</td>
<td>Coronary heart diseases</td>
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<tr>
<td>CMC</td>
<td>Carboxymethyl cellulose</td>
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<tr>
<td>DF</td>
<td>Dietary fibre</td>
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<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
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<td>FF</td>
<td>FF= functional food</td>
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<tr>
<td>FI</td>
<td>Functional ingredients</td>
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<tr>
<td>FOS</td>
<td>Fructooligosaccharide</td>
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<td>FUFOSE</td>
<td>Functional food science in Europe</td>
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<tr>
<td>FV</td>
<td>Final viscosity</td>
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<td>GG</td>
<td>Guar gum</td>
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<td>GI</td>
<td>Glycaemic index</td>
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<td>GL</td>
<td>Glycaemic load</td>
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<tr>
<td>GR</td>
<td>Glycaemic response</td>
</tr>
<tr>
<td>ILSI</td>
<td>International Life Sciences Institute</td>
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<tr>
<td>IDF</td>
<td>Insoluble dietary fibre</td>
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<tr>
<td>LV-SEM</td>
<td>Low Vacuum-SEM</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
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<td>mm</td>
<td>Millimetres</td>
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<tr>
<td>µm</td>
<td>Micrometre</td>
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<tr>
<td>PT</td>
<td>Peak time</td>
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<td>PTe</td>
<td>pasting temperature</td>
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<tr>
<td>PV</td>
<td>Peak viscosity</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolutions per minute</td>
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<td>R-SQ</td>
<td>Regression square</td>
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<tr>
<td>RSM</td>
<td>Response Surface Methodology</td>
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<tr>
<td>RVA</td>
<td>Rapid visco analyser</td>
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<td>SB</td>
<td>Setback</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>SD</td>
<td>Soluble dietary fibre</td>
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<tr>
<td>TPA</td>
<td>Texture profile analyser</td>
</tr>
<tr>
<td>Tg</td>
<td>Glass transition temperature</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>USA</td>
<td>United states of America</td>
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<tr>
<td>WWF</td>
<td>Waxy wheat flour</td>
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<tr>
<td>WHC</td>
<td>Water holding capacity</td>
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</table>
Chapter one
Introduction
1.1. Introduction

Obesity and diabetes mellitus are two primary nutritional diseases which are becoming a great concern to public health. There is growing consumer desire and awareness of functional foods (FF) with certain physiological roles in relation to health (Katina et al., 2005, Sivam et al., 2010, Scazzina et al., 2013). Therefore, many food products have been enriched with different food components such as vitamins, minerals and dietary fibre (DF) to increase their nutritional value. Foods supplemented with DF from different sources could be considered as functional food (Filipovic et al., 2007), which have a positive effect on human health.

Cereal products (i.e. Bread) in different forms are important in human daily meals (Simovi D.Š. et al., 2010). In terms of nutrition, cereal foods are essential as they confer food elements such as carbohydrate, protein, DF, vitamins, (Katina et al., 2005, Gellynck et al., 2009) antioxidants and phytochemicals (Sivam et al., 2010).

Cereal products with increased level of DF and antioxidants are desirable in food products because of their health benefits such as reducing the risk of diabetes (Weickert and Pfeiffer, 2008, Al-Dmoor, 2012), attenuation risks of high blood cholesterol (Anderson et al., 2009), reducing faecal transit time (Feldheim and Wisker, 2000), elimination of constipation, decrease in colorectal cancer, reductions in hypertension (Burke et al., 2001), diminishing incidents of cardiovascular diseases (Keogh et al., 2003, Esposito et al., 2005) as well as enhancing the growth of beneficial microflora (Tudorica et al., 2002, Pereira et al., 2004, Burke et al., 2001, Jenkins et al., 1979). These are primarily due to the advantage roles of DF in providing and supporting body with several physiological and metabolic influences on the gastrointestinal tract and
carbohydrate and fat metabolism (Wang et al., 2002, Park et al., 1997, Borchani et al., 2010). Furthermore, DF can be well-used as a fat replacement to re-obtain lost mouth feel, when starch-based carbohydrate is eliminated (Simovi D.Š. et al., 2010). Therefore, health experts suggest diminishing the consumption of animal protein and fat and increase of cereal intake, which is in the majority of European countries. Since the majority of western food contains less than 20g/day (Katina et al., 2005, Cummings and 2000). Meanwhile, the suggested DF amount for daily consumption is about 38g for male and 25g for female (Trumbo et al., 2002, Sendra et al., 2010, Anderson et al., 2009). So, enriching bread with DF might be a convenient mechanism to raise DF intake in the daily diet (Sangnark and Noomhorm, 2004b, Goesaert et al., 2005). Various DF can be added to bread including guar gum and modified cellulose (Pomeranz et al., 1977), cereal bran (Ranhotra et al., 1990), β-glucan (Knuckles et al., 1997), flaxseed (Koca and Anil, 2007), date seed fibre (Bouaziz et al., 2010), and date flesh fibre (Borchani et al., 2010). However, DF possibly causes some alterations in the quality, texture, dough rheological behaviour, properties and overall acceptability of bread (Simovi D.Š. et al., 2010, Aravind et al., 2012b, Ktenioudaki and Gallagher, 2012). Bearing in mind, good quality properties with high level of safety and convenience should remain a precondition for consumers. Several functional ingredients (FI) have been used in the bakery industry particularly in bread to find their influences on the final-product during producing steps to improve product characteristics regarding nutritional and technical aspects (Rodge et al., 2012, Morris and Morris, 2012, Mohammed et al., 2012, Qin et al., 2009, Angioloni and Collar, 2009b). These authors have acquired different results with dissimilar final-product quality, which apparently depends on the properties of the FI added into a particular
product. In bread, this might be ascribed to the impact of FI on flour pasting and visco-elastic properties, gel texture as well as dough rheology (Angioloni and Collar, 2009b).

FI like IN, GG and WWF have been predominantly utilised in the bakery industry as a result of their technical properties and nutritional and health benefits which they offer. Alongside their health benefits, studies have emphasised the influential effect of gum and IN on gelatinisation profile and pasting properties of flour in starch based food products. Additionally, they have been utilised in industry as stabilisers and thickeners to improve dough quality and rheology and extend shelf-life (Chaisawang and Suphantharika, 2006). For instance, it has been declared that adding gums to flour resulted in a less sticky dough and improved dough strength (Angioloni and Collar, 2008), which could develop handling properties along with increased bread volume through improving gas retention capacity. This is due to gum interaction with biopolymers in the flour which could work synergistically to alter the pasting parameters and rheological traits of the dough (Chaisawang and Suphantharika, 2006). IN is found to have an interesting role in some aspects of food technology due to its physiological properties such solubility and creaminess that can improve product texture and improve mouth-feel. In the bakery industry, particularly bread making, IN could ameliorate some aspect of bread crust and crumb quality for instance, although it perhaps reduces bread volume (Mandala et al., 2009). WWF is flour where the starch primarily comprises of amylopectin with less or no amylose. WWF has been widely used (fully or partially) in cereal products such as bread for instance, and can prolong shelf life through hindering retrogradation of starch molecules (Hung et al., 2007a). The assumption is that amylose foremost is responsible for the
retrogradation of starch after gelatinisation and cooling which could lead to a less soft product. So, lack of amylose could lead to delay retrogradation of starch and improve product quality through giving glutinous configuration as well as improving and extend shelf life of the end-product (Chakraborty et al., 2004, Hung et al., 2007a).
Chapter two

Literature review
2.1. Functional food and its importance

Due to changes in lifestyle and development in technology, in recent years, the amount of daily energy expenditure has been reduced. Likewise, the consumption of foods that are high in fats and carbohydrate and low in some micronutrients and DF that are crucial to health is steadily increasing (Kwak and Jukes, 2001). Consequently, many health problems could occur such as, cardiovascular diseases, diabetes, high blood pressure etc. The public desire for healthy foods is steadily increasing as nutrition directly contributes to human health, as well as well-being rather than merely satisfying hunger. Furthermore, due to public concern about health, the demand for a healthy diet is increasing to reduce mortality and improve life quality (Siró et al., 2008, Betoret et al., 2011).

Diet has a substantial role in sustaining human health as it meets the need of the body for essential nutrients and energy for normal physiology. Moreover, alongside providing satiety, food provides pleasant tastes and flavours. However, it is postulated that diet plays an important role in the body’s health maintenance through influencing risks of diseases. For these reasons, there is are new kinds of food which are commonly labelled as FF (also called ‘nutraceuticals’ ‘novel foods’, ‘pharmaceuticals’, ‘pharma foods’ and designer foods ). These have been developed to meet the demand of consumers to provide the body with certain physiological benefits. Although all food products might be functional to some extent, functional foods provide the body with some beneficial support beyond their role in providing the body with essential nutrients (Granato et al., 2010a).
Nutritionally, this type of product contains one or more FI such as DF, phenols, probiotics, vitamins and minerals which are created to carry certain metabolic and physiological benefits to reduce risks of diet-associated diseases (Granato et al., 2010b). The ingredients vary according to the type of fortified food, for instance; some dairy products are fortified with probiotics which are live microorganisms which have positive effects on colon health and reduce the number of pathogenic bacteria. In a similar way, soluble dietary fibre (SDF) are added to dairy products (Tudorica et al., 2004) to replace fats or to increase the DF content and thus reduce the energy and fat content. It can be concluded that the purposes of FF are to reduce the risks of diseases, improve life quality, extended the life span and finally produce healthy and easy to prepare products for consumers (Roberfroid, 1999, Arvanitoyannis and Van Houwelingen-Koukaliaroglou, 2005).

Commercially, market for FF is steadily flourishing in some countries like the Netherlands and Spain. Whilst in some other countries such as the United Kingdom (UK), Germany and France the market for FF is considerably increasing (Annunziata and Vecchio, 2011). However, FF production is a costly process which requires a comprehensive knowledge about the consumers’ attitude, database of marketing, quality (i.e. taste, appearance), functionality or/and physiological demand, appeal as well as the cost of a certain product (Betoret et al., 2011). So, before marketing food products as FF, it is necessary to take into consideration the previous points to make a sustainable and successful business.
2.2. Concept and definition of functional food

The concept of FF belongs to the middle of 1980s, when they were first promoted in Japan by some scientists who studied the connection between nutrition, supplementation, and alteration of food digestion. Then the interest for FF spread over the EU and US (Siró et al., 2008). The term of FF refers to the food that is enriched with a novel ingredient that possess positive physiological influences (Van Kleef et al., 2005). This concept has been described many times and there is no a single agreed definition to precisely describe FF (Alzamora et al., 2005). To date, various food scientists, organisations and academics have suggested different definitions for FF for example; “foods that provide health benefits beyond basic nutrition” (Lynnette R, 2009), Functional Food Science in Europe (FUFOSE) supported by International Life Science Institute (ILSI) defined FF as “a food product can only be considered functional if together with the basic nutritional impact effect on one or more functions of the human organs thus either improving the general and physiological condition or/and decreasing the risk of the evaluation of disease. The quantity and form of the FF should be as it is normally expected for dietary purposes. Therefore it could not be in the form of a pill or capsule just as normal food form” (Roberfroid, 2007). Alzamora et al, (2005) and Robertforid (2007) stated that the European Consensus on “Scientific Concepts of Functional Foods” have described FF as “a food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease”.
Even though there is no an agreed definition for FF, there are some features that should exist in a food in order to be labelled food as functional. According the previous literature, the following properties describe FFs (Roberfroid, 2002, Kwak and Jukes, 2001, Shahidi, 2004, Doyon and Labrecque, 2008);

- Possess ingredients that effectively improve a number of the body functions alongside its nutritive influence.
- They should be in the form of conventional food rather than tablet, capsules or pills.
- Their mission should be to decrease risks of diseases not to promote them.
- It should be safe and accepted by the food regulations.
- It should be consumed daily as a portion of normal daily diet.

2.3. How foods can be changed to functional

All kinds of food from different sources can be labelled as functional since they supply the body with some essential nutrients to meet daily requirements and maintain a body’s well-being. However, FFs perhaps have more benefits that discriminate them from conventional foods as they provide the body with some physiological and metabolic functions beyond the nutritive value. Therefore, there are numerous approaches to switch particular foods to functional. This is through different mechanisms (Roberfroid, 1999, Siró et al., 2008, Henry, 2010).

For instance

1- By increasing the amount of a natural food ingredient to become a quantity which has more potential to stimulate positive influences (e.g. enriching product with some components such as DF).
2- Supplementation of a constituent that does not exist naturally in foodstuffs, but has been proved to be salutary such as a non-vitamin antioxidant and adding a sterol or beneficial bacteria to margarine.

3- Through replacing or removing a component that is consumed more than recommended and could have an unhealthy effect by a component that has been proven to have a positive physiological effect in a positive way such as replacing fats with DF in dairy products yoghurt and ice cream.

4-Through naturally stimulating the proportion of an essential food component (enhancement of the bioavailability) either by special breeding or producing or feeding with a novel product and/or genetically modifying for instance; feeding chicken to produce eggs with a high content of omega-3 fats or tomato stimulated to produce a high lycopene content.
2.4. Functional food ingredients related to bakery products:

2.4.1. Dietary fibre

DF contains a large number of different elements that vary in chemical, physical and physiological characteristics. The concept of DF was initially used in 1953 by Hipsley after observing rare incidents of pregnancy toxaemia in those people who consumed high fibre foods (Brownlee 2011).

There is no unified description of DF that is agreed worldwide. This is attributed to the difference in plant-derived materials and the analytical procedures should in the definition (Buttriss and Stokes, 2008) as well as physiological description that is given (Ktenioudaki and Gallagher, 2012). Firstly, DF was defined by Hipsley in 1953 as “non-digestible component of plants that make up the plant cell wall for example cellulose and hemicellulose (Brownlee, 2011). Then, it was claimed that gums and mucilage (non-digestible polysaccharide) are related to plant cell wall but not strictly a part of it and contribute to the fibre diet content (Trowell, 1976). After that the definition remained the same until 2000s when the American Association of Cereal Chemists (AACC) - scientific review committee defined DF as the edible part of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Later, some scientists and experts redefined DF for instance; Weickert & Pfeiffer (2008) described DF as “any non-digestible carbohydrates and lignin that are not degraded in the upper gut”.

DF has been classified by different researchers based on various factors such as; chemical group, solubility in water and the source. According to the chemical group, DF has been classified into: non starch poly saccharides (i.e. cellulose, hemicellulose), polyfructose (i.e. IN ), gums (i.e. pectin),
oligosaccharides, (attached to polysaccharides and increased indigestibility) analogous carbohydrates (i.e. by-products of food production) and other plant substances (i.e. mucilage) (Papathanasopoulos and Camilleri, 2010). DF has also been classified according to the chemical structure, into two classes such as linear and branched molecular structure. Furthermore, classification by source is another classification which is less common than others and classifies polysaccharides into plant polysaccharides and animal polysaccharides (Chawla and Patil, 2010).

There is another classification which is simpler than the previous ones, and classifies DF into two types according to solubility in water into soluble, such pectin, gums and dextrin, and insoluble such cellulose and hemicellulose (Elleuch et al., 2011).

2.4.2. Sources of Dietary fibre
Currently, various types of DF are present in different sources of food in dissimilar amount. In addition, DF is accumulated in different places of the source. For example some of them are present in the plant cell wall, while some of them prevalent in roots and tubers of plants. The table (2.1) below shows types and sources of some DF.
Table (2.1) Common types of DFs and their sources (Buttriss and Stokes, 2008, Papathanasopoulos and Camilleri, 2010)

<table>
<thead>
<tr>
<th>Fibre components</th>
<th>Food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin</td>
<td>Fruits and vegetables cell wall and intracellular tissue. Vegetables, legumes and nuts represent 15-20% pectin fibre.</td>
</tr>
<tr>
<td>Gums and mucilage</td>
<td>Gums: plant exudates, seeds and seaweed extracts. Mucilage like psyllium: exist in the cell of the external layers of seeds.</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Nearly 1/3 fibres of fruits, vegetables, nuts and legumes comprise of hemicellulose. Mainly found in cereal grains.</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Essential component of plant cell wall, 1/4 of the grains and fruit fibre and nearly 1/3 in vegetables and nuts. Cellulose is abundant in cereal bran.</td>
</tr>
<tr>
<td>Lignin</td>
<td>External layer of cereal grains as well as woody component foods for instance; celery.</td>
</tr>
<tr>
<td>β-glucan</td>
<td>Essential compound of cell wall substances of barley and oats and small quantity observed in wheat.</td>
</tr>
<tr>
<td>Alginate</td>
<td>Present in cell wall of brown algae</td>
</tr>
<tr>
<td>Non-digestible oligosaccharide(NDOs)</td>
<td>Main sources include onion, Jerusalem artichokes and chicory.</td>
</tr>
<tr>
<td>Other synthetic Carbohydrate compounds</td>
<td>Polydextrose, for instance. It is used as a sugar replacement in some reduced energy products and as a bulking agent as well as to give texture.</td>
</tr>
<tr>
<td>Other small components</td>
<td>For example phytic acid is related with fibre in some products like cereal grain. Other components related with fibre are tannins, phytosterols and cutins.</td>
</tr>
</tbody>
</table>

2.4. 3. Daily requirement of DF intake
The relationship between DF intake and reduction of risks of non-communicable diseases such as CHD, hyperglycaemia, colorectal cancer and hypertension has been confirmed. This is after many scientific investigations where
researchers have found fewer incidents of those diseases in those populations who consume a diet with a high ratio of DF.

Because of the variance in methods and assay techniques for quantifying DF, the average of daily intake required of certain types of DF (soluble dietary fibre (SDF), insoluble dietary fibre (IDF), vegetables, fruits and cereals) is disputable (Pereira et al., 2004). Therefore several scientific researchers and food organisations have recommended different quantities. The majority of recommendations for the required amount of DF are proposed according to the results obtained from investigations linking DF with health outcomes (Kranz et al., 2012). Also, DF intake is suggested according to different parameters such as age, sex and calorie intake, for instance generally the recommended for an adult woman and man is 28 and 35 g/day and for each 1000kcal intake 14g DF is recommended (Anderson et al., 2009, Nicklas et al., 2011). Unfortunately, often people fail to consume the quantity of DF that has been recommended by health authorities (Ktenioudaki and Gallagher, 2012). For example in the USA the proposed daily intake is 20 to 30g/d, but the amount consumed is reported to be 14 to 18 g/d (Nicklas et al., 2011).

Due to the factors mentioned earlier, the recommended quantity of DF that should be taken daily is dissimilar worldwide (Table 2.2). Consequently, different amounts have been recommended by various countries as a daily requirement.
Table (2.2) Daily recommended quantities of DF for adults in some countries (Galvin et al., 2001, Manzi et al., 2001, Buttriss and Stokes, 2008, Pereira et al., 2004)

<table>
<thead>
<tr>
<th>Country</th>
<th>Amount of DF (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>United kingdom</td>
<td>18</td>
</tr>
<tr>
<td>Australia</td>
<td>25 to 30</td>
</tr>
<tr>
<td>America</td>
<td>20 to 30</td>
</tr>
<tr>
<td>New Zealand</td>
<td>25 to 30</td>
</tr>
<tr>
<td>Japan</td>
<td>20 to 25</td>
</tr>
<tr>
<td>Italy</td>
<td>30</td>
</tr>
<tr>
<td>Germany</td>
<td>30</td>
</tr>
</tbody>
</table>

2.4.4. The potential health benefits of some FI

There are numerous benefits of FI. Some potential health benefits of DF are shown below (Table 2.3).

Table (2.3) The potential health benefits of DFs; adapted from (Weickert and Pfeiffer, 2008).

<table>
<thead>
<tr>
<th>SDF</th>
<th>IDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-prandial glucose response</td>
<td>Risks of Type 2 diabetes</td>
</tr>
<tr>
<td>Total and LDL cholesterol</td>
<td>Insulin sensitivity</td>
</tr>
<tr>
<td>Gastric emptying time</td>
<td>Gut transit time</td>
</tr>
</tbody>
</table>

Short chain fatty acids (SCFA) that is produced from fermented SDF

<table>
<thead>
<tr>
<th>Upward effects</th>
<th>Downward effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulking effect</td>
<td>Gut hormones</td>
</tr>
<tr>
<td>Energy density</td>
<td>Weight gain</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
</tbody>
</table>
2.4.5. Health aspects of fibre
The desire for DF has steadily increased since “the dietary fibre hypothesis” suggested that DF is counted as a highly essential nutrient particularly, for preventing chronic diseases and helping in reducing their risks (Shin, 2012). DF provides an important role in the digestive system, and in fat and carbohydrate metabolism in the small and large intestine, thereby modulating the effect of enzyme activity and nutrient absorption. So health authorities recommend the public to consume a high proportion of DF in the part of daily diet and decrease consumption of animal fat and protein.

A high intake of DF has an inverse correlation with some chronic diseases CHD such as diabetes and colorectal diseases through influence the digestion (Ktenioudaki and Gallagher, 2012, Devries, 2003). It has been found that IDF can have many health benefits such as: insulin secretion enhancement, decreasing intestinal transit time as well as laxation (Aravind et al., 2012a). Similarly, it is been asserted that SDF can positively modulate the digestion of carbohydrate and reducing hyperglycaemia (Kaczmarczyk et al., 2012). Furthermore, it can protect the colon from diverticulosis and cancer (O’Callaghan et al., 2012).

2.4.5.1. Effect of DF on Diabetes
Diabetes mellitus is a complicated metabolic disorder that occurs due to imperfections in the insulin secretion and function (Bhathena and Velasquez, 2002, Willett et al., 2002). It has been postulated that food with a high glycaemic index could increase the risk of diabetes through two mechanisms; 1- long term intake of high glycaemic index foods requires more insulin and this leads to pancreatic fatigue and as a result causes glucose intolerance,2- high glycaemic index products could stimulate insulin resistance (Brand-Miller, 2003,
Willett et al., 2002). Thus, this all leads to an accumulation of glucose in the blood stream which causes hyperglycaemia.

Diabetes mellitus is one of the most widespread diseases over the world. According to the Center of Disease Control and prevention in 2010, there were approximately 19 million people in the US who were diagnosed with this disease and in 2010 approximately 2 million people who recently diagnosed with this disease were above 20 years old (Post et al., 2012).

Many studies have confirmed the ability of DF in diminishing risks of diabetes through reducing the effect on plasma glucose and insulin activity. A study was conducted on the effect of pectin on gastric emptying and sugar uptake. Seven healthy volunteers participated in the study and they took glucose solution with and without pectin. They found that pectin significantly lowered blood glucose (Holt et al., 1979). Weickert et al (2005) conducted a study among 14 healthy women volunteers. They consumed 3 matched pieces (10.4–10.6 g/portion) of enriched bread with oat fibre, wheat fibre and resistant starch for 6 hours on six to eight occasions. They discovered that consuming the daily recommended amount of IDF quickened early insulin response and decreased post prandial glucose (Weickert et al., 2005). The mechanisms by which DF decreased post – prandial glucose and glycaemic index are not fully understood. It is proposed that the viscous properties of SDF in the intestine reduced the rate of absorption of glucose (Butt et al., 2007). A review by Roberts (2011) reported that soluble fibre decreases starch gelatinisation as fibre competes with starch for available water thereby resulting in a reduction in starch gelatinisation. Furthermore, a physical barrier created by the fibre and protein mixture around starch granules and leads to the starch avoiding digestive enzymes (Parada et al., 2011). Ou et al. (2001) conducted an in-vitro study to observe the role of DF
in decreasing post prandial- serum glucose. They utilised two types of IDF; wheat bran and enzyme-resistant starch of maize amylose and three kinds of SDF; carboxy methyl cellulose (CMC), GG, and xanthan gum (XG). They found that the fibres decreased blood glucose through delaying the diffusion, and decreasing glucose concentration and constrained α-amylase action.

2.4.5.1.1. The potential mechanisms of the influence of DF on post-prandial blood glucose

There are numerous mechanisms that have been mentioned in the literature regarding the ability of DF to lower post-prandial blood glucose. Here are some of them.

✧ Producing short chain fatty acids
Short chain fatty acids (SCFA) are produced through fermenting SDF in the upper gut by the microflora. SCFA are believed to have a metabolic effect including regulating glucose secretion and GR. In a study looking at the on the relationship between fermentable DF and SCFA, the results showed that fermentable DF such as IN might help decrease risks of type II diabetes by increasing the concentration of SCFA and controlling gut hormones which may participate in regulating blood glucose (Tarini and Wolever, 2010).

✧ Slowing gastric emptying
Presence of viscous DF is postulated to reduce glucose absorption by slowing stomach emptying and slowing down gut transit time through the small intestine. Consequently, it probably can regulate the glucose absorption, hence produce a decline in post-prandial blood glucose (Dikeman and Fahey Jr, 2006).
Creating physical barriers

It has been postulated that viscous DF can act a physical barrier whereby glucose is avoided from intestine mucosal cells resulting in decreasing glucose diffusion and absorption (Papathanasopoulos and Camilleri, 2010). Furthermore, it was postulated that DF acts as physical barrier that reduces the opportunities of α-amylase to contact starch and degrade it. Furthermore viscous polysaccharides create an unstirred layer which attenuate and extends glucose absorption in the small intestine. This reduction in glucose absorption seems to attenuate glucose concentration in the blood stream (Tudorica, 2004).

Insulin enhancement

It is believed that IDF could play a beneficial role in lowering risks of type II diabetes by reducing post-prandial glucose through improving insulin sensitivity. In a randomized, controlled study among 17 obese individuals with normal glucose, after consuming intake IDF-supplemented bread (31.2 g/day) for three days, the insulin sensitivity was improved (Weickert et al., 2006). Whole grain products with a high proportion of β-glucan improved insulin response. However, using different types of SDF and IDF yielded different results (Kim et al., 2009).

2.4.5.1.2. Concept of glycaemic index (GI)

The term glycaemic index (GI) (comparing food with white bread or glucose) or glycaemic response (GR) (in case of comparing tested food with the same bread control) was firstly suggested by Jenkins and others in 1981 to describe the amount of carbohydrate absorbed after a meal (Jenkins et al., 1981). It is a physiological evaluation that is done to speed of carbohydrate absorption in a
food product via measuring its effect on the blood serum. So, GI is defined as an increased area under the curve of blood glucose after consumption of 50g carbohydrate from an observed food in relation to the area after ingestion of 50g of carbohydrate from a standard food (reference food), typically glucose or white bread (Ludwig, 2000, Willett et al., 2002, Alminger and Eklund-Jonsson, 2008). It can be deduced from the definition that GI does not consider the amount of eaten carbohydrate. Foods have been categorised into three groups according to the GI value, which are low GI food (<55), medium (55-69) and high (> 70). Furthermore, GI is used for people with diabetes for the purpose of guiding them to choose low GI foods (Venn and Green, 2007). Low GI foods have been recommended to protect and/or eliminate some serious diseases such as diabetes, obesity and heart diseases.

**2.4.5.1.3. Parameters modulating food glycaemic index**

There are numerous factors that affect the GI of food products. Differences in the biological and chemical attributes, physiological structure of foods, also the mechanic process, preparation and consumption technique could all lead to variation in the GI value. These parameters could change the structure, form and digestibility of foodstuffs. Hence they could change the GI value. Here are a few factors that could modify the GI of food and then impact on the post-prandial blood glucose:

**Mastication of food**

Long-periods of mastication lead to lower post-prandial glucose than usual mastication in healthy individuals. This could be attributed to initial insulin excretion (Suzuki et al., 2005). When fruit of fruits and vegetables and vegetables ripeness is developed, the GI is diminished. Because the starch
breaks down and converts to monosaccharides which have a lower GI, then it confers a lower GI (Englyst and Cummings, 1986, Arvidsson-Lenner et al., 2004).

**Botanical sources and type**

Differences in sources of DF could also lead dissimilar post-prandial blood glucose responses. This difference could be due to the botanical source which is related to the starch and the proportion of amylose to amylopectin as amylose has a linear structure which is slowly hydrolysed while amylopectin has a branched structure and easier to digest (Allen et al., 2012). The processes like grinding, rolling and pressing can modulate the structural integrity of products thereby affecting glycaemic response.

**Mechanical process and particle size**

Mechanical processes can deform the external coat of the starch granule and germ which leads to reduce particle sizes and hence increases the contact surface area and confer better chance for enzymes and acids to break down food into simpler sugar units, and this makes it easier for absorption by intestine. For instance, reducing the size of 1 inch of potato and make it mash increased GI by \( \frac{1}{4} \) (Wolever et al., 1991). In addition, processes like shearing and/or refining can also increase the GI value. These processes reduce the particle size and improve water absorption and thus also facilitate attack by enzymes. This is perhaps the same for food puree rather than whole foods since apple juice showed higher GI comparing with the whole apple (Vosloo, 2010).
Moisture and heating processes can facilitate swelling and disruption of the granules thereby easing food digestion and absorption of food starches. If a starchy food is cooked, the starch granules rupture and gelatinisation takes place which amylose and amylopectin in starch are changed to the amorphous form and become ready to hydrolyse. This could increase GI, but this could be changed based on the amount of water and cooking time (Pi-Sunyer, 2002, Arvidsson-Lenner et al., 2004). Then if the starch is cooled, it becomes a gel, hence the gel may convert to crystals (according to heat and moisture availability) and become resistance starch, causing reduction in GI (Vosloo, 2010).

The value of GI in compound foods depends on the ratio of each nutrient in a particular food. It has been stated that the amount of carbohydrate in food confers a high GI. Moreover, the proportion of carbohydrate, fat, protein determines the GI of food (Pi-Sunyer, 2002). The author elucidates that protein-rich foods are well-documented to enhance insulin secretion without increasing post-prandial plasma glucose. Furthermore, fats in the diet cause insulin secretion enhancement alongside a reduction in post-prandial glucose.

The presence of DF can effectively modulate carbohydrate metabolism throughout the digestive system. There is an argument about which type of DF (soluble or insoluble) is more involved in the reduction of GI. Some studies suggest insoluble dietary fibre (ISD) fibre modulates GI and some others suggest SDF has a stronger effect on GI. A study by some authors found a
negative correlation between whole grain and fibre consumption with type II diabetes (Montonen et al., 2003). Other studies have stated that SDF is more related to GI reduction (Arvidsson-Lenner et al., 2004, Vosloo, 2010, Pi-Sunyer, 2002). A study in 1990 conducted a survey of 25 types of food about the relationship between DF and GI. They discovered that total DF modulates the GI value (Wolever, 1990). In regards to the question, whether naturally occurring DF is effective in reducing glycaemic response, it has been suggested that added DF could have a more profound impact than naturally occurring DFs since they have a little affect (Pi-Sunyer, 2002, Vosloo, 2010).

2.4.5.1.4. Glycaemic response measurement
Different methods can be followed to measure GI according to the type of subjects (normal and diabetes). For normal subjects, GI could be implemented as follows; after subjects fast overnight, each test food and standard food (white bread or glucose) corresponding to 50g of carbohydrate, is served at different times in a random order. The standard food should be fed at least three times to reduce the variation. The blood sample should be taken from a finger prick at 15, 30, 45, 60, 90, and 120 min from the beginning of test meal. Hence, the area under the glycaemic response curve is calculated to produce the value of GR for each food (Wolever et al., 1991, Wolever, 2004). Arterial blood is another place that can be utilised to take blood samples from the subjects. However, this method is associated with followed by some potential risks that could harm the subjects during and after sampling. Therefore a capillary finger prick is more safe and feasible (Brouns et al., 2005). It is noteworthy that food and water have to be served to keep the balance of fat, protein and water (Tudorica, 2004). GR is varied between individuals according their body status. Thus, it is recommended to recruit healthy individuals to assess GR. Since healthy people
have GR smaller than people who have diabetes and glucose intolerance (Venn and Green, 2007).

2.4.5.2. Effect of FI on satiety and weight management

Satiety is described as a fullness feeling that occurs in between two meals. The level of satiety could determine the amount and time of the subsequent meals. So, inclusion of food with a high level of satiety in a meal could prolong the time between two meals and postpone the subsequent meal. Also, it could have an impact on the quantity eaten at the next meals (Hess, 2010). This could aid weight regulation in the overweight by reduction of food consumption.

Food varieties are quite different in terms of energy density, satiation and satiety which depend on the food constituents and their quantity i.e. water, lipid, sugar, protein and DF. A variety of factors affect and determine the degree of appetite and fullness sensation such as; customs and habit, temper, texture, appearance and form of the food stuffs (Hess, 2010). It is widely recognised that fibrous food could restrict and attenuate energy density in certain food products and enhance and confer satiety feelings sooner and for longer (Burton-Freeman, 2000). There are numerous factors that contribute in enabling DF to have an impact on the digestion and breakdown of food and giving fullness sensation.

DF could affect satiety and energy intake of a food through modifying quality and texture of some fibrous foodstuffs. DF could increase chewiness which requires more efforts, time and saliva for the mastication process. This chewing may produce in a variety of signals and cephalic and gastric response, causing a earlier fullness sensation and preventing over eating (Burton-Freeman, 2000). Furthermore, DF extends the gut transit time as some kinds of fibre
require more saliva in mouth and gastric digestive chemicals. Since it absorbs more liquid and creates a gel which promotes stomach destination (Howarth et al., 2001). The bulking effect and viscous features of fibre possibly results in prolonging the digestion in process the alimentary tract.

Viscous traits of DF cause a physical barrier that could hinder the approach of digestive enzymes in the small intestine and enhance and confer the fullness sensation faster. It has been shown that apples with its natural fibres extended the fullness sensation greater than its fibreless juice (Hess, 2010). Viscous fibre is hypothesised to enclose the nutrients in food which supposedly requires more time to break down and limits contact with the intestine villi to uptake the nutrients (Howarth et al., 2001). Viscous DF also may promote satiety through creating a great matrix in the small intestine which could slow the gastric emptying process (Galisteo et al., 2008). It is worthy of notice that the response to satiety in humans depend on the gender and weight status (obese or normal) (Carroll et al., 2007).

It has been found that females are more sensitive to the inclusion of fibre in foodstuffs. This is presumably associated to the difference and dissimilarity in the cholecystokinin response which conveys satiety signals to the brain. In addition, it is known that weight status is an important influence in relation to fibre consumption and satiety. Obese participants have more potential to decrease food intake with the existence of fibre in food, but further research is essential to confirm that (Burton-Freeman, 2000).

2.4.5.3. Effect of FI on quality of bread
Quality has generally been described as the physical properties of a product (Grunert, 2005). This could include colour, shape, volume and overall
appearance. However, taste, smell and texture have very important effects on the bread quality. Quality of products is one of the main characteristics that have a great impact on the acceptability, consumer perception and marketability of food products. Therefore, from the producers’ point of view, quality of products is considered as an effective factor for consumers which increase the market of certain product.

There are different definitions and descriptions of food quality. It has been reported that quality of food has four dimensions into perspective of consumers which encompasses; firstly flavour, taste, smell and physical appearance confer a pleasure and hedonic feeling to the consumers. Secondly, functionality is a characteristic that affects the consumers’ choice since health is an important issue that is directly related to the quality of life. The method of production is the third dimension that is of concern for consumers: this dimension includes organic production, animal welfare, and presence of genetically modified food. Finally, the convenience of the products to a certain consumers; there are some points that modulate the suitability of a product to certain consumers such whether this product is easy to buy, takes a lot of time and effort to prepare and easy to dispose of the waste (Brunsø et al., 2002).

Bread is a widely consumed commodity worldwide, and its quality is considered to influence its marketability and consumer perception. Quality of bread such as flavour, taste, texture, crumb firmness and crust colour primarily depend on the quality of flour (quantity and quality of gluten) as well as the interaction between starch and gluten. Therefore, removing and adding any ingredients could modulate this interaction thereby resulting in quality change.
Several studies have been carried out on the effects of FI (DFs and/or WWF) on bread quality (crumb and crust quality, colour, taste, and texture). Different research has concluded dissimilar results, which seemingly depend on the chemical structure of FI, their chemical interaction with flour bio-polymers and their behaviour while existing in the system. Wang et al (2002) used carob fibre, IN fibre and pea fibre enriched bread. They discovered that the DFs can impact on enriched bread quality, for instance; loaf volume of the control sample was 906(ml). Meanwhile, the volume of the samples containing carob fibre, IN and pea fibre were 861, 733, and 719 (ml) respectively. On the other hand, the crumb smoothness can be increased when the carob fibre and the pea fibre are added. Gomez et al (2003) studied the effect of pea fibre, cocoa fibre, coffee fibre, micro crystalline fibre, and wheat fibre on the quality of bread. They found that the enriched bread samples had a similar colour to the control, except the cocoa fibre and the coffee fibre. This is due to the reflection of cocoa and coffee fibre colour on bread colour. Also, the volume of the enriched breads was decreased. This is because of the reaction between fibre and gluten caused decreased gas retaining capability of dough which led to reduction of the volume of the bread. Filipović, (2007) and Simović et al. (2010) reported that sugar beet fibre could negatively influence bread quality. The crumb quality considerably deteriorated and the volume of the bread samples with DF was reduced by 35% in comparison to the bread samples without DF (Filipovic et al., 2007). Although WWF is a FI that is relatively new to the market, it has been used in different products to improve the end-product quality. It has been stated that partially adding WWF could upgrade final-product quality which leads to high volume, less firmness, (Van Hung et al., 2006) confer stability and prolong shelf life  (Yi et al., 2009). More details are given at the end of this chapter.
2.4.5.3.1. Bread making and role of some important constituents

Bread making is a staple food which is consumed daily and its history goes back to the 9th century (Mondal and Datta, 2008). Traditionally, bread is produced from wheat flour which mainly consists of gluten (wheat protein) and starch, with some other ingredients such as water, salt and yeast. There are some optional ingredients that are sometimes included in bread making that influence dough rheology and bread quality, such as oil, milk and sugar. Every basic component in bread has an essential role. Water helps other components to produce an appropriate dough consistency and it creates an appropriate condition for biochemical reactions during dough making and bread producing.

Yeast ferments saccharides during the dough making process, acts as a leavening agent, and produces gas and ethanol as a metabolite of saccharide fermentation. Hence it creates cells and converts dough to a stable foam structure (Borsuk et al., 2012). Baking powder (sodium bicarbonate) can be used to produce gases instead of yeast. The aim of adding of salt is to confer a flavour to the end-product and restrict the action of yeast.

Several biochemical interactions occur throughout the process of bread making, mixing ingredients, producing dough and baking (Haraszi et al., 2008). For example; water steams, volume increases due to the action of yeast, starch gelatinises and protein denatures. All these lead to yield a palatable product (Sivam et al., 2010). Starch and gluten are the main components that play an important role on the quality, shelf life and overall acceptability of the product.

Starch

Starch is one of the most prevalent components in many food commodities. Among them cereal grains, a significant amount of starch that effects the
nutritional and technical properties of starchy products. Starch is comprised of polymers of glucose residues as amylose and amyllopectin. Amylose possesses a linear structure of D-glucose residues linked with α-1→4 bonds while amyllopectin possesses a branched structure of D-glucose units linked with α-1→4 bonds and the branches linked α-1→6 glycoside bonds (Van Hung et al., 2006, Lee and Lee, 2012). The proportion of amylose to amyllopectin varies between starch sources, but general proportion is between 25-28% and 75-78% for amylose and amyllopectin respectively.

This ratio has a substantial impact on the physico-chemical, nutritional and technical traits of the grain thereby affecting the quality and appearance of the final product (Blazek and Copeland, 2008). Amylose is less soluble than amyllopectin and reduces peak viscosity and produces less resistance to retrogradation (Van Hung et al., 2006). Additionally, a high amylose to starch ratio produces nutritionally interesting properties due to the resistance to digestion by digestive enzymes so it confers fewer calories (Regina et al., 2006).

Starch is prevalent within granules different in magnitude and morphology according to botanical source (Goesaert et al., 2005). There are two common types of wheat *Triticum aestivum* starch granules which are A and B granules. The type A population have a spherical shape with a diameter nearly 1-10 μM. Meanwhile, the type B population have a lenticular shape with a diameter ranging between 15-40μM (Salman et al., 2009).

Starch undergoes gelatinisation with the presence of adequate heat and moisture. This causes which starch granules to uptake water and irreversibly disrupts crystalline form of starch. This relies on the temperature, quantity of
water and presence of shear rate, hence starch components leach out and the chemical structure of the starch changes from the crystalline to amorphous. When the cooked-starch is cooled down then the viscosity increases since molecules re-associate and form a gel and rearrange the structure into a more ordered crystalline. This process is known as retrogradation (Figure 2.1) (Copeland et al., 2009, Goesaert et al., 2005, Primo-Martín et al., 2007).

Starch absorbs the available water during dough making and swell thereby gelatinisation occurs which causes amylose and amylopectin to leach out. With the presence of high temperature, moisture and mechanical shearing, the mechanical mixing of dough components mainly starch and gluten owing to critical modulation in protein structure. This improves gluten network configuration via fracture of S-S bond and form hydrophobic- hydrogen bond, thus formation a starch- protein bi-network (Aït Kaddour et al., 2008).

The quality of this network depends on the quantity and quality of starch and gluten, mixing time, temperature and moisture. Extra water content could increase the time of dough development (Auger et al., 2008). Whilst over-mixing of dough yields a sticky dough (Aït Kaddour et al., 2008).

Baking is an important step in bread making since it is the final step which determines the quality of the final product. Heating bread ingredients results in several physic-chemical and biological events for instance; water steaming, cell configures, volume increases, changes in chemical and physical properties of protein occur and starch granules gelatinise (Mondal and Datta, 2008, Sivam et al., 2010). Shelf life and product quality rely on the baking procedure (i.e. baking period and temperature) as it determines the starch hydration, viscosity and crumb softness.
1-Native granules in raw food 2- starch granules absorbed water and swollen 3- Gelatinisation occurred after rapturing granules and leaching amylose 4- retrogradation after cooling the starch paste and reassociation amylose molecules 5- Amylopectin molecules configure a crystallins state

In a study to discover the influence of the heating process on the starch traits and bread crumb and firmness, it has been reported that using a low temperature (low heating rate) resulted in a lower amylose hydration rate, lower amylopectin retrogradation temperature and high bread softness (Patel et al., 2005). Starch gelatinisation and protein denaturation have an important role during the baking process as they control water uptake and also participate in converting dough into crumb (Hug-Iten et al., 2003, Mondal and Datta, 2008). When bread temperature decreases and it starts to cool, the gelatinised starch molecules re-crystallise and then re-associate, this is known as retrogradation (Primo-Martín et al., 2007, Hug-Iten et al., 2003). This causes another phenomenon called staling which takes place a few hours after bread is baked.
This is due to physic-chemical modulation in bread quality such as loses of crumb softness, aroma alteration and organoleptic quality deterioration (Mondal and Datta, 2008, Sourki et al., 2010).

- **Gluten**

Wheat proteins comprise of water soluble proteins such as albumin and globulin which make up a quarter of wheat protein, while water insoluble proteins such as glutenins and gliadins make up the rest of the protein content (Lee and Lee, 2012). Gluten is another main constituent of bread that influences and contributes to the dough rheology and bread quality. Gluten can be determined in flour by rinsing dough constituents to eliminate the soluble materials (i.e. starch), hence an elastic block remains which resists removed by the water, this is known as gluten (Wieser, 2007).

Gluten is primarily classified into two fractions; gliadins which are a group of heterogeneous monomeric proteins and glutenins are a group of heterogeneous and non-branched polymers based on extractability in aqueous alcohols (Goesaert et al., 2009). Gliadins are soluble in aqueous-alcohol solution (60-70% ethanol) and their molecular mass is between 30,000 and 60,000 g/mol. Whilst, glutenins are insoluble in aqueous ethanol and their molecular mass range from 500,000 g/mol to over millions g/mol (Nordqvist et al., 2012). Gliadins impart viscosity and plasticity. Meanwhile, glutenins confer extensibility and a rubbery texture to the products (Gómez et al., 2012). Quantity and quality of gluten in bread making can govern the rheology of dough and performance of the end-product because the viscoelasticity of dough and the volume expansion of bread depend on the strength of gluten and its ability to retain produced gases by yeast during fermentation. Due to the broken starch and protein during
milling which hydrolysis and ferments easily, hard wheat is more appropriate to make bread, while for producing cookies and pastries soft wheat is suitable (Pasha et al., 2010).

During mixing dough, gluten absorbs water because of the energy introduced from mechanical mixing and un-combined protein blocs rupture thereby gluten converts to a viscoelastic gluten network (Singh and MacRitchie, 2001, Goesaert et al., 2009). Furthermore, fractured disulphate bonds (-S-S) re-associate between protein molecules and play an important role in forming the protein network (Singh, 2005). The mixing process can also have an impact on dough quality since protein absorbs water and configures a cohesive visco-elastic network which reaches an ideal elasticity and extensibility and enables gluten to retain the gases (mainly CO₂) that are produced by yeast during the fermentation period. Hence transforms the dough to a stable firm structure (Veraverbeke and Delcour, 2002). Through retaining produced gases, the volume of bread expands (Peighambardoust et al., 2010). While mixing dough for a longer time influences dough rheology and causes the dough to be resistant dough to less resistance to extension thereby reducing ability of dough to retain and hold gases. A farinograph is one of the main techniques that is used to observe the development of dough during mixing (Miś et al., 2012). Another factor that modulates gluten network is the dissimilarity in the proportions of gliadins to glutenins or vice versa in flour. Glutenins confers elastic dough. On the contrary, gliadins lead to viscous dough with less elasticity. The elasticity of glutenins is presumably associated with high molecular sub-units and glutenins polymers can configure a big network comprising of long chain with several overlapping point among the chains (Veraverbeke and Delcour, 2002). So, imbalance between glutenin and gliadin
crucially affects dough rheology and quality which leads to either weak elasticity of the dough and low volume bread and/or strong elasticity, consequently retarding volume increment (Goesaert et al., 2005).

During baking, further chemical and physical modulations occur as starch gelatinises and protein denatures. This alters the hydrophobicity of gluten and sulfhydryl/disulfide exchange forming new S-S- crosslinks that change its form. As a consequence of that, besides starch gelatinisation, a foam body of bread is formed (Goesaert et al., 2005). At low temperature, no physical changes occur in the gluten network since it is already denatured, while when the temperature reaches above 70˚C, more moisture transforms from gluten to gelatinised starch. It has been reported that gluten becomes more denatured when heated at 80˚C hence a stronger matrix forms, (Kieffer et al., 2007, Mondal and Datta, 2008). So, protein forms a cross-link bond and more aggregates, it leads to more denaturation and coagulation of protein and then bread crumb begin to form (Singh, 2005). Due to temperature gas and steam transformation during baking, the cells are formed and the pressure of CO₂ causes bread expansion (Gandikota and MacRitchie, 2005). The expansion of the bread depends on the elasticity and viscosity. Additionally, gelatinisation is another factor that increases viscosity and hinders expansion. This is ascribed to the impact of the gelatinised starch on the membrane of close cells and merges them due to the raised pressure on the cells. Thus, some gases might move towards the outside resulting in decreasing the ability of gas retention as well as bread volume and expansion (Zhang et al., 2007, Mondal and Datta, 2008).

The Crust is where the most evaporation happens, predominantly retarding volume expansion. The crust is another factor that impacts bread volume via
hindering volume expansion. This is attributed to the inhibition of the migration of moisture and steam from the centre into the surface of bread. When the temperature reaches above 150°C, the maillard reaction happens. This results in forming browning colour on the outer layer of bread.

As we can see, starch and protein have a very important role within the bread system. Therefore adding any novel ingredient or/and removing these two components could have potential modifications on the bread quality. Adding DF to the ingredients could have an impact on the development of the gluten matrix during baking through altering the quality of the end-product such as texture, crumb structure and product volume. It has been postulated that adding DF could interact with gluten and reduce its ability to maintain and expand. This could be due to the several reasons. Firstly, DF addition could attenuate the concentration of gluten in the system. Secondly, DF probably competes for water thereby hindering the access of gluten to the water which results in a delay in gluten development. Thirdly, physical rupture of cells could be another cause that could ruin the ability of the cells to retain gas (Ktenioudaki and Gallagher, 2012).

Unlike starch, which has a role in bread staling, wheat protein on its own is not correlated to bread aging (Bhattacharya et al., 2002, Gerrard et al., 2001). However, it has been shown that the interaction between starch and gluten has an impact on bread staling. It has been postulated that the interaction between protein and starch has an inverse relationship on bread staling (Gray and Bemiller, 2003). This has been attributed to the ability of protein in absorbing and acting as a moisture reservoir. In some studies looking at the role of gluten on bread crumb softness, the results have shown that the presence of wheat gluten reduced bread staling owing to a high water absorption (Salehifar, 2011,
Furthermore, the addition of soy flour to rice bread improved the aging and staling. It has been suggested that the interaction between amylopectin and soy protein could delay the recrystallisation of amylopectin (Sciarini et al., 2010).

### 2.4.6.4. Effect of FI on Shelf life of bread

Bread is a staple product that is consumed daily and its freshness is important to consumers. Bread is considered a perishable cereal product (Goesaert et al., 2009) and its palatability (which is also called shelf life) does not last more than a few days after baking. The shelf life of a product can be defined as the period of time that a product is considered safe to health and possess an acceptable organoleptic quality. The shelf life of bread is limited by some parameters such as quantity and quality of the recipes (Mandala, 2005), storage conditions (Galić et al., 2009), the interaction between bread ingredients (Sciarini et al., 2010) and method of baking (Purhagen et al., 2012). Alongside these parameters, microbial activity could shorten the shelf life, particularly fungi as they grow in low water activity. These parameters could reduce shelf life through some changes such as microbial growth, chemical spoilage (i.e. rancidity) and physical change (i.e. bread staling) (Smith et al., 2004).

Different mechanisms such as freezing and modified atmosphere packaging have been designed to reduce potential shelf life deteriorations (Rodríguez et al., 2000, Latou et al., 2010, Bhattacharya et al., 2003). Moreover different formulations and ingredients (i.e. gums) are used to change and improve the interaction of the components and prolongs shelf life (Rodge et al., 2012). The major problem encountered for bread shelf life and quality is staling. Staling is described as changes that take place after a short time baking bread. These
changes lead to reduce quality and undesirable organoleptic traits such as increasing crumb and crust firmness, changing flavour and aroma as well as bread magnitude (Skendi et al., 2010, Curti et al., 2011). The reasons for staling are not entirely understood. Several investigations have proposed various reasons such as starch gelatinisation, wheat protein and the interaction between gluten and starch (Gray and Bemiller, 2003). Despite that, staling has been primarily attributed to the gelatinisation of starch since the starch granules uncover, leach out and viscosity of the slurry increases. After cooling down, amylose in the starch starts to crystallise, loses its amorphous structure and metamorphoses to a crystalline form. However, amylopectin re-crystallises rather gradually and over time its form alters to a more ordered structure (Goesaert et al., 2009). Alongside starch retrogradation, moisture transformation from inside to outside of the product is another factor that leads to staleness (Smith et al., 2004, Katina et al., 2005).

There are two types of staleness; crust staling takes place owing to the movement of moisture from inside to outside and causes to a leathery and soft texture (Gray and Bemiller, 2003, Primo-Martín et al., 2006). Goestaert et al (2009) suggest another cause of staling when bread starts to cool, interactions between amylose molecules take place and they start to crystallise. Furthermore, amylose is able to interact with the presence lipid and form a complex mixture. This interaction produces a perpetual network with partly crystallised regions which acts as a cross regions. These amylose networks with the formed gluten network are responsible for fresh bread softness. Furthermore, over time, gelatinised amylopectin slowly converts to a grid like form and the presence of large amount of amylose in swollen granules may increase the onset re-arrangement of amylopectin side chain to a more ordered
form. Subsequently, partially crystallised networks with the inclusion of starch chains that crosses via amorphous and crystalline zones spread throughout the bread crumb. This network is responsible for the crumb staling and develops over time. Hence, increasing the quantity and magnitude of crystalline junction regions could result in reducing crumb softness. Furthermore, starch retrogradation could affect the water movement within the bread system during aging. Water moves from the non-crystallised zones towards the crystallised regions, so it becomes stuck and freezes within the amylopectin side chains. This migration of moisture is sometime known as crumb to crust moisture migration. As a result, the glass transition temperature of the gluten network and non-crystallised zones raise and the networks can not be elasticised by rehydration due to the loss of gluten resilience. This increases crumb firmness over time during storage. Therefore, it can be noticed that the interaction between wheat protein and gelatinisation of starch play a significant role in the bread crumb firmness phenomena and staling.

A variety of additives have been utilised in bakery products and the aim of them is to improve dough rheology, preserve freshness and improve quality of the final product such as using DF and WWF. Amongst them, DF is known to have an important influence on the chemical and physical interaction with bread constituents such as an effect on moisture content and interaction with gluten. For example SDF and hydrocolloids (or gums) are widely used to prolong bread shelf life (Rosell et al., 2001, Collar et al., 2007, Skendi et al., 2010).

This is due to the advantageous roles of SDF in improving water distribution within products. It has been shown that addition of DF decreased bread firmness and retarded bread staling (Nilufer-Erdil et al., 2012). This might be attributed to the ability of DF to retain water, resulting in modulating changes in
flour and then dough texture, pasting properties, retrogradation and starch crystallisation. Also DF plays a significant role in distributing moisture throughout the bread system during bread storage. In an investigation about the modulations of pectin and gum on corn pan bread quality, it has been reported that the additives improved staling of bread via improving bread extensibility, strengthen, volume and water holding capacity (WHC) (Yaseen et al., 2010). Gomez et al (2003) studied the influence of DF from various sources such as pea, orange, cocoa, coffee, wheat and microcrystalline cellulose on dough and bread quality. They found that through improving bread characteristics, the shelf life of the bread was improved (Gómez et al., 2003). In another study, Brewer’s spent grain (BSG) was added to bread as a source of DF with some enzymes. The authors stated that replacing wheat flour with 20% and 30% of the BSG fibre considerably improved crumb firmness. While 10% did not significantly improve crumb firmness (Stojceska and Ainsworth, 2008).

Another factor that possibly improves shelf life of bread is WWF. As explained earlier, amylose is speeds up the staling process. There is a relatively recent product (WWF) that has been used to prolong shelf life since it contains mostly amylopectin with nearly no amylose. Alteration of the starch component which means modulating the portion of amylose to amylopectin such as with WWF, could retard starch retrogradation (Bhattacharya et al., 2002).

This might be ascribed to the ability of amylopectin to enhance moisture intake and reduce the enthalpy of amylopectin retrogradation, consequently leading to reducing hardness and softening the texture of the bread (Goesaert et al., 2009, Park and Baik, 2007, Garimella Purna et al., 2011). However WWF could reduce bread volume and lead to a poor crumb quality (Park and Baik, 2007).
2.5. Some examples of FI:

2.5.1. Inulin
IN is a member of SDF family. Chemically, it consists of β-D fructose linked by β-2→1 bond often ended by a glucose unit (Figure 2.2) (Roberfroid, 2005, Haraguchi, 2011) and ideally ranges a degree of polymerisation (DP) from 2 to 60 (Mantzouridou et al., 2012). IN is non-digestible polysaccharide and resistant to degradation in the small intestine. However, it undergoes fermentation by microflora that is present in the large intestine. IN exists in a wide variety of roots and tubers of plants such as Jerusalem artichoke, chicory, leeks, and onion occurring as non-structural storage carbohydrate (Ramirez-Farias et al., 2009). IN has an unusual collection of nutritional and technical benefits. Therefore, it is added to different types of food products to improve functionality and quality of food products (Franck, 2002). IN and fructooligosaccharides (FOS) are of great interest to food industries and food authorities because of the unique characteristics of IN, as it interconnects between dietary and technical traits. Additionally, IN can be exploited as fat replace up to 100% in some food products to improve mouth-feel and selectively induce the growth and activity of colonic micro flora (i.e. Bifidobacterium) and consequently, impart positive influences on the host's health (Pawel, 2009, Glibowski and Wasko, 2008).

Technically, IN is white and odourless and has slightly sweet taste. When it is entirely blended with water, it creates a three-dimensional network resulting in a white, creamy and gel formation that can be supplemented into food product (Nair et al., 2010). Temperature could effectively alter its solubility (Chawla and Patil, 2010). Due to its physical stabilisation, IN is widely used in foamy, emulsions-based products, dairy mousses (chocolate, and fruit) (Franck, 2002),
dairy products (Meyer et al., 2011) as well as cereal products (Brennan et al., 2004).

This is because it keeps the stability of the products, thereby improving organoleptic quality. Similar to that, IN promotes the quality and sensory attributes of cereal products due to the water-retention capability which keeps of bread and cake appearance fresh and humid hence prolonging shelf life. A study about the rheological properties of IN enriched yog-ice cream revealed that IN significantly influenced structure and texture of the yog-ice cream combination. Moreover, it was concluded that an acceptable yog-ice cream quality and rheology was achieved when 5% IN was incorporated into the mixture (El-Nagar et al., 2002). The effect of IN on the texture traits and starch degradation was studied. The results showed that IN reduced texture firmness as well as inhibited the degradation (Brennan et al., 2004).

Figure (2.2) Chemical structure of Inulin (Borneo and Leon, 2012)
Nutritionally IN can be regarded as a functional fibre (Azorín-Ortuño et al., 2009, Auger et al., 2008). Several epidemiological studies have elucidated that has a
positive influence IN on health by improving the functions of the digestive tract. It is a natural prebiotic that extends many desirable and beneficial influences including reduction of calorie intake and post-prandial blood glucose, reduction of lipid absorption as well as reducing risks colonic diseases. It has also been shown that IN increases mineral uptake (i.e. Calcium and Magnesium) (Yeung et al., 2005) and immune system function (Gourbeyre et al., 2011). The presence of B (2-1) bond linkages in turnovers IN is undigested and reaches the upper gut intact.

It creates a viscous solution that leads to a decrease in post-prandial glucose. In a study carried out by Brennan et al (2004) IN -incorporated into pasta decreased rate of sugar release by almost 50% (Brennan et al., 2004).

IN undergoes fermentation by colonic microflora leading to selectively stimulating the growth and activities of colonic microflora and diminishing the proportion of pathogenic bacteria. Additionally, fermentation of prebiotics results in the production SCFAs (i.e. propionate, acetate) which decreases colonic pH hence reduces risks of colorectal diseases (Slavin and Feirtag, 2011). The exact mechanisms of lowering cholesterol by the prebiotic are not clear. A study paper reviewed by Ooi et al (2010) showed that prebiotics could affect serum cholesterol through two mechanisms: decreasing lipid absorption accompanied by enhanced cholesterol excretion through the feces and fermentation by colonic microflora followed by SCFAs production. In similar way; prebiotics increase the viscosity of the chyme in gastrointestinal tract thus preventing cholesterol absorption. A study over 4 weeks about the influence of IN on the cholesterol content in blood stream of some rats was carried out. The results showed a reduction in blood cholesterol as well as higher excretion of faecal
cholesterol in compare to the control (Ooi and Liong, 2010). Similar to that, IN has potential to decline post-prandial blood glucose.

2.5.2. Guar gum

GG is a type of SDF which belongs to a group of gums (i.e. Arabic gum, locust bean gum). They are polysaccharides and called hydrocolloids. Chemically, it consists of high molecular weight polysaccharides ranged between 50,000 to 8,000,000 DA. GG comprises of a chain of B-D mannopyranosyl units joined by (1-4) glycosidic bond. Every second residue carries α-D- galactopyranosyl residue linked to the man chain by α-(1-6) linkages (Figure 2.3) (Belitz et al., 1999, Theuwissen and Mensink, 2008, Rodge et al., 2012). GG is derived from the seed endosperm of the guar plant *Cyamopsis tetra-gonoloba* (Aravind et al., 2012c, Chawla and Patil, 2010) and originates from Pakistan and India. It also can be found in Australia, South Africa and South America (Rodge et al., 2012, Yoon et al., 2008). GG acts a nutritional and technical ingredient as it confers flexibility to a product, keeps stability and consistency of texture as well as being beneficial for health. GG is well-documented to have a high viscous trait that makes it preferable to be used as a thickener and stabiliser in a wide variety of food products (Selomulyo and Zhou, 2007, Mandala, 2005) due to its water holding capacity (WHC) and retention ability, controlling water movement, reducing ice crystal formation (Rodge et al., 2012) and moisturising products. Furthermore, the solubility and gel forming ability of GG in cold water have shown to be appropriate in several applications in foodstuffs such as sauces, ice cream, beverages and bread as a thickener and texture improver (Butt et al., 2007). However, adding more quantity probably leads to a poor and unacceptable quality as it imparts a sticky mouth feel making the product difficult to chew (Roberts, 2011).
GG is used worldwide in food industry (Yoon et al., 2008). Particularly in bakery products, GG is commonly used due to unusual functionality. It improves rheology texture and final product quality. This is correlated to the potential of GG in WHC increasing viscoelastic properties and loaf volume (Rodge et al., 2012). In addition, GG perhaps acts as an anti-staling agent which can prolong the shelf life of bread through improving the mixing system between ingredients (Selomulyo and Zhou, 2007), softening bread crumb and keeping quality of product during storage (Rodge et al., 2012). A study completed by Ribotta et al (2004) reported that GG improved texture and volume of bread produced from frozen and non-frozen dough.

In another investigation, it has been found that adding GG to chapatti, unleavened flat Indian bread when fresh and in storage (at ambient temperature about 30 and fridge 4) improved the overall quality of bread, in particular extensibility and crumb firmness (Shalini and Laxmi, 2007). Such a polysaccharide like GG can be considered as a FI. This is due to the effectiveness of GG in reducing the risk of diet related diseases such cardiovascular diseases and diabetes. Many studies have confirmed the influence of SDF like GG in lowering risks of these diseases (See health effect of dietary fibre).
2.5.3. Waxy Wheat Flour

Starch is one of the polysaccharides present in the nature in different botanical sources used as an energy source. Starch is one of the most abundant components in nature. It can be widely found in cereals grains such as rice and wheat; in fruit such as banana and in some vegetables such as beans and peas. It can be used in different aspects of the food industry which might be connected to the ability of starch in moisture holding and its action as a thickener and gelling properties (Nashed et al., 2003).

It is been presumed that amylose is majorly responsible for bread staling due to the linear structure of amylose which gelatinises and retrogrades relatively quickly in comparison with amylopectin after been heated and cooled, hence produces a stable and relatively rigid paste (Qin et al., 2009). Meanwhile amylopectin resists and retards retrogradation and leads to a sticky and viscous
gel. Therefore, presence of a high proportion of amylopectin which retain moisture could be useful in retarding bread staling and extending shelf life (Chakraborty et al., 2004).

There were efforts to produce a starch with less proportion of amylose for the purpose of correcting the defects that occur due to the existence of amylose. For the first time, WWF was developed in by some Japanese scientists using a conventional breeding technique (Graybosch et al., 2003, Reddy and Seib, 2000). This is another type of wheat, its starch is known as a waxy starch which refers to a starch that mainly consists of amylopectin (Chakraborty et al., 2004). It can be also called as amylose-free starch (Hung et al., 2007a).

Producing such a modified wheat that contains an abnormal starch because of its chemical structure is supposed to play different actions during processing as well as modulates quality, texture and appearance of the final products (Hung et al., 2007b). Due to the properties of waxy starch it has the ability to absorb and hold water as well as creating viscous and gel formation (Garimella Purna et al., 2011). WWF has several implementations in the food industry and bakery productions in particular (Hung et al., 2007a). Its pasting properties confer more stability to the products that are stored at low temperature (Yi et al., 2009). It is preferable to acquire a product with high volume and soft texture (Van Hung et al., 2006, Hung et al., 2007a) and improve shelf life (Graybosch et al., 2003). WWF has been widely used partially or fully in bakery products for different purpose such as improving quality and/or extending shelf life. Using partial WWF in producing sponge cakes resulted in holding moisture and led to a softer product (Acosta et al., 2011).
In a study to investigate the effect of WWF on bread making, the results showed that partially replacing bread flour by WWF improved quality and led to a bread with less crumb firmness (Morita et al., 2002b, Hung et al., 2007a). Conversely, not all the studies confirmed the above results. In another study, WWF was used to investigate its influence on tortillas quality. The results indicated that the dough was less sticky and elastic and the final product characterised with a soft appearance. However, the additive darkened the colour, reduced springiness after a period of storage and needed extra force to separate (Guo et al., 2003). WWF possesses a wide range of applications and functionality in the food industry, however more research is needed to understand it more comprehensively (Chakraborty et al., 2004).
2.6. The rationale

The interest in DF consumption is increasing because of its nutraceutical benefits that. Despite the fact that there are dissimilar findings regarding the influence of DF upon the body (because of the difference in type and amount of DF), research has generally shown that high DF consumption could reduce risks of diet-related diseases. Therefore, health professionals recommended an increase in intake of DF consumption foods and reduction in protein and animal fat in the diet. A way of achieving this goal is enriching popular products like bread with DFs. However, there are a lot of potential detrimental effects come across in relation to the quality, sensory, texture and edibility of the DF-supplemented food products.

Most research reported so far has focused on nutritional aspects instead of quality and palatability. Studies on the addition of DF’s on bread have not covered processing properties such as cooking properties, gel texture, dough rheology and microstructure of the products. Studying these processing steps could give very useful information about the influence of the FI on quality of the final product. In addition, there is not much research using some types of FI like IN, GG and WWF on common products like pita and flat Arabic.

The combinations of these ingredients with different physical properties may potentially work together to improve all the aspects of the breads. Additionally, not much literature is available on the potential impacts of the preparation process in SEM on the microstructure of food, since sometimes the preparation method could influence the food structure. This procedure modification impact could orientate the researchers into a wrong direction and give misinformation. Therefore, a design was used to select twenty different combinations of FI from dissimilar sources to scan and visualise their influences on the bread quality,
shelf life and functionality. It was expected that the FI would influence the parameters used throughout the study.

The novel ingredients were expected to alter the cooking properties, dough rheology and bread rheology quality due to their effects on starch gelatinisation and gluten strength. Furthermore, they were also expected to postpone the staling of the products as they have a good ability to absorb water and moisture. Finally, because of the influence of the DFs on the starch gelatinisation, they were expected to reduce the products’ post-prandial blood glucose response.

2.7. Research objectives

The overall aim of this study is to develop, and create flat breads rich in DF and FI with quality similar to the conventional flat bread quality, but with extended physiological benefits to the consumers.

Objectives:

1- To assess the impact of functional ingredients alone and in combination on the cooking and pasting properties of wheat flour.

2- To evaluate the effect of the functional ingredients on dough rheology and machinability (i.e. extensibility and stickiness).

3- To establish connections between the functional ingredients substitution in combination and alone in flour on physically and instrumentally measured quality of pita and Tandoori bread varieties.

4- To visualise the influence of functional ingredients on the microstructure of the Pita and Tandoori breads using SEM.
5- To study the influence of the functional ingredients alone and in combination on the sensory attributes of pita and Tandoori breads.

6- To determine the shelf life of the supplemented products and to study the influence of the additives on the Pita and Tandoori breads performances during storage over a period of time.

7- To investigate the influence of DF on post-prandial blood glucose (in vivo)
Chapter three

The influence of the three novel functional ingredients on cooking properties, dough machinability and handling quality and breads quality

(General materials and methods)
3.1. Introduction

Bread is generally deemed as staple food that is eaten daily and its consumption exceeds tens of kilograms per capita annually, in particular in western countries (El-Khoury, 1999). There are various types of bread with a variety of different names. One type is pita/Tandoori bread which is flat, mostly rounded and two-layer bread. This type of bread has attained desire and acceptance over the world especially in Middle East and North Africa households. This is because of having pocket that is created during baking, this bread can mainly be used to be wrapped around other food products or used with other food products in the pocket (Borsuk et al., 2012). However, some people might be worried about bread’s carbohydrate content. It has been highlighted that bread is one of the food stuffs that contains a large quantity of carbohydrate (Tudorica, 2004). Consuming excessive carbohydrate over the time could make serious problems and leads to several food related chronic diseases. Therefore nowadays, the era of only making a food to fill stomach and give sense of fullness has almost passed since the health aspect of food have been highly emphasised on by public and food professionals.

Thus the desire and interest for healthy food is steadily increasing due to the consumers awareness regarding the role and the impact of food on the body’s well-being (Rosell et al., 2006). Food scientists and technologists progressively endeavour to create functional products that are relatively high in necessary nutrients; for instance DF rich products. DF offers enormous nutraceutical and physiological benefits. It has been used for a long time for health benefits in different products (Tudorica, 2004, Arismendi et al., 2013). But, a concern that decreases the feasibility of DF as FI and its supplementation to food products is the influence of FI on the quality potentially changing the efficacy production
process. Adding FI could influence the cooking and viscoelastic properties of starch. Furthermore, FI could change the dough processability/machinability and quality characteristic. This could lead to change the production process and productivity. Particularly if this change would go to a negative direction, since dough quality primarily determines the quality of the end-product. This potentially affects the quality, texture and palatability of the end-product. Finally, adding novel FI could influence the sensory attributes of the final product. Thereby, the marketability and desirability of the product could be decreased. A compromise solution thus is necessary to create a functional product that possesses a quality similar to the conventional product.

Therefore, the objective of this work was to study and understand the impact of different ranges of FI alone and in combination on cooking and viscoelastic properties. The study also aimed to unravel the machinability properties and quality of dough. Furthermore, the impact of the FI on two types of breads was observed. Moreover, the study aimed to investigate the effect of different baking process of bread varieties on the behaviour of the FI and then quality of the breads and choose the best dose of the FI to create functional products. In addition, the microstructure pita and Tandoori supplemented with different types of FI was also intended to image. Finally, two types of SEM (Cryo-SEM and LVSEM) were used to discover any potential modification of preparation method on the microstructure of bread.
3.2. Experimental design

Response surface methodology (RSM) is a statistical and mathematical technique helps to optimise, develop, and improve products. It has also a substantial role in formulating new products. Therefore, this statistical design was aided to develop two types of breads by adding FI. Three novel FI different in their physic-chemical properties were selected in order to be used in this study. The range of the FI was IN up to 8%, GG up to 2% and WWF up to 15% utilised in this study. The ranges were selected based on the data available in the literature about the influence of FI and their doses on the quality and health aspect (Only IN and GG) of products. In the RSM, central composite design (Minitab 16.2.2) was used to select 20 different combinations between three FI and then select the best dose of the FI that does not change the production process step (cooking properties, dough quality and product quality) (Table3.1). The first 12 runs should be done in the first day and the second 8 runs should be completed in the second day (according to the statistical design recommendations). The WWF bread would not be used for the GR and satiety test as there is no evidence that WWF confirming its health benefits. The graphs are presented in the form of surface plots and counter plots which illustrate the influence of the FI alone and in combinations on each parameter. The extra parameter (the third parameter) is held at zero point. The table data exhibits the influence of the FI ingredients alone, in interactions and in combination for each parameter. The constant is the point where the factors have no influence, any change far below or far above this point may show significant effect. $R^2$ is a description of the amount of variation in the observed responses that is explained by the statistical model.
<table>
<thead>
<tr>
<th>Run</th>
<th>IN %</th>
<th>Guar gum %</th>
<th>Waxy wheat flour %</th>
</tr>
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<td>4</td>
<td>1</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0</td>
<td>0</td>
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</tr>
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<tr>
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<td>7.5</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
3.3. Materials and methods:

3.3.1. Basic ingredients
These ingredients were used in this study to make dough and bread samples; commercially milled flour (Medium chapatti flour-its chemical composition is presented as supplied by the company), commercially compressed dry yeast, salt, water, and three FI; Inulin (Fruitafit HD-Calleva Ltd., UK), guar gum (E412) (Calleblend GUA-Calleva Ltd., UK), waxy wheat flour (HOMECRAFT™ Create 765 National Starch Corn Products Ltd., UK). The proportions are presents in table 3.2.

Table (3.2) Nutritional information of flour used in this study as provided by the supplier

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity per 100g flour</th>
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<tr>
<td>Protein (g)</td>
<td>12.0</td>
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<tr>
<td>Fat (g)</td>
<td>1.9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>64.5</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>7.1</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>337</td>
</tr>
<tr>
<td>Sodium</td>
<td>Trace</td>
</tr>
</tbody>
</table>

3.3.2. Visco-elastic properties
The dough pasting profiles, peak viscosity (PV), breakdown (BD), SB, pasting temperature (PTE), peak time (PT) and final viscosity (FV) were investigated using a Rapid Visco Analyser (RVA) (RVA-, Newport Scientific, Warri wood, Australia) following a standard method and 25ml of water was added to a canister followed by 3g of sample. The samples were prepared by replacing a part of flour by the proportions presented in the table 3.1 and then 3g of the
flour and the FI was weighed. The samples then mixed with a paddle 4-5 times. Then, the canister placed in the machine and heated to 50˚C and stirred at 160 rotations per minute (rpm) for 10s in order to be dispersed entirely. The slurry was held at 50˚C for up to 1 min, and then heated to 95˚C over 3 min 42s and held at 95˚C for 2 min 30 s, and finally cooled to 50˚C over 3 min48 s, and held at 50˚C for 2 min. The pasting temperature (when viscosity first increases by at least 25 CP over a 20-Sec period), PT (when PV occurred), PV (maximum hot paste viscosity), breakdown (PV minus holding strength or trough viscosity, FV (end of test after cooling to 50 ˚C and holding at this temperature), SB (FV minus PV), and total SB (FV minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software.

3.3.3. Flour gel preparation
The flour slurry was directly gained after the flour visco-elastic properties assessment ended. The slurry of each sample of the viscoelastic test was poured into two sample containers (bowls), left for nearly 1 hour to set and kept for the next day to do further assessment on the gel texture and/or properties.

3.3.4. Gel colour measurement
The colour of the gel samples was evaluated with colorimeter (Minolta Ltd.; Model, CM2600d, UK) which was calibrated with 10° standard observer D65 (room light) and calibrated with a white ceramic plate to define the product CIE lab value $a^*$, $b^*$ and $L^*$ value which represent redness/greenness, yellowness/blueness and brightness respectively. Six replicates were run per sample and the whiteness was calculated according to the following equation (Borsuk et al., 2012):

$$\text{Whiteness} = 100 - \left( [100-L^*]^2 + a^{*2} + b^{*2} \right)^{0.5}$$
3.3.5. Texture analyser
After the slurry from RVA was cooled and stored for 24 hours at ambient temperature, Texture Analyser Profile (TPA) was used to assess the texture (hardness, adhesiveness, springiness) of the gel produced from, using texture analyser (TA-TX2-Stable Micro System, UK) calibrated with a 5Kg weight. The settings were; pre-test speed: 1.0mm/s; test speed: 1mm/s; post-test speed: 1 mm/s; distance: 3mm, with 12.7 millimetres (mm) diameter hemispherical Delrin cylinder probe. Six replicates for two containers were performed per sample.

3.3.6. Dough making
Dough samples were prepared following the procedure described by Borsuk et al. (2012) with some modifications. These ingredients were used based on 100g flour; 1% active dry yeast, 1.5% salt and 53g of water. The ingredients were put in a blender (kitchen aid, model 5KPM5, USA) and then water added and mixed at 140 rpm for 2 minutes. The dough then was placed in a slightly oiled container for 1 hour at 37°C in a fermentation room (incubator). The dough then was taken out from the cabinet, cut into 2 equal pieces and put in the cabinet again for 10 min. The dough was then machinery sheeted (Rondo Econom STM-Swiss, Chessington) 3 times rotating 90° in between to give 3mm thickness. To give a circular shape, the dough was cut to confer 10cm diameter with metal ring. The dough was then put on the tray to proof in the fermentation room for half an hour before baking. For Tandoori bread, dough was produced using the same recipe and additives as used for pita only the amount of water was increased to 69g. The dough was then fermented for 1 hour in fermentation room at 37 °C and transferred to local restaurant to bake.
3.3.7. Dough evaluation:
Texture analyser TA.XT2 (stable micro system, UK) with a 5kg load cell was used to evaluate for dough quality and handling properties such as dough extensibility and resistance to extension and dough machinability/processability such as stickiness, cohesiveness and adhesiveness.

3.3.7.1. Dough quality and handling properties
Dough quality was assessed using standard Kiefer dough and extensibility rig, the settings were; pre-test speed: 2.0mm/s; test speed: 3.3mm/s; post-test speed: 10.0mm/s; distance: 40mm (Tudorica, 2004). The 25g of the dough was put onto the grooved base of the form and the top block of the form placed onto the sample and pressed strongly until the block come together. The excess dough was removed using a spatula and then the dough was left in this status for 40 min in order to convert the dough into strips. Then, the strips were taken out from the press and placed under the texture analyser. The dough resistance to extensibility was evaluated by recording the maximum force when the elasticity of dough exceeded and the strips fracture, and the distance when the fracture occurred was used an indication to calculate dough extensibility. The test was run in ten replicates.
3.3.7. 2. Dough processability/machinability
Dough machinability/processability was determined using the SMS/Chen-Hoseney Dough stickiness cell (A/DSC) and 25mm Perspex cylinder probe (P/25P), the test settings were; pre-test speed: 2.0 mm/s; post-test speed: 10.0 mm/s; distance: 12mm; force: 40g; time: 0.1s (Tudorica, 2004). Dough samples were divided into 5g portions and put in the dough stickiness cell. The dough was then extruded through the holes present on the die by rotating inside screw and the first extrusion discarded by a spatula. The screw was then rotated once again until a 1mm height of dough sample extruded through the die. The screw was then rotated a little backwards to reduce the stress on the sample and stop more extrusion. The surface of the sample then was covered for 30s with the Perspex cap to reduce moisture migration. Then after, the stickiness cell was set under the machine to commence the test. The highest positive force reading, positive area and the distance between two anchors represent the dough machinability properties. Ten replicates were run per sample.
3.3.8. Bread baking
Pita breads were baked at 228°C ± 5 for 3 min using lab oven (Bartlett, Model: E9E, G.E.E Barlett & son Ltd. Hampshire, UK). The bread was cooled and assessed for pocket height, weight; diameter and weight loss. For Tandoori bread, baking was carried out using a traditional clay oven (Tandoor) at 300 ± 5 °C for 70 sec in a local restaurant in Plymouth city where Tandoori flat bread is routinely produced. Then after, the bread cooled down, placed in plastic bags and transferred back to the lab to conduct product assessment.
Figure (3.4) Bread baking process; pita bread using lab oven and Tandoori bread using clay oven

3.3.9. Physical evaluation of bread quality
Physical assessment of pita/Tandoori bread included; pocket height (thickness), bread volume, weight loss, diameter and colour.

3.3.9.1. Weight loss
Weight loss of pita and Tandoori bread samples as a result of baking, each sample weighed before and after baking. Weight loss was calculated according to the equation below (Borchani et al., 2010). Three readings per batch were run.

\[
\text{Weight loss} = \frac{\text{weight before baking} - \text{weight after baking}}{\text{weight before baking}} \times 100
\]

Figure (3.5) Weighting dough before and bread after baking
3.3.9. 2. Diameter
The diameter was measured twice using a ruler and turned around 90° in between. Three readings were run per batch.

![Diameter measurement](image)

Figure (3.6) Diameter measurement

3.3.9. 3. Pocket height (thickness)
After the samples were cooled down to room temperature, pocket height of each sample was evaluated using a ruler. Three readings per batch were run and the mean value calculated. For Tandoori breads the thickness of each sample batch was measured and ten readings were run per bread.

![Height measurement](image)

Figure (3.7) Height (thickness) measurement

3.3.9. 4. Crust colour
Minolta colorimeter which calibrated with a white paper was used to measure the crust colour of the bread. The colour of sample was denoted by the 3 dimensions $L^*$, $a^*$, and $b^*$. When the $L^*$ represents the value of a product lightness which gives 100 for perfect white and zero for dark. But $a^*$ and $b^*$
represent redness and yellowness colour of products respectively. Nine readings for pita bread and twelve readings from Tandoori bread per batch of three samples were run. The whiteness of bread was calculated according to this following method (Borsuk et al., 2012):

\[
\text{Whiteness} = 100 - [(100 - L^*)^2 + a^*^2 + b^*^2]^{0.5}
\]

Figure (3.8) Measuring bread colour

3.3.9.5. Loaf volume
Pita/Tandoori bread volume of bread was measured with a standard method rapeseed displacement method. A container of known size was filled with rapeseed and then samples placed in the container followed by rapeseed. The extra rapeseed which is no longer able to fill the container is deemed as loaf volume. Three readings per sample batch were run.

Figure (3.9) Loaf volume measurement
3.3.10. **Instrumental evaluation of bread quality**

Texture profile analyser (TPA) from Texture analyser was used to evaluate pita bread quality parameters (hardness, springiness and chewiness). Circles of bread were taken from the bread and tested, the settings were; pre-test speed: 1.0mm/s; test speed: 1mm/s; post-test speed: 1 mm/s; distance: 40%, with 36mm cylinder probe with radius (P/36R). Eight replicates per each sample batch were assessed. For Tandoori bread, penetration test from texture analyser was used to measure the quality parameters; toughness and extensibility (elasticity). The bread was cut into four equal pieces and a penetration test was conducted using the settings; pre-test speed: 2.0mm/s; test speed: 1mm/s; post-test speed: 10 mm/s; distance: 60mm, with 1 inch plastic ball probe with radius (P1/S). Six replicates were run per batch of three samples.

![Figure (3.10) Instrumental measurements of the bread qualities; Pita (hardness, springiness and chewiness), Tandoori (toughness and extensibility)](image)

3.3.11. **SEM (Cryo-SEM)**

SEM (Cryo–SEM - Jeol 6610- Oxford instruments) was utilised to visualise the microstructure of breads with and without additives. A piece of specimen was placed on the specimen holder and stuck using Tissue-Tech and carbon (colloidal graphite) before being frozen by placing in liquid nitrogen (-190°C). Afterwards, the specimens were directly transferred into Cryo-unit where they
were sublimated for 5 min at from -140°C -90°C and coated with gold 90 mA for 90 seconds and then transferred into SEM chamber where they were observed and imaged at 5 kV. LVSEM (LVSEM. Jeol 5600, Oxford instruments) was also used to compare it with the Cryo-SEM method. For that reason the pita bread samples (control bread and bread with all the ingredients) were frozen and left overnight to dry. Then the specimen was stuck on a specimen holder coated with gold and tested.

3.4. Statistical analysis

The results from viscoelastic properties experiment, dough quality, dough handling properties and breads quality were analysed using RSM. However the results from the sensory evaluation assessment submitted to statistical analysis using analysis of variance (ANOVA) and Tukey’s test from Minitab (16.2) software (Minitab Ltd, Coventry-UK) to discover any statistical differences for sensory attributes of all the types of the breads.
Chapter four

The influence of the three novel functional ingredients on cooking properties, dough machinability, handling quality and bread quality

(Results and discussion)
4.1. Results and Discussions:

4.1.1. Visco-elastic properties

The real values of the pasting parameters are presented in table 4.1 and the analysed values are presented in table 4.2. The analysis of the data in RSM showed that substituting flour with FI such as IN (8%), guar gum (2%) and waxy flour (15%) considerably affected some slurry viscoelastic characteristic (PV, breakdown, SB, and PT and PTe). Firstly, GG linearly significantly increased (p<0.05) PV. Increasing PV of starch by GG has been confirmed by some previous studies (Achayuthakan and Suphantharika, 2008, Rodge et al., 2012). It can be attributed to the solubility and viscous traits of GG which could have a synergistic influence with flour constituents on starch to enhance viscosity (Brennan et al., 2008). Another potential factor is that GG extends in a sheet shape which allows granules a chance to swell and raise viscosity before granule rupturing occurs (Achayuthakan and Suphantharika, 2008). GG also significantly (p<0.05) accelerated breakdown (Figure 4.1). As previously discussed, addition of the GG to starch in the presence of heat and mechanical shearing could increase breakdown as a result of the force that is imposed upon the starch granules and subsequent disruption. This is due to the mechanical shear resulting in diminishing viscosity and this decrement known as breakdown (Rodge et al., 2012). Set back is the period when the temperature goes down and starch molecules start to re-associate and increase viscosity.
Table (4.1) Influence of FI on pasting properties of wheat starch

<table>
<thead>
<tr>
<th>Factors</th>
<th>PV (cP)</th>
<th>BD (cP)</th>
<th>SB (cP)</th>
<th>FV (cP)</th>
<th>PT (min)</th>
<th>PTe (°C)</th>
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<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>862</td>
<td>392</td>
<td>524</td>
<td>994</td>
<td>5.4</td>
<td>87</td>
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<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>820</td>
<td>385</td>
<td>494</td>
<td>929</td>
<td>5.4</td>
<td>86</td>
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<td>IN (4) GG (1) WWF (7.5)</td>
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<td>493</td>
<td>933</td>
<td>5.3</td>
<td>88</td>
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<td>IN (8) GG (0) WWF (0)</td>
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<td>434</td>
<td>785</td>
<td>5.2</td>
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<td>354</td>
<td>669</td>
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<td>89</td>
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<td>IN (8) GG (2) WWF (15)</td>
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<td>465</td>
<td>934</td>
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<td>756</td>
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<td>839</td>
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<td>533</td>
<td>960</td>
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<td>84</td>
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<td>561</td>
<td>1043</td>
<td>5.5</td>
<td>84</td>
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<td>405</td>
<td>485</td>
<td>963</td>
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<td>527</td>
<td>587</td>
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<td>375</td>
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<td>555</td>
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<td>498</td>
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<td>5.2</td>
<td>86</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>859</td>
<td>402</td>
<td>537</td>
<td>994</td>
<td>5.5</td>
<td>86</td>
</tr>
</tbody>
</table>

*cP; centipoise
It can be seen from table 4.2 that GG considerably increased SB. This result is in agreement with that found by Chaisawang, and Suphantharika (2006). It has been reported that increased breakdown and SB is associated with the increase in PV and FV (Brennan et al., 2008). The pasting temperature of the wheat starch linearly decreased with the increase of GG. Brennan et al (2008) revealed similar results when they added GG to wheat flour. The decrease in pasting temperature is ascribed to the interaction of gums with the small quantity of leached amylose from some swollen granules (Huang, 2009). Although this factor has been confirmed by a group of researchers, they have postulated that the reduction in the pasting temperature of starch is as a result of altering the starch granule form by gums (Liu et al., 2006).

FV is the viscosity which occurs after decreasing the temperature of the slurry so the starch molecules come together due to the decreasing energy and this leads re-association of starch molecules (amylose and amylopectin). This represents the texture and quality of the end-product. GG also raised the FV of the starch considerably.

Similar results were observed by (Chaisawang and Suphantharika, 2006) postulated that the increase of FV is due to the interaction between polymer and gum particles. The increase of FV by gums is possibly due to two mechanisms which are depletion and bridging flocculation: Polymers enhance gum particles to come close and approach each other. Depletion flocculation is the process whereby gum particles aggregate after the solvent that exist between particles diffuses out due to the osmosis pressure. Meanwhile, bridging flocculation is the process by which free ends of polymers are adsorbed by the gum particles and come together forming a bridge between them (Achayuthakan and Suphantharika, 2008).
PT is the time when maximum PV occurs. This time could be expressed as the time required for gelatinising. It can be seen that addition of GG increased peak time.

Table (4.2) significant coefficients (confidence interval 95%) for cooking properties parameters

<table>
<thead>
<tr>
<th>Factors</th>
<th>PV (cP)</th>
<th>BD (cP)</th>
<th>SB (cP)</th>
<th>FV (cP)</th>
<th>PT (min)</th>
<th>PTe (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>847.7</td>
<td>393.1</td>
<td>504.1</td>
<td>958.7</td>
<td>5.4</td>
<td>86.1</td>
</tr>
<tr>
<td>IN</td>
<td>-122.6</td>
<td>-77.9</td>
<td>-54.4</td>
<td>-99.1</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Guar</td>
<td>169.5</td>
<td>99.8</td>
<td>64.0</td>
<td>133.7</td>
<td>0.16</td>
<td>-6.2</td>
</tr>
<tr>
<td>WWF</td>
<td>n.s.</td>
<td>-48.4</td>
<td>-43.3</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>IN *IN</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Guar*Guar</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>WWF*WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-7.6</td>
</tr>
<tr>
<td>IN *Guar</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>IN *WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Guar*WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>R-SQ</td>
<td>80.9%</td>
<td>82.7%</td>
<td>79.3%</td>
<td>77.4%</td>
<td>75.9%</td>
<td>80.7%</td>
</tr>
</tbody>
</table>

n.s. = no significant influence of factors at p<0.05

PV= peak viscosity, BD=breakdown, SB=setback, FV=final viscosity, PT=peak time, PTe=pasting temperature

*cP; centipoise

R-SQ= Regression square coefficient of the fitting model
Figure (4.1) The influence of FI on the pasting profile of flour starch; 1, (IN 0*, GG 2, WWF 0), 2 (IN 0, GG 0, WWF 0), 3 (IN 4, GG 1, WWF 7.5), 4 (IN 0, GG 0, WWF 15), 5 (IN 8, GG 0, WWF 0)

It might be due to the interaction of starch molecules and gums which probably lead to longer time to gelatinise. Some studies have shown similar outcome such those found by Brennan et al (2008). However, no significant difference was observed by Chaisawang and Suphantharika (2006). The results from the data analysis showed that IN inclusion in the slurry complex modified the pasting parameters, for example; it decreased PV; breakdown, SB and FV. However, IN exhibited no appreciable influence on PT and pasting temperature.

In research about the influence of DF on starch degradation and texture
properties of biscuit, it has been reported that IN decreased PV and FV (Brennan and Samyue, 2004). Reduction of the viscoelastic parameters of starch by IN is possibly due to substituting a part of starch with IN. This either plays a role as attenuator or it could have a restrictive impact on starch gelatinisation (Brennan and Samyue, 2004). Furthermore, it has also been found that IN unlike GG does not establish synergistic cooperation with starch to enhance viscosity (Zimeri and Kokini, 2003). IN alone also reduced SB and breakdown significantly. This could be due to the reduction in SB, PV and FV which resulted in the decrement in SB and breakdown as discussed earlier.

WWF singly showed an appreciable decrease (p<0.05) in the breakdown and SB linearly and pasting temperature in a quadratic manner (Figure 3.2). This is more likely to be due to the absence of amylose, since amyllopectin potentially is more sustainable and endures shear rate and temperature changes. WWF contains a high amount of amyllopectin with relatively no amylose and lipid which modifies the pathway of starch gelatinisation. So, this amount of amyllopectin could have tolerated the change in the temperature and hinders the re-association of molecules and gel formation.

This property can be deemed as an outstanding trait of WWF and useful for bread making since it detains retrogradation of starch, thereby improving shelf life and producing persistent softness of bread (Morita et al., 2002b). WWF also caused alteration in the pasting temperature of wheat starch in a quadratic way. The addition of a high level of WWF (15%) led to a diminished pasting temperature. The result is congruent with some previous studies (Abdel-Aal et al., 2002, Morita et al., 2002a).
This might be due to the absence of amylose and lipid owing to rapid uptake of water and facilitating gelatinisation of starch at a lower temperature. Exclusion of amylose and phospholipids and their interactions to form combinations amylose-phospholipid in wild-type wheat starch could limit the water absorption ability and gelatinisation. Therefore with the absence of these limitations, starch swells at lower temperature (Yoo and Jane, 2002, Hung et al., 2007b).

More interestingly and it’s noteworthy that combinations of all the ingredients IN, GG and WWF, showed no significant effect on the viscoelastic profile of starch.
Figure (4.2) Response surface plots for influence of the FI on the pasting profile
Extra factor held at zero level; Pasting temp=pasting temperature
4.1.2. Gel Colour assessment

Amongst all the interactions that have been used between all the three additives no changes on the whiteness of flour gel was noticed except with WWF which quadratically influenced gel whiteness. Addition of 7.5 % of WWF led to decrease whiteness to the lowest point. While increasing WWF to 15% greatly increased whiteness of slurry. The increase in flour whiteness is assumed to be due to the replacement of flour contains small brown particles with white WWF. Hence it could have increased gel transparency and whiteness.

4.1.3. Gel properties assessment with texture analyser (TPA)

The real values of the gel texture and quality; hardness, adhesiveness, springiness and whiteness are presented in table 4.3 and the analysis of the data is shown in table 4.4. It is clearly evident that only IN and WWF alone and in combination altered hardness. While no change on the adhesiveness was found in the interactions alone or in combination was found.

It has been reported that the hardness that develops in starch based products during storage is due to the retrogradation of starch molecules (Goesaert et al., 2005). WWF was observed to have a notable effect on gel hardness reducing it significantly reduced (p˂0.0001). If flour is substituted with WWF which contains almost no amylose, this could hinder initial re-association of starch molecules thereby intercepting retrogradation of starch and improving stability. The same result is expected when adding WWF to flour, as endured WWF the different temperature treatments during pasting. This is a great indication that WWF improves stability of the product through hindering starch retrogradation and then improved firmness (Morita et al., 2002b).
Moreover, it is mentioned in the literature that existence of amylose in starchy foodstuffs could increase a product's firmness. A study was conducted to establish a link between the amount of amylose and the properties of noodles. The authors discovered that a reduction in quantity of amylose significantly decreased the product hardness (Heo et al., 2012).
IN demonstrated a significant effect on the hardness of the wheat flour gel similar to WWF. With increasing IN substitution, the hardness of the gel linearly decreased (Figure 3.3). This is seemingly due to the effect of IN on the starch gelatinisation properties, as explained earlier. IN showed a restrained impact on starch pasting characteristics which led to the hindering impact on the leaching amylose and amylopectin. Consequently, this could have delayed molecular re-association of the starch and stability persistence. Springiness (which is also termed as elasticity) is described as the capability of pressurised-food products to return to their pre-deformed condition after the pressure is removed (Cavani and Pinnavaia, 2009).

Table (4.4) Data analysis of the influence of FI on the texture and colour of flour gel

<table>
<thead>
<tr>
<th>Factors</th>
<th>Hardness (N)</th>
<th>Adhesiveness (N)</th>
<th>Springiness (%)</th>
<th>Whiteness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>10.4</td>
<td>-1.3</td>
<td>1.2</td>
<td>60.23</td>
</tr>
<tr>
<td>IN</td>
<td>-1.5</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>GG</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>WWF</td>
<td>-3.3</td>
<td>n.s.</td>
<td>0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>IN * IN</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>GG*GG</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>WWF * WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>9.2</td>
</tr>
<tr>
<td>IN * GG</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>IN * WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.011</td>
<td>n.s.</td>
</tr>
<tr>
<td>GG* WWF</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>R-SQ</td>
<td>95.6%</td>
<td>35.2%</td>
<td>95.4%</td>
<td>46.98%</td>
</tr>
</tbody>
</table>
IN (p<0.05) and WWF (p< 0.0001) alone and in combination enhanced product springiness. It can be implied that IN and WWF could confer elasticity and decrease brittleness of a product. It is probably related to the influence of these two ingredients hindering the re-association of starch molecules. Alternatively, it could be due to the amylose reducing quality. The previous study by Hoe et al (2012) also showed that reduction of amylose increased springiness.

Figure (4.3) Response surface and counter plots for the influence FI on flour gel properties
4.1.4. Dough Evaluation:

4.1.4.1. Dough quality and handling properties
Traditionally dough is made from the basic ingredients flour, salt, yeast and water. After mixing, fermentation, and proofing, the dough is converted into a porous mass. After this, the dough is converted into bread through the setting of cells in the dough by heating / baking. The dough making process is a substantial step in bread production which can dramatically influence the quality and processability of the bread. Having dough with adequate rheology properties (i.e. extensibility and resistance to extension) is important in bread production, technically and economically. Technically, it ensures the production process can go smoothly. Economically, it could improve the production efficacy.

Resistance to the extension is a point to recognise the strength and potency of dough particularly gluten to sustain and retain gas (Tudorica, 2004). Extensibility is an indication of the handling and processing characteristic of certain types of dough (Wang et al., 2002). Therefore, from this point of view, the dough was assessed to view the influence of the FI on the dough rheology and handling properties.

The actual values of the dough quality parameters (dough extensibility and resistance to extension) are shown in table (4.5) and data analysis are presented in table (4.6).

FI changed dough quality parameters in different ways. It can be derived from the statistical analysis that extensibility and resistance to extension was considerably modified with substituting flour with additives. WWF and GG alone and in combination by IN led to significant increases (p<0.05) in dough
extensibility. On the other hand, resistance to extension was linearly decreased by IN alone and in combination with GG (figure 4.3).

However, it can be noticed that resistance to extension was increased in dough containing by GG (Table 4.6). Therefore, it is obvious that GG, IN and their interaction interfered to some extent with the starch-gluten matrix and then modulated dough properties. GG alone increased the rate of both dough rheology and resistance to extension. GG strengthened the rheology and visco-elastic properties of gluten. This could imply that GG possesses a viscous trait and enhances the visco-elastic properties of dough. This result is congruent with the previous studies (Smitha et al., 2008, Sharadanant and Khan, 2003, Sim et al., 2009). The influence of GG on the dough could possibly be due to the interaction between GG from galactomannan and gluten (Rodge et al., 2012). Thus, GG could have offered a synergistic effect with gluten. Consequently, it increased dough rheology and handling parameters and perhaps gas retention ability (Smitha et al., 2008). Even with SEM imaging, it was observed that GG had a strong connection with starch and gluten and gave a smooth structure to the microstructure of bread. This implies that GG could cooperate with the flour bio-polymers matrix rather than weakening them. On the other hand, IN reduced resistance to extension. Even sensory evaluation revealed that IN made the dough weaker during processing. The reverse effect of IN on dough rheology has been ascribed to its attenuation influence upon the gluten system instead of offering a synergistic cooperation to flour bio-polymers (Wang et al., 2002, Nadir et al., 2011).
Table (4.5.) The actual values of the impact of blends of FL on the dough rheology and handling properties

<table>
<thead>
<tr>
<th>Factors</th>
<th>Resistance to extension (N)</th>
<th>Extensibility (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>30.0</td>
<td>-19.5</td>
</tr>
<tr>
<td>IN(4) GG(1) WWF (7.5)</td>
<td>28.8</td>
<td>-16.9</td>
</tr>
<tr>
<td>IN(4) GG(1) WWF(7.5)</td>
<td>32.4</td>
<td>-16.1</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (0)</td>
<td>22.6</td>
<td>-19.3</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (15)</td>
<td>19.0</td>
<td>-17.4</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (15)</td>
<td>27.7</td>
<td>-11.6</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (0)</td>
<td>49.4</td>
<td>-16.0</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (15)</td>
<td>51.4</td>
<td>-12.3</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>32.3</td>
<td>-17.5</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (15)</td>
<td>27.9</td>
<td>-13.8</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (0)</td>
<td>32.3</td>
<td>-18.1</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (0)</td>
<td>30.5</td>
<td>-13.0</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (15)</td>
<td>34.4</td>
<td>-14.9</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>31.5</td>
<td>-16.0</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>29.5</td>
<td>-15.4</td>
</tr>
<tr>
<td>IN (8) GG (1) WWF (7.5)</td>
<td>29.7</td>
<td>-15.5</td>
</tr>
<tr>
<td>IN (4) GG (2) WWF (7.5)</td>
<td>44.0</td>
<td>-14.4</td>
</tr>
<tr>
<td>IN (0) GG (1) WWF (7.5)</td>
<td>33.0</td>
<td>-15.2</td>
</tr>
<tr>
<td>IN (4) GG (0) WWF (7.5)</td>
<td>30.3</td>
<td>-15.0</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>38.0</td>
<td>-18.6</td>
</tr>
</tbody>
</table>
It has been found that the addition of 6.8 % IN extracted from Jerusalem artichoke greatly reduced the elasticity of dough compared with the control (Morris and Morris, 2012). Moreover, in an investigation into the effect of IN dough rheology, it was found that the addition of IN reduced the strength of the pasta dough (Nadir et al., 2011). This could be attributed to the attenuation effect of IN on the gluten matrix system and weakening the strength of system which reduces the gas retention capacity. Alternately, it is known that IN restricts the water uptake by starch and gluten due to its greater affinity to water and this could have altered the dough development process (Ktenioudaki and Gallagher, 2012). Therefore, it probably conferred dough a weak texture. However, to study the influential modification of IN on dough rheology, it has been reported that adding 1-5% of IN did not produce any significant difference on dough quality and rheology (Collar et al., 2007).

Regarding WWF, it can be seen that substitution of wheat flour with WWF linearly increased dough extensibility (Table 4.6). This is probably related to the change in starch-protein matrix due to the alteration in amylopectin and amylose content, and then dough rheology parameters become altered. A previous study revealed that WWF addition increased dough extensibility (Yi et al., 2009). They have suggested that this increment is probably due to the flour constituents, in particular glutenin. Although the ingredients affected dough quality, their combinations showed no influence on the dough quality. Based on this result, it is better using combination of these ingredients to obtain a dough quality similar to the control.

Table (4.6) The data analysis for the effect of ingredients on the dough extensibility and resistance to extension
<table>
<thead>
<tr>
<th>Factors</th>
<th>Resistance to extension (N)</th>
<th>Extensibility (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>32.29</td>
<td>-16.58</td>
</tr>
<tr>
<td>IN</td>
<td>-6.44***</td>
<td>-0.13</td>
</tr>
<tr>
<td>GG</td>
<td>7.08***</td>
<td>1.62**</td>
</tr>
<tr>
<td>WWF</td>
<td>-1.23</td>
<td>1.47**</td>
</tr>
<tr>
<td>IN *IN</td>
<td>-3.27</td>
<td>0.76</td>
</tr>
<tr>
<td>GG*GG</td>
<td>2.56</td>
<td>1.41</td>
</tr>
<tr>
<td>WWF*WWF</td>
<td>1.59</td>
<td>-0.64</td>
</tr>
<tr>
<td>IN *GG</td>
<td>-3.03*</td>
<td>1.07*</td>
</tr>
<tr>
<td>IN *WWF</td>
<td>-0.49</td>
<td>-0.58</td>
</tr>
<tr>
<td>GG*WWF</td>
<td>0.90</td>
<td>0.13</td>
</tr>
<tr>
<td>R-SQ</td>
<td>89.6%</td>
<td>80.7%</td>
</tr>
</tbody>
</table>

R-SQ: Regression square for the fitting model

p<0.05, p<0.01 and p<0.001 are represented by *, ** and *** respectively.
Figure (4.4) Response surface plots for the influence of FL on the dough quality and handling properties

Extra factor held on zero level

R. to extension = resistance to extension
4.1.4.2. Dough processability/machinability traits
Dough processability/machinability parameters included stickiness; cohesiveness and adhesiveness. They refer to the dough characteristics under mechanical action. For instance; stickiness refers to the difficulty in cutting, sheeting and flattening dough during bread production, which might have a negative impact on mass production efficacy as a result of gluing dough to the dough processor (Rathod, 2008). Furthermore, cohesiveness indicates the capability of food materials to keep their integrity under mechanical pressure.

Processability trait values are shown in figure 4.5 and data analysis are shown in table 4.7, where it can be seen that FI also could modify dough processability. Statistical analysis has shown that substitution of flour with IN increased stickiness, adhesiveness (p<0.0001) and cohesiveness (p<0.001). Whilst in an opposite manner, GG significantly decreased (p<0.05) all traits. Only combinations of GG and IN reduced (p<0.05) stickiness and adhesiveness lower than with IN alone and higher than with GG alone. Finally, none of the interactions affected dough cohesiveness. FI therefore seem to have dissimilar interactions with biopolymers resulting in variable results regarding dough processability properties.
Table 4.7 The actual values for the effect of factors on the dough machinability/processability traits

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stickiness (N)</th>
<th>Adhesiveness (N*s)</th>
<th>Cohesiveness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>8.7</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>IN(4) GG(1) WWF (7.5)</td>
<td>8.2</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>IN(4) GG(1) WWF(7.5)</td>
<td>10.2</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (0)</td>
<td>22.7</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (15)</td>
<td>14.4</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (15)</td>
<td>7.5</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (0)</td>
<td>4.4</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (15)</td>
<td>3.7</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>11.8</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (15)</td>
<td>5.2</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (0)</td>
<td>6.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (0)</td>
<td>10.6</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (15)</td>
<td>10.9</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>3.4</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>12.8</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>IN (8) GG (1) WWF (7.5)</td>
<td>14.3</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>IN (4) GG (2) WWF (7.5)</td>
<td>5.1</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IN (0) GG (1) WWF (7.5)</td>
<td>4.8</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IN (4) GG (0) WWF (7.5)</td>
<td>12.4</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>10.6</td>
<td>0.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Adding IN to dough could increase dough stickiness which might reduce the handling quality of the dough. It is believed that some DFs could change dough processability traits when added. This has been attributed to the strong affinity of the DFs towards water and moisturising the surface of the dough (Angioloni and Collar, 2008). Consequently, it could cause sticky dough. In a study to investigate the influence of IN on dough rheology, it has been demonstrated that increasing the IN proportion in the dough system decreased water absorption of gluten (O'Brien et al., 2003). This means that IN moisturises the dough and offers more free water on dough surface, thereby increasing dough stickiness (Sangnark and Noomhorm, 2004a). In a review about IN, it has been reported that IN powerfully configures a white jelly and sticky structure with the existence of water (Nair et al., 2010). This could be an indication that using extra amount of that IN potentially confers negative attributes by increasing dough processability. In contrast, GG seems to be reducing processability traits. During an investigation about the effect of gums on dough machinability traits, similar results were found i.e. gums significantly reduced dough processability traits (Angioloni and Collar, 2008).

Regarding the combination of IN and GG, probably GG had a dominant and diminishing effect on IN. Similar to our results, it has also been reported that different combination of carboxyl methyl cellulose (a type of gum) and fructooligosaccharide reduced dough stickiness (Angioloni and Collar, 2008). Our results are in agreement with this finding, revealing that the interaction of GG and IN diminished dough stickiness.
It is worth pointing out that extreme increasing of the dough processability traits might have undesirable consequences. The opposite could also be true; extremely reducing them could have negative impact on the integrity and consistency of the dough system. Thereby, it could influence the gas retention capacity and hence bread quality, in particular bread magnitude. Thus it is so important to choose an appropriate type and amount of DF to add to dough to ensure dough with acceptable machinability properties. But, all the dough enriched with the additives could be processed comfortably.

Figure (4.5) Contour plots for the influence of FI on dough processability properties

Extra factor held at zero level
Table (4.8) Result from RSM analysis of the effect of FI on dough processability

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stickiness (N)</th>
<th>Adhesiveness (N*s)</th>
<th>Cohesiveness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>9.38</td>
<td>0.36</td>
<td>0.63</td>
</tr>
<tr>
<td>IN</td>
<td>4.50***</td>
<td>0.25***</td>
<td>0.17**</td>
</tr>
<tr>
<td>Guar</td>
<td>-2.96**</td>
<td>-0.17**</td>
<td>-0.10</td>
</tr>
<tr>
<td>WWF</td>
<td>-1.28</td>
<td>-0.07</td>
<td>-0.03</td>
</tr>
<tr>
<td>IN * IN</td>
<td>-0.11</td>
<td>0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>GG*GG</td>
<td>-0.96</td>
<td>-0.05</td>
<td>-0.08</td>
</tr>
<tr>
<td>WWF*WWF</td>
<td>0.019</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>IN * GG</td>
<td>-1.96*</td>
<td>-0.14*</td>
<td>-0.06</td>
</tr>
<tr>
<td>IN * WWF</td>
<td>-1.20</td>
<td>-0.07</td>
<td>-0.02</td>
</tr>
<tr>
<td>GG*WWF</td>
<td>0.67</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>R-SQ</td>
<td>84.4%</td>
<td>88.2%</td>
<td>79.5%</td>
</tr>
</tbody>
</table>

R-SQ: Regression square for the fitting model

p<0.05, p<0.01 and p<0.001 are represented by *, ** and *** respectively.
4.1.5. Bread evaluation

4.1.5.1. Physical measurement of bread quality

Bread production comes after a series of processes which commence with dough making and end with baking, through changing a foamy phase of dough to spongy phase of bread. During mixing and then fermentation air cells are produced and these cells are surrounded by walls of starch-protein matrix. More gases are produced then during fermentation and proofing stages causing the cells to stretch and expand. Baking causes starch gelatinisation and cross-linking of proteins which causes the gases to break out the cells and permit the gas to move towards crust and the cells become disclosed (Garimella Purna et al., 2011). Adding novel ingredients to flour therefore potentially changes this process and impacts on bread quality. For instance, it has been presumed that the presence of WWF in dough doesn’t allow gas to move and the cells become impermeable owing to the integrity of waxy starch granules. Thus, the gases generated during baking leads the cells to stretch and expand but the cells are unable to rupture and break, hence sustaining its foamy form and shrinkage occurs while cooling down (Garimella Purna et al., 2011). Meanwhile, bread quality (i.e. volume, colour, texture, and overall appearance) is very important to consumers; hence it has a substantial influence on the marketability and desirability. From that point of view, the bread types were assessed.

The actual values of the influence of FI on pita bread quality are presented in table 4.9 and the analysed data are shown in the table 4.10. As experienced in the earlier chapters, once again the functionally altered bread affected some quality parameters in different ways.

IN showed a quadratic effect on bread height. Increasing IN to 5% reduced pita height to the lowest point but then showed a tendency to increase. Reduction of
bread volume has also been reported by some previous studies (Meyer and Peters, 2009, O’Brien et al., 2003). They reported that the addition of 5% IN caused reduction in the bread volume. The potential explanation for that the influence of IN addition on glutens, capacity to retain gases generated during fermentation and the baking period. This could be due to the interaction of IN with the gluten matrix (Rosell et al., 2001). This could weaken the gluten matrix and increase gas permeability. But, the addition of IN at 7.5% into the bread increased bread volume and height (Peressini and Sensidoni, 2009). This is possibly due to the inclusion of fructose, sucrose and glucose which are postulated to help improve bread height and volume via retarding starch gelatinisation (Roberts et al., 2012). Similarly, it has been suggested that IN postpones starch gelatinisation which probably provokes dough expansion while baking (Peressini and Sensidoni, 2009). The microstructure of bread with IN confirmed this assumption. It can be observed that the bread volume was affected when GG added (Figure 4.6). GG considerably increased pita bread volume and the second maximum volume was recorded when 2% GG added. Moreover, GG marginally increased bread height. Similar result were observed when GG was added into wheat bread (Rodge et al., 2012). The authors argued that GG increased bread volume from 728ml to 820ml by increasing the proportion of GG from 0.25 to 1%. This might be owing to the synergistic effect of GG with the gluten-starch matrix which could lead to increased gas retention and consequently increasing final product height (Rodge et al., 2012). Alternatively, it could be as a result of changing cooking and gelatinisation properties. Gomez et al (2007) showed that the increasing volume of cake enriched with GG is associated with increased starch pasting, viscosity and its gas retention capacity, which restricts gas diffusion. Moreover, GG could
enhance gluten development and improve its potential to retain gas. Consequently GG leads to increased product volume increasing. But it can be reasonably expected that the volume of bread primarily depends on the quality and strength of wheat protein, in particular gluten (Roberts et al., 2012). The weight loss before and after baking was quadratically (p<0.05) modified with the substitution of flour with GG. With 1% substitution of flour with GG the lowest loss of moisture was recorded but then this tends to increase again. Several studies have pointed out the ability of hydrocolloids to bind water and increase the moisture content of products (Guarda et al., 2004, Tavakoli-pour and Kalbasi-Ashtari, 2007, Rodge et al., 2012, Rosell et al., 2001). It is believed that the volume of bread increases as a result of water evaporation during baking time which could raise the pressure in the bread and enhance bread volume (Rodge et al., 2012). Alternatively, it is perhaps owing to the increased volume by GG which increases the contact surface area, giving temperature a chance to hit the surface and increase evaporation. Accordingly, this could cause a decrease in weight loss after baking.

Pita bread has a flat form with two thin layers that are totally exposed to heat. Accordingly, this could cause a reduction in weight loss after baking. Because both sides are exposed to temperature and this could promote moisture loss during baking. Colour is substantial product acceptability. This implies it has a main role in the decision of rejecting and/or accepting the food product (Angioloni and Collar, 2009a). It can be seen that none of the ingredients neither alone nor in combination influenced bread colour. Similarly, no change was noticed regarding the diameter of the bread produced.
Table (4.9) The actual value of the influence of FL on the pita bread quality (physical assessment)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Volume (ml)</th>
<th>Diameter (mm)</th>
<th>Height (mm)</th>
<th>Weight loss (g)</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>73</td>
<td>90</td>
<td>17</td>
<td>13</td>
<td>59</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>91</td>
<td>90</td>
<td>21</td>
<td>12</td>
<td>67</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>88</td>
<td>91</td>
<td>25</td>
<td>9</td>
<td>69</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (0)</td>
<td>84</td>
<td>87</td>
<td>23</td>
<td>10</td>
<td>65</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (15)</td>
<td>85</td>
<td>89</td>
<td>21</td>
<td>12</td>
<td>68</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (15)</td>
<td>133</td>
<td>89</td>
<td>32</td>
<td>11</td>
<td>69</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (0)</td>
<td>117</td>
<td>89</td>
<td>23</td>
<td>13</td>
<td>67</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (15)</td>
<td>108</td>
<td>89</td>
<td>24</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>98</td>
<td>93</td>
<td>24</td>
<td>11</td>
<td>70</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (15)</td>
<td>99</td>
<td>89</td>
<td>21</td>
<td>13</td>
<td>69</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (0)</td>
<td>105</td>
<td>90</td>
<td>23</td>
<td>13</td>
<td>68</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (0)</td>
<td>106</td>
<td>91</td>
<td>23</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (15)</td>
<td>85</td>
<td>90</td>
<td>18</td>
<td>10</td>
<td>67</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>100</td>
<td>91</td>
<td>23</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>103</td>
<td>89</td>
<td>24</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>IN (8) GG (1) WWF (7.5)</td>
<td>143</td>
<td>90</td>
<td>35</td>
<td>12</td>
<td>70</td>
</tr>
<tr>
<td>IN (4) GG (2) WWF (7.5)</td>
<td>113</td>
<td>91</td>
<td>27</td>
<td>31</td>
<td>66</td>
</tr>
<tr>
<td>IN (0) GG (1) WWF (7.5)</td>
<td>100</td>
<td>91</td>
<td>24</td>
<td>13</td>
<td>68</td>
</tr>
<tr>
<td>IN (4) GG (0) WWF (7.5)</td>
<td>83</td>
<td>92</td>
<td>18</td>
<td>13</td>
<td>69</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>112</td>
<td>90</td>
<td>25</td>
<td>13</td>
<td>65</td>
</tr>
</tbody>
</table>
Table (4.10) The data analysis of the influence of FI on the pita and Tandoori bread qualities (physical assessed)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Pita bread</th>
<th>Tandoori bread</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (ml)</td>
<td>Diameter (cm)</td>
</tr>
<tr>
<td>Constant</td>
<td>96.5</td>
<td>9.84</td>
</tr>
<tr>
<td>IN</td>
<td>2.3</td>
<td>-0.175</td>
</tr>
<tr>
<td>GG</td>
<td>12.2*</td>
<td>0.22</td>
</tr>
<tr>
<td>WWF</td>
<td>-1.3</td>
<td>-0.02</td>
</tr>
<tr>
<td>IN *IN</td>
<td>18.9*</td>
<td>-0.07</td>
</tr>
<tr>
<td>GG*GG</td>
<td>-4.74</td>
<td>0.03</td>
</tr>
<tr>
<td>WWF *WWF</td>
<td>-4.41</td>
<td>-0.10</td>
</tr>
<tr>
<td>IN *GG</td>
<td>6.17</td>
<td>0.07</td>
</tr>
<tr>
<td>IN *WWF</td>
<td>5.48</td>
<td>0.01</td>
</tr>
<tr>
<td>GG * WWF</td>
<td>2.98</td>
<td>-0.05</td>
</tr>
<tr>
<td>*R-SQ</td>
<td>57.73%</td>
<td>53.59%</td>
</tr>
</tbody>
</table>

*P value <0.05

*R-SQ = Regression square coefficient of the fitting model
All the ingredients alone altered some of the bread quality characteristics. However, when it comes to combinations of the ingredients, the effects seem to be ameliorated and less profound and even cannot be noticed sometimes. So, the quality of bread with combinations of FI is likely to be similar to the control. Generally, combinations of FI did not demonstrate any appreciable impact on pita bread quality properties.

Regarding physical parameters of the Tandoori bread; the actual data for volume, diameter, thickness weight loss and whiteness are shown in the table 4.11 and the analysis data is presented in the table 4.10. The weight loss as a consequence of baking indicated that the combination of IN and GG tended to produce moisture loss. Some previous observations have claimed that the moisture content of bread increased as a consequence of adding hydrocolloids and due to their capability in water holding and retaining (Ghodke, 2009, Rosell et al., 2001, Rodge et al., 2012). This has been attributed to the chemical structure of hydrocolloids and presence of hydroxyl group which increases the chance of interaction between hydroxyl group and water molecules (Guarda et al., 2004), whereas IN could reduce this effect. So when it comes to the combinations of IN and GG the weight loss was greater. Adding IN could have decreased the water uptake potential of GG and lead to evaporation during the baking period. So, the difference could be in the mechanism and the mode of action of different DF in terms of binding and interacting with moisture. The volume of bread was also affected by some of the FI for instance; WWF significantly influenced the volume of bread. Substituting 15% flour with WWF seems to decrease bread volume.
Table (4.11) The actual value of the influence of FI on Tandoori bread quality (physical assessment)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Volume</th>
<th>Diameter</th>
<th>Thickness</th>
<th>Weight loss</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>348</td>
<td>28</td>
<td>32</td>
<td>15.8</td>
<td>63</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>310</td>
<td>28</td>
<td>35</td>
<td>15.9</td>
<td>63</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>300</td>
<td>28</td>
<td>35</td>
<td>16.5</td>
<td>65</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (0)</td>
<td>353</td>
<td>27</td>
<td>35</td>
<td>16.9</td>
<td>61</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (15)</td>
<td>357</td>
<td>28</td>
<td>33</td>
<td>15.9</td>
<td>61</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (15)</td>
<td>318</td>
<td>27</td>
<td>32</td>
<td>14.8</td>
<td>60</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (0)</td>
<td>329</td>
<td>27</td>
<td>32</td>
<td>14.5</td>
<td>66</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (15)</td>
<td>247</td>
<td>25</td>
<td>35</td>
<td>12.2</td>
<td>60</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>350</td>
<td>27</td>
<td>33</td>
<td>16.0</td>
<td>62</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (15)</td>
<td>293</td>
<td>26</td>
<td>33</td>
<td>17.4</td>
<td>64</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (0)</td>
<td>317</td>
<td>29</td>
<td>32</td>
<td>18.1</td>
<td>62</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (0)</td>
<td>313</td>
<td>28</td>
<td>34</td>
<td>14.8</td>
<td>62</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (15)</td>
<td>327</td>
<td>28</td>
<td>32</td>
<td>16.9</td>
<td>66</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>340</td>
<td>28</td>
<td>33</td>
<td>16.1</td>
<td>62</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF(7.5)</td>
<td>343</td>
<td>29</td>
<td>35</td>
<td>17.3</td>
<td>60</td>
</tr>
<tr>
<td>IN (8) GG (1) WWF(7.5)</td>
<td>400</td>
<td>29</td>
<td>38</td>
<td>17.5</td>
<td>61</td>
</tr>
<tr>
<td>IN (4) GG (2) WWF(7.5)</td>
<td>362</td>
<td>27</td>
<td>32</td>
<td>15.2</td>
<td>63</td>
</tr>
<tr>
<td>IN (0) GG (1) WWF(7.5)</td>
<td>337</td>
<td>28</td>
<td>31</td>
<td>16.2</td>
<td>61</td>
</tr>
<tr>
<td>IN (4) GG (0) WWF(7.5)</td>
<td>388</td>
<td>28</td>
<td>33</td>
<td>18.0</td>
<td>59</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>293</td>
<td>28</td>
<td>31</td>
<td>14.8</td>
<td>63</td>
</tr>
</tbody>
</table>
To maintain the volume of bread, it is important to have a continuous channel to sustain the product after removing it from the oven (Rodríguez-García et al., 2012). WWF showed a similar result on Tandoori bread volume as discussed in pita bread. This means that WWF failed to rupture and fracture the cell wall to allow the gas to escape and increase the volume of bread. Thus the bread shrinks after cooling down (Garimella Purna et al., 2011). Furthermore, it was also noticed that bread with WWF could not produce bubbles on the surface of Tandoori bread. This could be related to the decrease of bread diameter, as it is also affected by WWF addition. Regarding diameter, WWF addition quadratically reduced bread diameter. Conversely, combination of IN with GG and IN with WWF increased bread diameter. This could be the result of IN addition which made the dough easy to flatten and stretch. Alternatively, combinations of these ingredients could strengthen dough rheology so allowing the bread to stretch more, hence, it increased bread diameter. Finally, crust colour and thickness of bread was not influenced significantly with the addition of the FI neither alone nor in combination. From the aforementioned results, it could be argued that the FI at some stage affected the quality parameters, though the affects did not dramatically change the bread quality. This could be a positive point, since we possibly could produce fibre enriched bread without changing the physical characteristic drastically. Furthermore, it can be noted that the effect of FI alone and in combination demonstrated less influence on the Tandoori bread quality parameters. This could imply that changing the amount of water and following a different mechanism of baking bread could influence the behaviour of FI and then bread physical quality parameters.
Figure (4.6) Response surface plots for the influence of FI on the Pita bread quality

Figure (4.7) Response surface plots for the influence of FI on the Tandoori bread quality
4.1.5.2. Instrumental measurement of bread texture

The actual values of influence of Fl on the bread quality from texture analyser are presented in the table 4.12 and the analysed values are presented in the table 4.13. The instrumental assessments of the pita bread samples enriched with the ingredients were comprised of hardness, springiness and chewiness. Hardness or firmness is defined as the energy required abolishing a given deformation. Springiness (which is also termed as elasticity) is described as the capability of pressurised-food products to return to their pre-deformed condition after the pressure is removed. Whereas, chewiness is deemed as the force needed to chew a food product into a form that would be ready to swallow, but in the texture analyser it is associated with hardness, cohesiveness and elasticity (Balestra, 2009). However, in fact chewiness is a more sophisticated process since it involves several parameters such compression, piercing cutting and saliva to lubricate.

The results showed that the addition of the Fl had a profound effect on hardness, springiness and chewiness. Hardness was significantly (p<0.05) increased and springiness decreased (p<0.05) and chewiness marginally increased when pita contained IN. The results are in agreement with an earlier study which reported that adding IN into gluten-free layer cakes considerably increased hardness and decreased springiness (Gularte et al., 2012, Morris and Morris, 2012). It can be seen that the addition of IN as a source of DF could have a negative affect and increase product firmness in comparison to the control. In a review about the effect of FOS and IN enrichment on bread in terms of texture and quality of the final product, it has been stated that IN could lead to a firmer product quality as a result of tightening product structure (Morris and Morris, 2012).
Table (4.12) The actual values of the instrumental measurement of pita/Tandoori bread qualities (instrumental measurements)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pita bread parameters</th>
<th>Tandoori bread parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (N)</td>
<td>Springiness</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>395.1*</td>
<td>0.846</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>378.2</td>
<td>0.861</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>336.7</td>
<td>0.863</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (0)</td>
<td>242.1</td>
<td>0.914</td>
</tr>
<tr>
<td>IN (8) GG (0) WWF (15)</td>
<td>406.7</td>
<td>0.839</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (15)</td>
<td>381.1</td>
<td>0.869</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (0)</td>
<td>303.2</td>
<td>0.904</td>
</tr>
<tr>
<td>IN (0) GG (2) WWF (15)</td>
<td>374.6</td>
<td>0.875</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>351.9</td>
<td>0.851</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (15)</td>
<td>191.3</td>
<td>0.902</td>
</tr>
<tr>
<td>IN (0) GG (0) WWF (0)</td>
<td>284.4</td>
<td>0.923</td>
</tr>
<tr>
<td>IN (8) GG (2) WWF (0)</td>
<td>518.9</td>
<td>0.835</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (15)</td>
<td>372.0</td>
<td>0.836</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>357.9</td>
<td>0.868</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (7.5)</td>
<td>307.2</td>
<td>0.873</td>
</tr>
<tr>
<td>IN (8) GG (1) WWF (7.5)</td>
<td>395.0</td>
<td>0.874</td>
</tr>
<tr>
<td>IN (4) GG (2) WWF (7.5)</td>
<td>319.3</td>
<td>0.861</td>
</tr>
<tr>
<td>IN (0) GG (1) WWF (7.5)</td>
<td>322.4</td>
<td>0.889</td>
</tr>
<tr>
<td>IN (4) GG (0) WWF (7.5)</td>
<td>285.0</td>
<td>0.891</td>
</tr>
<tr>
<td>IN (4) GG (1) WWF (0)</td>
<td>304.7</td>
<td>0.883</td>
</tr>
</tbody>
</table>

Chewiness= hardness x cohesiveness x springiness
Values are average of eight replicates
This tightening probably causes these changes in hardness, springiness and chewiness. Likewise GG inclusion significantly enhanced (p<0.05) bread hardness, chewiness and reduced springiness. It has been found that GG addition increased bread hardness from 1.11g to 2.86g merely by increasing the level of GG in bread from 0.25% to 1% (Rodge et al., 2012). In another study to investigate the influence of GG on the technical and textural traits of pasta, it was found that the addition of 2.5% GG was enough to enhance hardness of cooked pasta (Aravind et al., 2012a). The presence of DF in the bread is suggested to increase strength of the gas cell wall. Consequently, it could increase hardness of bread (Skendi et al., 2010). Alternatively, the influence GG on the pita bread textural attributes could potentially be due to weight loss as discussed earlier which could reduce softness and elasticity of bread and increased chewiness.

In terms of WWF, reduction in the bread springiness is evident when WWF was added to pita bread and a significant impact on springiness was observed. Adding WWF into muffin to investigate its effect on the quality of muffin, WWF seemed to decrease springiness but not significantly (Acosta et al., 2011). This indicates that WWF could modify crumb structure in a way would be less springy. The addition of WWF into wheat bread results in a crumb described as “keyhole”, and with an increasing level of WWF, the crumb cells were found to be bigger but fewer in number. Furthermore it was found that increasing the proportion of WWF resulted shrinkage of crumb bread (Garimella Purna et al., 2011). So, it can be summarised with having this kind of crumb, the first bite (compression) possibly breaks the crumb cells walls and the cells may not be able to recover for the second bite due to the breakage. Consequently, it causes decrease in springiness.
Table (4.13) Data analyse for the instrumental measurements of pita/Tandoori breads qualities (instrumental measurements)

<table>
<thead>
<tr>
<th>Factors</th>
<th>pita bread</th>
<th>Tandoori bread</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (N)</td>
<td>Springiness</td>
</tr>
<tr>
<td>Constant</td>
<td>347.59</td>
<td>0.862</td>
</tr>
<tr>
<td>IN</td>
<td>46.78*</td>
<td>-0.016*</td>
</tr>
<tr>
<td>GG</td>
<td>48.77*</td>
<td>-0.013*</td>
</tr>
<tr>
<td>WWF</td>
<td>7.24</td>
<td>-0.014*</td>
</tr>
<tr>
<td>IN *IN</td>
<td>21.48</td>
<td>0.016</td>
</tr>
<tr>
<td>GG*GG</td>
<td>-35.04</td>
<td>0.011</td>
</tr>
<tr>
<td>WWF *WWF</td>
<td>1.15</td>
<td>-0.006</td>
</tr>
<tr>
<td>IN *GG</td>
<td>6.14</td>
<td>-0.001</td>
</tr>
<tr>
<td>IN *WWF</td>
<td>6.08</td>
<td>0.001</td>
</tr>
<tr>
<td>GG * WWF</td>
<td>-17.2</td>
<td>0.012</td>
</tr>
<tr>
<td>R-SQ</td>
<td>58.54%</td>
<td>77.37%</td>
</tr>
</tbody>
</table>
Figure (4.8) Response surface plot for the influence of FI on pita/Tandoori bread qualities (Instrumental measurement)
Regarding Tandoori bread, it can be seen that IN significantly altered the bread texture and conferred a harder texture. IN seemed to have a quadratic influence on the bread texture particularly toughness. Substituting 4% flour with IN increased the product firmness, whereas increasing to 8% seems to provoke product hardness. This could refer to the fact that the potential modification impact of IN depends on the amount of fortified IN. This result is similar to those found by a previous study that found that adding 3% IN decreased bread hardness while raising this level to 8% resulted in a harder bread texture compared to the control (Korus et al., 2006).

Assessing freshly baked bread enriched with IN using a puncture test showed that the product crumb and texture is harder than the control (Mandala et al., 2009). The influence of a prebiotic on the fortified product quality and texture depends on the added quantity of prebiotic (Morris and Morris, 2012). The increase in the bread firmness produced by addition of IN is reported by some earlier studies. Fortifying bread with FOS enhanced bread firmness (Mujoo and Ng, 2003). So it is hypothesized that adding DF into bread could tighten the bread structure and then increase firmness (Mujoo and Ng, 2003).

It is clearly evident that neither GG nor WWF demonstrated any influence on the freshly baked Tandoori bread. It is obvious that only with the single additives demonstrated any changes on bread quality instrumental measurements. More interestingly, combinations of the ingredients seem to possess less influence on the bread quality. It could be beneficial to create fibre rich bread which has similar quality to the control. As experienced in the physical assessment of bread, once again FI showed less influence on the freshly baked Tandoori bread. It can therefore be suggested that the behaviour and influence of the FI differs from one product to another, even in the same group of product. Dissimilarity could be as a result of different methods in the dough rheology baking mechanism.
4.1.6. SEM (Cryo-SEM)

The SEM helped to visualise the microstructure of samples supplemented with the FI; IN 8%, GG 2%, WWF 15%, all ingredients together (IN 4%, GG 1% and 7.5%) and control (without additives). The micrographs of the Cryo-SEM helped to observe and understand the interaction between the additives and biopolymers within the bread system, hence their influences on the whole process of product creation can be observed. The micrograph of pita samples are presented in the Figure 4.9.

It can be seen from the microstructure of control bread (4.9 control) that there is a strong linkage between flour constituents particularly the starch-protein matrix. Starches appear to be gelatinised partially and swollen. However the starch granules retained identity and the bread structure kept its integrity. Furthermore, it can also be observed that the outline of granules is detectable. Finally, starch granules seem to be stuck the in protein matrix. The micrograph of bread supplemented with all the ingredients is presented in figure 4.9 All. It is interesting that the image shows that microstructure of bread seems to possess a similar microstructure to control. Starch granules are gelatinised partly and retained their identities. However some granules appeared to be gelatinised fully. Moreover, starch granules are embedded in protein and the additives matrix and a connection between them can be observed. However, the starch granules are more detectable than in the control. This could be attributed to the competition between FI and starch granules, since they absorb moisture quicker than the starch.

The microstructure of bread with IN seems to have a discontinuous and less consolidated starch-protein matrix, and starch granules are prevalent, discrete and less wrapped by IN. It is also evident that IN tends to make strips in between starch granules which could be due to the starch-fibre interaction. Furthermore, starch granules appeared to be less swollen and/or less gelatinised (Figure 4.9 IN). This
presumably is due to the competition between starch and IN for moisture, but the stronger affinity of IN to water than starch, owing to absorbing water quicker, has led the starch granules to be less gelatinised. IN is a water soluble fibre at ambient temperature and takes up moisture fast which is assumed to restrict starch swelling and consequently change its integrity. Tudorica et al (2004) found that the addition of IN to bread caused starch granules to be less swollen and have less interaction with their neighbour granules. In a study about the influence of different proportions of IN, addition on pasta microstructure, it has been concluded that IN caused an incoherent structure and modified the integrity of starch-protein matrix. This was attributed to the impact of IN as IN competes with starch and protein and due to the greater affinity of IN to water; it alters the protein-starch matrix. This consequently leads to an incoherent structure. Additionally, IN competes with starch to combine with protein and results in producing a weak protein-starch matrix (Manno et al., 2009). Similar results have been stated by some authors regarding the effect of IN on pasta structure (Aravind et al., 2012d).

The micrographs of pita bread enriched with GG showed a different impact (figure 4.9 GG). It can be observed that the starch granules are wrapped and covered with GG. This could provide evidence that the starch granules are immersed in GG and protein throughout the network, and some indistinct outlines of starches are barely visible due to the smooth structure and no cavities. Furthermore, unlike IN, GG not only did not seem to weaken the starch-protein matrix, but also appeared to form a continuous and dense network of protein-starch. This observation is congruent with the earlier finding which suggested that starch from GG bread was coated with galactomannan from GG and the granules extended in a continuous network as a result of gluiness traits of gums (Brennan et al., 1996). Similar to that, some authors have stated that GG could create a dense structure when added to bread dough
(Ribotta et al., 2004). More recently, a piece of research was carried out on the influence of different hydrocolloids on the properties of puri bread. It was reported that the addition of GG enveloped and left starch granules partially hydrolysed (Parimala and Sudha, 2012). The microstructure of pita samples replaced with 15% waxy flour is presented in the figure 4.9 (WWF). It can be seen from the image that WWF modifies bread microstructure in a way dissimilar to GG and IN. The bread microstructure looks altered and relatively disintegrated. Furthermore, some granules look gelatinised fully, but the majority of the starches are partially swollen. So, some of the starch outlines can be determined. Finally, the microstructure seems to be less smooth and it contains more cavities.

It has been cited that that waxy starch starts absorbing water and swelling at a lower temperature to the wild-species of starch granules and around 70°C the morphology of starch modifies and disassociates into small fractions. Whereas, normal wheat starch granules endure temperature even beyond 90°C and persist while keeping their form (Guan, 2008).

Some researchers looked at the influence of different levels of WWF on bread texture and quality attributes, they saw that the addition of WWF into bread led to a microstructure that looked fused and this was clearly evident with the increased addition WWF. Moreover, the image also showed that protein the network seemed to be outstretched in between starch granules in the crumb (Garimella Purna et al., 2011). To understand the influence of WWF on dough, it has been found that the addition of WWF resulted in a discontinuous and disintegrated matrix structure which surrounded starch granules (Hung et al., 2007a). From the bread microstructure it can be seen that the additives altered the microstructure of bread in dissimilar ways and caused different modifications. This probably is due to their physico-chemical properties and behaviour within the bread system.
Figure (4.9) Pita breads microstructure with Cryo-SEM: control (no additives), All (IN 4%, GG1% and WWF 7.5%), IN (8%), GG (2%) and WWF (15%)
The microstructures of Tandoori breads supplemented with FI are presented in Figure 4.10. It is worth pointing out that changing the recipes quantity and baking process to some extent changed the microstructure of bread. The starch of control bread (4.10 control) is mostly gelatinised and to some extent outlines of granules can be hardly recognised. Whereas, the granules of bread with all the ingredients, despite being swollen, they seem to be less gelatinised and have kept their identity compared to the control. Moreover, they seem to be stuck in a complex matrix of GG, IN and gluten. This presumably could be as a consequence of competition for the water availability. As pointed out previously the FI have a stronger tendency and affinity towards moisture than starch. As a result of that, the gelatinisation of starch is restricted.

The microstructure of bread enriched with IN (4.10 IN) and GG (4.10 GG) showed the embedment of starch granules in the gluten and FI. It is apparent that starch granules are less gelatinised and swollen in comparison to the control. However IN shows less of an integrated and smooth structure. GG seems to wrap the granules and shows a more coherent and smooth structure. The microstructure of bread with WWF (4.10 WWF) seems to be less integrated than the control and the granules are more gelatinised than WWF in pita bread which gives a relatively smooth structure.

It can be derived from the aforementioned points that the amount of water added to bread could change the structure and shape of the starches. Also the temperature could influence starch gelatinisation. Hence it may provoke the rupture of granules during baking. So, we can now conclude that changing the amount of water could have an impact on the starch-protein matrix, hence the probable quality, texture and shelf life of the final-product. Similarly, temperature perhaps enhances the rapture of starch granules during baking.
Figure (4.10) Tandoori breads microstructure with Cryo-SEM; Control (no additives), All (IN 4%, GG1% and WWF 7.5%), IN (8%), GG (2%) and WWF (15%)
4.1.7. The influence of different preparation methods on starch microstructure

Cryo-SEM is a relatively recent technique that has plenty of applications in the field of food science and technology. It is interesting to compare variations of this recent technique such as LVSEM to see whether the freeze-drying technique would impact on the starch microstructure or not as a result of drying and moisture loss through leaving sometimes overnight. The microstructures of control bread and bread with all the additives imaged with Cryo-SEM are presented in Figures 5.14b and 5.12b respectively and the bread with the same recipe was repeated and imaged with freeze-dryer method Figures 5.14g and 5.14g.

It can be seen from the results (Fig4.11f and 4.11g) that the starch imaged with LVSEM exhibits shrinkage. Additionally, the starch granules seem to be more tightly packed and tightened, while this is not clearly evident in the Cryo-SEM (Fig 4.11a and 4.11b). A potential reason could be the removed of water in the sample which causes shrinkage in the microstructure. It was noticed that the physical appearance of the specimens were relatively shrunk and become fragile after the freeze-drying step. Despite this, further investigation could confirm this influence on the starch granules.
Figure 4.11 Microstructure of the control bread and bread with all the ingredients with two SEM techniques: Cryo-SEM; a) control; b) bread with the all ingredients. LVSEM; f) control bread, g) bread supplemented with the all ingredients.
4.2. Conclusions

To summarize, the aim of this work was to discover the influence of three FI alone and together on the cooking characteristics, dough quality and handling properties on two types of bread. The results showed that the addition of the FI modified cooking process and gel texture. IN alone decreased some pasting parameters. Contrarily, GG increased all the parameters apart from pasting temperature which decreased. But, WWF alone decreased SB and breakdown. More interestingly, neither combination of two ingredients nor mixing all the ingredients altogether showed appreciable change on the pasting properties. Regarding the gel, texture, IN and WWF addition alone reduced the hardness of the gel and alone and in combination considerably increased gel springiness. So gel texture results showed that the FI could be useful for reduction of the staling of bread.

The results of the dough processability and handling properties showed some changes with addition of FI. But none of the FI showed drastic detrimental change. Seemingly the combination of the FI showed closest results that of to the control. The outcomes of the breads’ quality also showed similar result to the dough results. The FI ingredients alone caused some modifications, but, combinations of all the ingredients produced similar results to the control. Despite showing some changes, none of the parameters caused dramatic modification even when the highest dose of the FI was used. Based on these results, it was found that none of the FI drastically changed the cooking characteristics, dough processability, handling properties and bread quality. So it was concluded that using the highest doses of the FI alone and in combinations will be used for further study to reveal the influence of the FI on the sensory attributes, shelf life and satiety response glycaemic response.
Chapter five

The impact of FI on the sensory attributes shelf life and glycaemic and satiety indices of the breads
5.1. Introduction

At present the demand for healthier food products like bread is steadily increasing, particularly bread high in FI (i.e. DF). Nevertheless, people expect good sensory characteristics with longer shelf life. However these characteristics sometimes cannot go together hand in hand. This is because the sensory properties of bread are likely to be altered by FI addition in particular flavour and overall acceptability. This could be due to the influence of FI on the breads texture and crumb quality (Purhagen et al., 2012).

There are different types of FI have been used in bread to improve its nutritional and/or technical properties. The data from literature showed that there are different results regarding the influence of SDF on the sensory and storage properties of bread, and these changes result from the physico-chemical properties of the SDF. But, commonly there is more likelihood that they will affect chewiness, flavour and overall acceptability. The presented information also suggests that the influence of DF on breads post-prandial glucose is various. For instance, IDF has less influential impact on GR. But, SDF is well-documented for its ability to positively influence food digestion especially carbohydrate (Tudorica, 2004). Therefore, based on the data obtained from the previous chapter, it is therefore the aim of this study to use the highest proportion of the FI alone and in combination to; firstly evaluate the sensory properties of the breads. Since dramatic modification of FI on the bread quality was not observed, the study aimed to see the perception of the consumers about the supplemented breads. Secondly, the study also intended to discover the influence of the FI on the shelf life properties of pita and Tandoori breads. Finally, the functionality of the products was assessed through measuring the
influence of the functional products on the post-prandial glucose and satiety response.

5.2. Materials and methods:

5.2.1. Sensory Evaluation
Samples of pita and Tandoori breads; control (no additives), All (IN 4%, GG1% and WWF7.5%), IN (8%), GG(2%) and WWF(15%) were subjected to a double-blind sensory evaluation by a panel comprised of 31 and 33 untrained volunteers respectively recruited from students and staff members of Plymouth University. The experimental protocol was approved by the University of Plymouth-Faculty of Science and Technology-Human Ethics committee. The samples were coded with three random digit numbers prior to the test. Then, the samples were served on white plates to be evaluated, for the following parameters appearance, texture, elasticity, flavour chewiness and overall acceptability using a nine point balanced hedonic scale ranging from 1 (strongly disliked) to 9 (strongly liked). An example of the evaluation form is presented in the appendix 5.

5.2.2 Bread shelf life measurement (staling)
After the samples were baked, cooled down and placed in plastic bags, they were stored at room temperature which was recorded with a temperature monitor (temperature data logger). The recorded temperature as graph is presented in the appendix 4. The microbial growth was also observed and tested with light microscopy (Stereo microscope, Olympus Tokyo-Japan) throughout the test.

For pita bread samples, four circles 20mm in diameter were taken in the middle of the samples to evaluate bread firmness, springiness and chewiness at 1, 3,
6,9 and 12 days using texture analyser TA- XT2 (TPA) 5kg loading cell, the test settings were: pre-test speed: 1mm/s; test speed: 1mm/s; post-test: 1mm/s; strain 60%. Ten replicates were run for each sample. For Tandoori bread, the bread was cut into four pieces and placed under texture analyser to measure toughness and extensibility at 1,3,6 days with the following settings: pre-test speed: 2.0mm/s; test speed: 1mm/s; post-test speed: 10 mm/s; distance: 60mm, with 1 inch plastic ball probe with radius (P1/S). Eight samples were run for each batch.

5.2.3 In vivo measurement of bread GR
After the ethical approval of the protocol by the Plymouth University Faculty of Science and Technology – Human Ethics committee, nine healthy and non-insulin dependent subjects were recruited from students and staff of Plymouth University. In this study, details of the research protocol were explained and written consent was received from all the volunteers before commencing the test. The exclusion criteria were self-declared knowledge of diabetes, kidney problems, allergies to food and body mass index (BMI) more than 30 kg/m². Therefore, a health questionnaire was given to ensure they were not included. After this, the participants were put through a screening process and anthropometric measurement. A stadiometer (Seca Ltd., Birmingham, UK) was utilised to measure height of the participants with no shoes. The weight of the participants was screened using a Tanita BC-418MA (Tanita UK Ltd., Yiewsley, Middlesex,UK) and body fat content and body fat index was calculated.

Bread quantity which is corresponding to 50g of available carbohydrate was served at morning after the subjects had fasted overnight. The meals were eaten in 15min. Blood samples were taken from the finger by the subjects
themselves at 0, 15, 30, 45, 60, 90 and 120min. Nine test samples for the supplemented breads (All, IN and GG) and three replicates for the controls (no additives) were run. The AUC was calculated following standard method by FAO and comparing with the control (FAO, 2013). The GR was calculated according the equation below.

GR value for the sample (%) = \frac{\text{mean AUC for the sample}}{\text{mean AUC for the reference food}} \times 100

Table (5.1) Body screen and BMI measurement for the participants in the GR and satiety response

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender</th>
<th>Age (year)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>30</td>
<td>174</td>
<td>58.6</td>
<td>19.4</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>35</td>
<td>157</td>
<td>53.3</td>
<td>2.16</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>27</td>
<td>177</td>
<td>78.8</td>
<td>25.2</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>30</td>
<td>165</td>
<td>69.5</td>
<td>25.5</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>33</td>
<td>173</td>
<td>73.4</td>
<td>24.5</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>33</td>
<td>180</td>
<td>81.1</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>29</td>
<td>173</td>
<td>89.6</td>
<td>29.9</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>30</td>
<td>174</td>
<td>60</td>
<td>19.8</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>50</td>
<td>172</td>
<td>85</td>
<td>28.7</td>
</tr>
<tr>
<td>Total</td>
<td>2 females</td>
<td>35.7±10.7</td>
<td>171.6±6.4</td>
<td>71.9±12.00</td>
<td>22.4±7.8</td>
</tr>
</tbody>
</table>

The exclusion criteria were body mass index (BMI) more than 30, people with diabetes, kidney failure, and self-declared allergy
5.2.4. Satiety assessment
The satiety response (SR) was assessed after serving the isocaloric meals. The participants were asked to record their satiety levels after each GR sampling time throughout the experiment (0-120 min) on a seven point scaling system from extremely hungry to extremely satisfied. Zero time was considered as the baseline. The incremental area under satiety curve was calculated for all the breads and compared with the control. The satiety form is presented in the appendix 8.

5.2.5. Statistical analysis
The results from the sensory evaluation assessment obtained submitted to statistical analysis using analysis of variance (ANOVA) and Tukey’s test from Minitab (16.2) software (Minitab Ltd, Coventry-UK) to discover the statistical differences for sensory attributes of all the types of breads. The data were calculated as means ± SD for the GR and satiety. Then ANOVA was used to find significant differences between the treatments and the controls.

5.3. Results and discussion:

5.3.1. The influence of FI on the sensory characteristics
It is an important step in product development to evaluate the successfulness and acceptability of any product by consumers to predict consumers perception which could impart an idea about palatability and then marketability of the products (ElÍÁ, 2011). Therefore the current sensory evaluation was undertaken to assess appearance, texture, elasticity, flavour, chewiness and overall acceptability of the breads with no additives (control), bread with all the combinations, bread with 8% IN, bread with 2% GG and bread with 15% WWF. In a ranking test the higher scores refers to a food with high acceptability.
The results showed all the types of bread scored more than 5 which are considered acceptable. Furthermore, the statistical analysis indicated that there is no significant difference between the attributes of appearance, texture, and elasticity of pita bread. On the other hand, there is a considerable difference between flavour, chewiness and overall acceptability. In terms of flavour bread with IN recorded the highest score with average of 7.3 and that was significantly different (p˂0.05) than GG bread 5.7, bread control 5.6 and WWF bread 5.5, but it was not significantly different than bread with all the ingredient which scored 6.6. It worth mentioning that IN can impart a special taste and smell to the products, and this has made it suitable to be used as a fat replacer in some products (O’Brien et al., 2003, Zahn et al., 2010). This also could be a reason for chewiness and overall acceptability.

In both chewiness and overall acceptability respectively, the ranking was same and IN came first with scores 7.1, 7.3. This was followed by bread with all the combinations 6.8 and 6.6, GG 6.0 and 6.1, WWF 5.7 and 5.7 and control 5.6 and 5.7. In both attributes, only bread with IN was significantly different than WWF and control, and the rest were not significant. It is worth pointing out that all the types of bread with and without additives were considered acceptable since all of them scored more than 5.

For Tandoori bread, there was no significant difference in terms of appearance, texture, elasticity and chewiness, despite the difference in the scored-ranking. In terms of flavour and overall acceptability, bread with all the ingredients scored first rank with 7.3 and 7.2 followed by GG 6.3 and 6.6, control 6.3 and 6.4, IN 6.0 and 6.2 and WWF 6.0 and 6.2 respectively. Furthermore, the statistical analysis showed that only in flavour and overall acceptability bread
with all the ingredients was different to bread WWF and there was no real
difference between the rests. It can be understood that similar to the physical
and instrumental measurement, sensory evaluation revealed that substitution of
flour with FI alone and in combination had less profound influence on the bread
sensory attributes. Moreover, the substitution of flour with FI has less sensorial
influential modification on Tandoori bread than pita bread. It can also be noticed
that partially substituting flour with FI did not deteriorate the quality. But also
improved some sensory attributes and palatability of both types of bread
particularly pita bread.

Figure (5.1) Sensory evaluation of pita bread with and without FI score means±
SEM (n=31)
Figure (5.2) Sensory evaluation of Tandoori bread with and without FI score means ±SEM (n=33)
5.3.2. Shelf life: texture properties of bread during storage time

There are potential changes which may occur during the storage period of bread such as changes in texture. The change in texture is largely linked to the phenomenon which is known as bread staling. It is the point where physicochemical properties of the product are changed between baking and consuming. Generally, the texture of bread is deteriorated since hardness and elasticity increase and springiness decreases and the product becomes chewy. The aroma and flavour of the product is also lost. Unfortunately, the bread consequently ends with the end-product depreciation.

The main reasons for this phenomenon could be associated to the moisture content, its distribution within the bread system and starch molecular reassociation (retrogradation). Wheat flour starch is comprised of branched and straight chains. During cooking, the starch granules absorb water and swell and the linear chains leach out from the granules to give initial form to the bread. Whereas, the branched chains require time to set and over a period of time, these chains could come together to make the crumb hard, water also could immigrate from crumb to the crust then evaporates (Angioloni and Collar, 2009b). Over time the bread gets firmer and loses its softness, this is the start of staling.

From this perspective, bread properties were assessed over storage period of time which included texture such as hardness, springiness and chewiness for pita bread and toughness and extensibility for Tandoori bread. The analysis of variance (ANOVA) for pita bread is presented in table 5.2.

In the first day, for pita bread, hardness and chewiness recorded same effect which showed that WWF significantly (p<0.05) increased these factors. In
contrast IN significantly decreased them comparing to the control. No changes happened to the springiness of the bread with and without additives. Day 3, only IN considerably reduced hardness and chewiness. The rest of the ingredients showed no impact. WWF significantly decreased (p<0.05) springiness. In the day 6, IN again decreased both hardness and chewiness parameters noticeably (p<0.05) comparing to the control. GG also decreased hardness and the rest of the parameters did not show any significant differences. Springiness also was not altered by any of the parameters. In the day 9, despite the fact that almost all the additives decreased the texture parameters, only IN and GG significantly (p<0.05) had reduced hardness and chewiness. One again, springiness recorded no changes by the additives. In the day 12, microbial growth was observed on the surface of breads with IN and bread with all the combinations. So they were discarded and removed from the test. However, in terms of texture they appeared to be still valid. The results of the day 12 suggested that bread with GG and WWF significantly reduced hardness and chewiness, comparing to the control. For Tandoori bread, in the day 1, no significant difference was observed comparing the control except for bread with all the ingredients, chewiness was increased. For the day 3, the scenario is changed in the statistical point of view. Since toughness was reduced with the addition of GG, combination of all the ingredients and WWF.
Figure (5.3) The influence of the FI on the pita bread hardness

Figure (5.4) The influence of the FI on the pita bread springiness
Moreover, extensibility was only reduced when combinations of all the ingredients and WWF were added. In the day 6, not many changes were recorded, none of the factors showed significant difference on hardness and extensibility except IN which increased extensibility comparing to the control. After day 6, mould growth was observed on the surface of the bread. Therefore the breads were discarded and discounted from the test. It can be seen quite clearly that the addition of FI alone particularly IN and GG and in combination could reduce hardness and chewiness in general to a good extend. However, not all of them showed difference statistically. This could be due to the high standard deviation because of the variation in the bread.
Table (5.2) The influence of the FI on the breads shelf life parameters

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Treatment</th>
<th>Pita bread</th>
<th>Tandoori bread</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardness (g)</td>
<td>Springiness</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>553+63&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.92+0.03</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>506+157&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.92+0.03</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>300+64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92+0.03</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>705+131&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.91+0.01</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>787+470&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92+0.03</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>925+212&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87+0.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>681+107&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.89+0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>551+156&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88+0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>983+481&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91+0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>724+217&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.06+0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>1084+181&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89+0.04</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>863+126&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.89+0.19</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>755+234&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84+0.06</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>712+242&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.91+0.05</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>1123+257&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89+0.03</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>1122+138&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87+0.04&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>1019+267&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81+0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>803+234&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83+0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>665+149&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92+0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>928+103&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.89+0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>1358+319&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86+0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>923+119&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85+0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>551+198&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92+0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Looking at the graphs of texture parameters, it is not just surprising but also was expected that the bread became firmer over storage period of time. This clearly points out to the physical changes and staling phenomenon. The second trend which was expected was the influence of the additives on the bread system. It can be obviously noticed that presence of FI could reduce the rate of staling and retard starch molecules re-association. Also it is evident that the FI showed dissimilarity in retarding starch gelatinisation. In a study on the effect of GG on the stored chapatti bread at room temperature, it has been reported that the addition of GG decreased the tear force and also extensibility of stored chapatti (Ghodke, 2009). This result is congruent with our finding which showed that GG decreased both toughness and extensibility force.

It has been mentioned that the water content of bread with GG was more comparable to the control. It has been reported that GG acts as an anti-staling agent can retard retro gradation of starch (Selomulyo and Zhou, 2007). The anti-staling influence of GG has been ascribed to its ability to distribute moisture in the bread constituents and system (Rodge et al., 2012). IN also could help in retarding the staling process. It has been reported that IN was also used in a different aspects of food technology including baked product to extend shelf life (Aravind et al., 2012d).

WWF also demonstrated an ability to retard staling. Therefore, WWF has also been added to baked product as an anti-staling substance since its composition is mainly amylopectin. The amylopectin molecules re-associate after baking, slower than normal wheat. Hence it could retard staling process (Bhattacharya et al., 2002). Alternatively, this could be a consequence of the ability of amylopectin to enhance moisture intake and reduce the enthalpy of amylopectin
retrogradation, consequently leading to staling postponement (Goesaert et al., 2009, Garimella Purna et al., 2011).

The influence of the additives on the shelf life of Tandoori bread is less profound as no significant difference was recorded. This could be due to the following factors. Firstly the property of Tandoori bread which has a thinner crumb comparing to pita bread which has a relatively thicker crumb. Secondly, because of the properties of the bread which needs more water comparing to pita, most of the starches are gelatinised and leached out of the granules as showed in the microstructure of Tandoori bread. This probably limits the restriction impact of the additives on the re-association of the starch molecules.

Thirdly, due to the thinness of the bread most of the moisture possibly evaporates then there will be less water kept in the system and this could not be enough to retard re-association of the starch molecules during storage time. Finally, the bread did not last long because of the microbial growth; it could not be stored for a long time to notice the full differences.
Figure (5.6) The influence of the FI on the Tandoori breads toughness

Figure (5.7) The influence of the FI on the Tandoori breads extensibility
5.3.2. In vivo measurement of the GR of breads

The results from the data analysis shown in the table 5.3 and in the figures of 5.8 and 5.9 represent GR for pita and Tandoori breads respectively. The results from the graphs show that the post-prandial blood glucose for all the types of breads seems to be similar in the first 30 min. However, beyond this time the post-prandial blood glucose seem to be reduced by the FI.

DFs alone and in combination significantly reduced the post-prandial blood glucose. GG greatly lowered (p<0.05) the GR of pita bread by 45%. IN also greatly decreased the GR by 43%. But, the combination of all the ingredients resulted in a reduction by 37%. The GR and post-prandial blood glucose significantly declined (p<0.05) on Tandoori bread with GG and IN., statistically it did not show any significant difference.

It can be understood from the above results that the SDF can be used to reduce the post-prandial blood glucose and GR of breads. It can also be noticed that the AUCs for Tandoori bread are slightly higher than the pita breads. This could confirm the same results as found in the bread microstructure, since it was discovered that the starches of Tandoori breads were more gelatinised.

This could imply that they are easier and quicker than the starch of pita for digestion for digestive enzyme to attack the granules. GR for combination of all the ingredients also was anticipated to be closer to the control comparing to the bread with GG and IN. This is because the microstructure of the breads showed that there is similarity between the control breads and bread with all the ingredients.
There are several studies regarding the food GR, particularly on the influence of DFs on the bread GR. In an in vitro study to discover the influence of some DFs including GG on the post-prandial serum glucose, it was seen that the blood serum glucose was declined by the addition GG (Ou, 2001). In a review, the impact of GG on post-prandial glycaemia has been listed when a single dose of GG managed to reduce the post-prandial blood glucose by 10%. It has been reviewed that GG could reduce post-prandial serum glucose by 26.56% after a liquid test meal was given to four subjects and blood glucose declined from 114± mg/dL to 86±3 mg/dL (Butt et al., 2007). Corporation of GG and CMC into spaghetti, it has been concluded that both gums appreciably decreased starch hydrolysis, which presumably reduced post-prandial blood glucose (Aravind et al., 2012c).

IN has also showed that it could reduce the post-prandial blood glucose. It has been stated that in an in vitro study that IN reduced the amount of released sugar from pasta (Brennan et al., 2004). In a study about the influence of two types of IN dissimilar in DP and crystallisation on pasta texture and structure, it has been found that IN can change starch digestibility which could potentially reduce the GR. They ascribed this to formation a matrix between protein and fibre which could act as a physical barrier in front of the digestive enzyme (Aravind et al., 2012a). The addition of 5%, 10% and 15% of IN to pasta could change the microstructure of starch comparing to the control which eventually alters the starch digestibility. It has been demonstrated that with an increase of IN the starch granules seem to be less gelatinised.
This has been ascribed to the greater affinity of IN which competes on the water availability with starch granules. Hence, the granules become less gelatinised (Manno et al., 2009). It has been concluded that supplementing 4% of soluble β-glucan into chapatti bread could considerably reduce its GI (Thondre and Henry, 2009).

A few mechanisms have been proposed by which DFs could affect and reduce the GR of food and post-prandial blood glucose. Firstly, DF could lengthen the process of digestion of starch in the stomach. Secondly, alteration of gastrointestinal movement and increase gut transit time and prolong digestion process. Thirdly, DFs could limit the amylase action and starch hydrolysis since they could cover starch granules and lengthen the distance between amylase and granules. Finally, DFs could create viscous layers which could work as physical barriers and will restrict the diffusion of saccharides in the small intestine (Papathanasopoulos and Camilleri, 2010, Vosloo, 2010, Ou et al., 2001). In addition to that, it has also been shown that through producing SCFA from DF by microflora presence in the gut, carbohydrate metabolism could be changed (Carter, 2012).
Figure (5.8) The glycaemic response for the pita breads

Figure (5.9) The glycaemic response for the Tandoori breads
Table (5.3) The incremental area under curve of GR and satiety of pita and Tandoori breads

<table>
<thead>
<tr>
<th>Bread types</th>
<th>Treatments</th>
<th>Time of the meal ingestion (min)</th>
<th>AUC</th>
<th>GR %</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Pita</td>
<td>Control</td>
<td>5.0</td>
<td>5.8</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>4.8</td>
<td>5.3</td>
<td>6.3</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>5.0</td>
<td>5.5</td>
<td>6.7</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>4.9</td>
<td>5.4</td>
<td>6.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Tandoori</td>
<td>Control</td>
<td>5.8</td>
<td>5.7</td>
<td>6.6</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>5.5</td>
<td>6.5</td>
<td>6.2</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>5.2</td>
<td>5.3</td>
<td>6.5</td>
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</tr>
<tr>
<td></td>
<td>All</td>
<td>4.9</td>
<td>5.6</td>
<td>6.6</td>
<td>6.8</td>
</tr>
</tbody>
</table>

AUC; area under curve, GR; glycaemic response, SR; satiety response

5.3.3. Satiety test

The satiety results are presented in table 5.3 and graphs for AUCs for pita and Tandoori breads are presented in figures 5.10 and 5.11 respectively.

The statistical analysis of satiety showed that the DF neither alone nor in combinations demonstrated any significant change in pita breads. Although, it seems that satiety was improved by the additives, but statistically no difference was seen between the control and the treatments. Similar results were also observed for Tandoori breads as they improved satiety but not significantly (p>0.05).

The benefit of satiety is due to the fact that high satiation foodstuffs could regulate energy intake and consequently body weight management. This is through giving fullness sensation and postpones the following meals. There are several investigations about the influence of DFs on the satiety response of foodstuffs. It is believed that the presence of DF could influence gut hormone
excretion which could play a key role as a satiety inducer. However the results might largely depend on doses and types of the DF. It has been highlighted that SDF plays a main role in improving the satiation capacity of the food. Adding 2.5% SDF for solid and semi-solid meals discovered that the satiety was improved (Brouns, 2002). In a very recent review about the influence of DFs on the appetite, it has been mentioned that GG and IN at high level could improve satiety and reduce energy intake (Clark and Slavin, 2013).

A few potential mechanisms have been implicated with the influence of DFs on satiety of fibrous food. First, naturally fibre-rich products could have low energy and sometimes low palatable characteristics. This could act as physical barrier to energy intake. Secondly, in terms of time, it could take a longer time to chew and digest. Consequently the satiety could be provoked. Thirdly, because of having viscous traits, some DFs could be digested and processed more slowly than non-fibrous foods. This also could obstruct digestive enzymes and impart the fullness sensation after ingestion. It has also hypothesized that SCFAs which are produced from fermented DFs could alter mobility in the gastrointestinal tract through stimulation of the secretion of some appetite and satiety regulating hormones such as ghrelin, cholecystokinin glucagon like peptide-1 (Hess, 2010, Fiszman and Varela, 2013).
Figure (5.10) The satiety response for the pita breads

Figure (5.11) The satiety response for the Tandoori breads
5.4. Conclusions

To summarise, the aim of this study was to investigate the influence FI on types of bread sensory evaluation, shelf life, GR and satiety. The data showed that the sensory evaluation was not changed dramatically. Likewise, some of the attributes were improved by the addition of FI. All the breads were deemed successful as they scored more than 5. The FI improved the shelf life of the bread, particularly pita bread. But, although the FI seems to improve Tandoori bread shelf life parameters, it could not be seen clearly as it did not last for long because of mould growth. The in vivo assay for GR showed that the DFs alone and in combination significantly reduced GR and post-prandial blood glucose except for bread with all the combination in Tandoori bread. It was a good indicator that the GR of bread can be reduced to a large extent through adding DFs without changing the quality of the products drastically. The data also pointed out that GR of food products can be reduced to almost half through adding the DFs. The data analysis also showed that the additives improved satiety; however, they were not significant in statistical point of view. The results may suggest that using DFs alone and in combinations could be used to create breads with positive quality and sensory attributes with medium GR.
Chapter six

General comments and conclusions
6.1. General comments and conclusions

Some health transitions have happened worldwide during the last few years because of the life style change. This presumably is the cause of so many non-transmitted epidemic diseases. Obesity, diabetes, cardiovascular diseases and hypertension have steadily increased which undoubtedly lead to the life style and quality depreciation. It could also cause a huge number of early stage deaths.

Therefore, in the community, health professional and the academic society there is a serious concern about this problem. For that reason, there are different ways that have been attempted to address this issue. For instance, creating food rich in DF with less saturated fatty acids and carbohydrate is one of them. The idea behind is reducing the uptake of fat and carbohydrate and boost the consumption of DF to reduce the risks of the chronic diseases. Because we get a feeling that altering of risk factor could possess a noticeable positive effect in the long term.

This study was an endeavour to get this issue addressed through using a wide range of three natural FI with different functional properties in cereal formulation intending not just creating fibre-rich functional breads useful nutritionally, but also acceptable in terms of quality and edibility.

Firstly, the behaviour of different FI alone and in combination was investigated in relation to the cooking and pasting properties. Also the flour gel texture was examined and assessed with texture analyser. The outcomes showed that the FI possess different impacts on the cooking and pasting qualities of the flour starch. For example, GG recorded the highest influence on the pasting parameters and seems to be incorporating with starch granules. In contrast, IN
and WWF alone showed an opposite manner which implies that they decreased the pasting parameters. This could suggest that they either did not cooperate with the granules to increase pasting parameters or they restricted starch gelatinisation. The combination of all the ingredients has another impact. It showed no significant influence on the pasting parameters since it was comparable with the control. It was observed that because of the difference in physic-chemical properties of the FI, they appeared to have different impact on the pasting properties. So it is important to take into consideration the properties of the additives into products. Since, they could not just change the cooking quality but also the whole product quality and shelf life.

The flour gel texture results showed that the FI had different impacts on the flour gel texture parameters (hardness, springiness, adhesiveness and whiteness). The results indicate that IN and WWF decreased significantly (p<0.01) gel hardness and increased springiness. In opposite, GG alone had no appreciable influence on the gel texture parameters. The FI neither alone nor in combination showed any considerable impact on adhesiveness. Finally, only WWF increased whiteness quadratically.

These results also confirmed the dissimilar influences of the FI on the starch gelatinisation within flour pasting system and could have different influence on the starch molecular re-association. The results of IN and WWF suggested that these two ingredients could have positive influences on the starch gelatinisation which presumably can extend shelf life of the products. This is through delaying the retrogradation of the starch molecules and ultimately keeping the product’s softness and retarding staling. The outcomes suggested that none of the
parameters showed a drastic influence on the viscoelastic properties of wheat the design, so the design was remained same for the next test.

Secondly the influence of the FI on the dough quality and handling properties (i.e. resistance to extension and extensibility) and machinability traits (i.e. stickiness, adhesiveness and cohesiveness) was studied. The results suggested that there was a difference in terms of dough quality and handling properties in relation to the functional properties. IN reduced (p<0.01) resistance to extension but no influence on extensibility. GG alone and in combination with IN significantly increased (p<0.01) both parameters. Regarding, dough machinability parameters IN increased all the parameters significantly (p<0.01). Furthermore, adding more IN could create sticky dough which could be difficult to process. In opposite, GG alone and in combination with IN reduced them. Combination of all the ingredients seems to have no noticeable changes on the dough quality and machinability.

Despite these influences, the enriched dough with different ranges of FI performed reasonably well during the breads production process. The most promising results on dough properties were linked to the utilisation of all the combination together in dough making since they did not show any changes in the dough quality and resulted in similar dough to the control. Once again none of the parameters showed dramatic change in the quality of the dough, so the design remained same.

The quality of the enriched breads was also assessed both physically and instrumentally. Physical assessment included; weight loss, colour, diameter, loaf height (thickness) and volume and instrumental measurement composed of hardness, springiness and chewiness for pita bread and toughness and
extensibility for Tandoori bread. The results exhibited that there is a little influence on the pita bread quality. Height was increased with both GG and IN addition. Similarly, GG and IN alone significantly (p<0.05) increased volume. However, GG induced moisture loss during baking. No influence was recorded in relation to bread colour and diameter. GG and IN alone also increased (p<0.05) hardness and chewiness but significantly reduced (p<0.05) springiness of pita bread. These impacts get even attenuated on Tandoori bread, since only WWF decreased volume and diameter. The combination of IN and GG noticeably increased weight loss and diameter and only IN quadratically increased bread toughness. It is evident that the FI did not change the product quality considerably. It was found that none of the FI neither alone nor in combination dramatically changed the quality of the breads. This led to a point where the highest proportions of the FI will be used. Thus, for the sensory evaluation, the highest proportion from the FI (IN 8%, GG 2% and WWF 15%) alone and in combination (IN 4%, GG1% and WWF 7.5%) was utilised and supplemented.

For the sensory evaluation, the results appeared to be similar to the quality assessment which suggested that none of the sensory characteristics were affected drastically by FI addition. For pita bread, only flavour, chewiness and overall acceptability were altered with the ingredients addition. IN scored the highest score (7.3) for flavour and was significantly different than bread with GG, control and WWF. Again for chewiness and overall acceptability IN scored the highest score and followed by bread with all the ingredients, bread with GG, bread with WWF and control. However, for Tandoori bread only flavour and overall acceptability were affected by FI addition. Bread supplemented with all the ingredients scored the highest score for flavour which was different than
bread with WWF and the rest was not. The same scenario was repeated for over all acceptability. Flavour and overall acceptability in both of the breads were affected by the FI. It seemed to be that IN is more useful and convenient for pita bread in terms of sensory attributes, but combination of all the ingredients showed to be more appropriate for Tandoori bread. It was commented in sensory sheet that bread that Tandoori bread enriched with IN was not even. In spite of that, it can be summarised that all the supplemented bread were acceptable since minimum score to refuse any bread character was 5, and all of them scored more than 5. Therefore, all the bread was deemed acceptable. One of the comments on the sensory evaluation that all the breads were similar in terms of sensory attributes.

The pita and Tandoori breads were also submitted to shelf life assessment. The results showed that staling rate was generally slowed down with the addition of the FI alone and in combination. Particularly IN and GG in pita bread which demonstrated a more efficacious impact on bread staling. However, for Tandoori bread because of the shorter period of shelf life, the influence cannot be noticed as much as pita bread. But, generally the FI slowed down the staling rate. This could suggest that having FI in the functional products could be useful for a longer shelf life and a softer texture.

In the final part the GR and satiety for both types of bread enriched with GG and IN alone and GG, IN and WWF all together were studied and measured. It seems that all types of bread alone and in combination reduced GR and AUC of the breads except for Tandoori bread combinations of all the ingredients was not significantly different. It was found that the GR can be reduced up to 45% through adding DFs. Also, the FI showed to increase the satiety but not
significantly. This was a positive point that functional breads can be created through adding DFs without changing the quality of the products drastically.

To conclude what has been presented:

1- FI can be used to create and design functional products with acceptable quality and sensory attributes. But, it is crucial to take into consideration the type, amount and physico-chemical properties of the ingredients should be suitable to the product to be supplemented with. This is important to make sure that the product could satisfy the need of the public in terms of quality and sensory characteristics.

2- Products with FI could also have sensory attributes and perform well during shelf life period of time. This could be useful to replace conventional products with functional products that would have better sensory attributes with longer shelf life.

3- The functional products supplemented with DF could bring more nutraceutical benefits because of having lower GR compared to the product without DF as seen in both types of breads. This could also reduce the risks of diabetes and other chronic diseases which come from over consuming energy. It was proved that DF could lower GR of the products. But the effect could be changed according to types of DF and the way of using them (alone or in combination) and the type of the product. In both breads, DFs showed better performance alone and decreased GR and AUC better than adding together.
6.2. Future Work:

There are other areas that can be investigated more as a completion of this study.

For examples the following areas can be studied:

1- In vitro digestion of the supplemented bread and then imaging with Cryo-SEM could be an interesting area to discover the influence of the FI on the starch digestibility. This allows viewing starch digestion in different breads and then comparing with the GR test.

2- Macrostructure scanning of the breads could be studied to discover if there is any difference in terms of the macrostructure of bread.

3- Using different packaging methods and some antimicrobial agents is another possibility to be researched. This enables us to stop microbial growth on the surfaces of the breads and go further with the shelf life measurement.

4- Using edible films is an option to be used on the surface of the bread to stop microbial growth and extend shelf life.

5- Kinetic study of bread staling using differential scanning calorimetry techniques to measure amylopectin retrogradation value.

6- In terms of satiety, different utensils such as plastic and metal can be used to see their influences on the satiety level and panellists perception.
6.3. Appendices:

a)

Appendix 1. Texture analyser - typical test to evaluate; a) flour gel texture, pita bread quality and pita shelf life parameters (hardness, springiness and chewiness)

Appendix 2. Texture analyser - dough quality and handling properties and Tandoori bread shelf life measurement
Appendix 3. Texture analyser - dough processability/machinability properties assessment temperature

Appendix 4. Shelf life storage temperature recorded.
Sensory evaluation of bread enriched with dietary fibre

Panellists code/initials: ……………… Product code: ……………

Please evaluate and indicate your opinion about each attribute by marking (✓) in a suitable box for each attribute. Please make sure that your results are placed under the correct code.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Liking scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strongly dislike</td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9</td>
</tr>
<tr>
<td>Appearance</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td></td>
</tr>
<tr>
<td>Elasticity</td>
<td></td>
</tr>
<tr>
<td>Flavour</td>
<td></td>
</tr>
<tr>
<td>Chewiness</td>
<td></td>
</tr>
<tr>
<td>Overall acceptability</td>
<td></td>
</tr>
</tbody>
</table>

Additional comments:

Appendix 5: The sensory evaluation sheet for both types of breads
Appendix 6: sensory evaluation a) pita breads and b) tandoori breads

a)

Sensory attributes for pita breads

b)

Sensory attributes for tandoori breads
RECORDING SHEET

Volunteer’s code: ...........................................

Sex: ..........  Age: ............

Weight (kg): .............  Height (cm): .............

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sugar level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Appendix 7: The recording sheet for the glycaemic response test
Assessment of Satiety test

Volunteer’s Code: ………………………………

Date: ………………………………….

After each blood sample check, Please tick the appropriate box, which corresponds to your feeling of hunger.

<table>
<thead>
<tr>
<th>Time in fasten state (0 min)</th>
<th>Level of Satiety ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extremely Hungry</td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
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<td>90</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

Appendix 8: The satiety response recording sheet for both types of the breads
Chapter seven
Reference List
7.1. References


dietary red meat than white meat and is attenuated by resistant starch. *Clinical Nutrition*, 31, 1-5.


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