UNIVERSITY
of
GLASGOW

Developing an index of dietary estimation of fermentable carbohydrate to allow dietary analysis for epidemiological studies

Sulaiman Alharbi

BSc, MSc

A thesis submitted for the degree of Doctor of Philosophy
to
The University of Glasgow

October, 2014

From research conducted at the
School of Medicine, Human Nutrition
University of Glasgow
Glasgow, Scotland

Dedication

I would like to dedicate my thesis to my wife and my kids, Alhanouf, Faisal and Turki. They are the ones who supported me with my education for the past four years when I was abroad. I will be eternally grateful for their support, love and prayers.

I would also like mention my brother, Sultan, who was always a phone call or an email away.

Lastly, I would like to express my appreciation to King Abdullah for all his support in our education abroad.

Abstract

It has been proposed that long-term consumption of diets rich in non-digestible carbohydrates (NDCs), such as cereals, fruit and vegetables might protect against several chronic diseases, however, it has been difficult to fully establish their impact on health in epidemiology studies. The wide range properties of the different NDCs may dilution their impact when they are combined in one category for statistical comparisons in correlations or multivariate analysis. Several mechanisms have been suggested to explain the protective effects of NDCs, including increased stool bulk, dilution of carcinogens in the colonic lumen, reduced transit time, lowering pH, and bacterial fermentation to short chain fatty acids (SCFA) in the colon. However, it is very difficult to measure SCFA in humans *in vivo* with any accuracy, so epidemiological studies on the impact of SCFA are not feasible. Most studies use dietary fibre (DF) or Non-Starch Polysaccharides (NSP) intake to estimate the levels, but not all fibres or NSP are equally fermentable.

Epidemiological and intervention studies use traditional dietary analysis methods, which depend on food composition databases that may not have been updated to include non-digestible oligosaccharides (NDOs), resistant starch (RS), and so do not provide accurate data on NDCs intake whether or not it is fermented. Therefore, this thesis attempted to develop an index of dietary estimation of fermentable carbohydrate (FC) from the amount of carbohydrate in diets that bacteria in the colon ferment to produce SCFA.

The first aim of this thesis (Chapter 3) was the development of the equations used to estimate the amount of FC that reaches the human colon and is fermented fully to SCFA by the colonic bacteria. Therefore, several studies were examined for evidence to determine the different percentages of each type of NDCs that should be included in the final model, based on how much NDCs entered the colon intact and also to what extent they were fermented to SCFA *in vivo*. Our model equations are

FC-DF or NSP[$] 1: 100 % Soluble + 10 % insoluble + 100 % NDOs[¥] + 5 % TS**
FC-DF or NSP 2: 100 % Soluble + 50 % insoluble + 100 % NDOs + 5 % TS
FC-DF* or NSP 3: 100 % Soluble + 10 % insoluble + 100 % NDOs + 10 % TS
FC-DF or NSP 4: 100 % Soluble + 50 % insoluble + 100 % NDOs + 10 % TS
*DF: Dietary fibre; **TS: Total starch; [$]NSP: non-starch polysaccharide; [¥]NDOs: non-digestible oligosaccharide

The second study of this thesis (Chapter 4) aimed to examine all four predicted FC-DF and FC-NSP equations developed, to estimate FC from dietary records against urinary colonic NDCs fermentation biomarkers. The main finding of a cross-sectional comparison of habitual diet with urinary excretion of SCFA products, showed weak but significant correlation between the 24 h urinary excretion of SCFA and acetate with the estimated FC-DF 4 and FC-NSP 4 when considering all of the study participants (n = 122). Similar correlations were observed with the data for valid participants (n = 78). It was also observed that FC-DF and FC-NSP had positive correlations with 24 h urinary acetate and SCFA compared with DF and NSP alone. Hence, it could be hypothesised that using the developed index to estimate FC in the diet form dietary records, might predict SCFA production in the colon *in vivo* in humans.

The next study in this thesis (Chapter 5) aimed to validate the FC equations developed (chapter 3) using *in vitro* models of small intestinal digestion and human colon fermentation. The main findings in these *in vitro* studies were that there were several strong agreements between the amounts of SCFA produced after actual *in vitro* fermentation of single fibre and different mixtures of NDCs, and those predicted by the estimated FC from our developed equation FC-DF 4. These results which demonstrated a strong relationship between SCFA production *in vitro* from a range of fermentations of single fibres and mixtures of NDCs and that from the predicted FC equation, support the use of the FC equation for estimation of FC from dietary records. Therefore, we can conclude that the newly developed predicted equations have been deemed a valid and practical tool to assess SCFA productions for *in vitro* fermentation.

In conclusion, this thesis demonstrated several relationships between estimated dietary FC using our equations and SCFA products *in vivo* and *in vitro*. These need to be further validated, for example by using FC from well-designed interventions and comparing these with fermentation in humans *in vivo.* The most reliable method that could be used to measure SCFA in humans' *in vivo* is the isotope dilution method. Once better validated the FC equations developed in this thesis will be a powerful epidemiological tool for future cohort, case control and intervention studies investigating the role of fermentation in a range of chronic conditions more clearly that using DF alone.

Table of Contents

List of Tables

List of Figures

Published abstract

Developing the concept of dietary estimation of fermentable carbohydrate (FC)

By S. Alharbi[1,2], A.L. Garcia[2],D. J. Morrison[2] and L. Hanske[2], C. A. Edwards[1], Human Nutrition, School of Medicine, College of Medical, Veterinary & Life Sciences University of Glasgow, New Lister Building, Glasgow Royal Infirmary, 10-16 Alexandra Parade, Glasgow City, G31 2ER, Scotland, United Kingdom (OC19), 48I

Acknowledgement

I wish to express my sincere gratitude to Allah for providing me with health, commitment and patience for completing my PhD studies. I am deeply grateful to my supervisor, Professor Christine Edwards for her knowledge, expertise, encouragement, support, and overall kindness. Many thanks to my joint supervisor Dr Ada Garcia for her advice, valuable insights and help. Without my supervisors I could not have done it, thank you for being so thoughtful. I would like also give big thanks to Dr Douglas Morrison for his help and advice.

I would also like to give a special thanks to Professor Tom Preston, Dr Laura Hanske and Alexandra C Small.

I would also like to give a special thanks to Dr Kostas Gerasimidis, who helped me every time I needed help.

I would like to acknowledge the lab technicians Frances Cousins and Graeme Fyffe for helping me with ordering and organizing our lab.

I would also acknowledge my colleagues (Anas Malik, Salma Malik, Clare Clark, Jaffar Khan, Muhammad Shahzad, Majid Alkhalaf, Olga Biskou, Hannah Harris, Min Hou and Bahareh Mansoorian) for their help and support throughout my PhD.

Author’s Declaration

I declare that the work in this thesis is the work of the author Sulaiman Alharbi. I am responsible for the organisation, lab work, analysis and data processing, unless otherwise cited. The research for this thesis was carried out between October 2010 and October 2014.

Sulaiman Alharbi

Abbreviations

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
BCFA	Branched chain fatty acid
BMI	Body mass index
CHO	Carbohydrate
CODEX	the international CODEX Alimentarius Commission
CRC	Colorectal cancer
CVD	Cardiovascular disease
DF	Dietary fibre
DM	Diabetes mellitus
DP	Degree of polymerization
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FAO	Food agriculture organization
FC	Fermentable carbohydrate
FDA	Food and Drug Administration
FFAR	Free fatty acid receptors
FFQ	Food frequency questionnaire
FOS	Fructo-oligosaccharides
GI	Glycaemic index
GLC	Gas Liquid Chromatography
GOS	Galacto-oligosaccharides
GPRs	Orphaned G-coupled protein receptors
HFD	High fat diet
HPMC	Hydroxy-propylmethylcellulose
IBD	Inflammatory bowel diseases
IOM	Institute of Medicine
LN	Lean
NDC	Non-digestible carbohydrate
NSP	Non-starch polysaccharide
OWO	Overweight and obese
RAG	Rapidly available glucose
RO	Resistant oligosaccharide
RS	Resistant starch
RS1	Physically inaccessible starch
RS2	Resistant starch granules
RS3	Retrograded starch
RS4	Chemically modified starch
SAG	Slowly available glucose
SCFA	Short chain fatty acid
WCRF	World Cancer Research Fund
WHC	Water holding capacity
WHO	World Health Organization
24-hr recall	24-hour recall

1 Chapter One

Literature review and background

1.1 Introduction

The role of non-digestible carbohydrates (NDCs) in disease prevention has been studied for many years, but it has been difficult to fully establish their impact on health in epidemiology studies. This is due, in part, to the wide range of properties of the different NDCs, where combining them all in one category for statistical comparisons may dilute their impact in correlations or multivariate analysis (Jones, 2014a). In addition, the potential role of short chain fatty acids (SCFA) in promoting health has received much attention; this has included recently the role of propionate in satiety (Arora et al., 2011) and the role of SCFA in obesity (Schwiertz et al., 2010, Rahat-Rozenbloom et al., 2014). Schwiertz et al. (2010) and Rahat-Rozenbloom et al. (2014) observed that overweight and obese individuals produce more colonic SCFA than lean due to differences in colonic microbiota, which may promote obesity through increased colonic energy availability for absorption. However, it is very difficult to measure SCFA production in humans *in vivo* with any accuracy, faecal SCFA levels may be misleading as most of those produced are absorbed quickly making epidemiological studies of the effects of SCFA on chronic disease very difficult. This thesis therefore describes the development and testing of an index for the estimation of potential SCFA production from dietary intake records which should improve the data available for epidemiological studies.

Carbohydrates can be classified nutritionally into digestible and NDCs, based on the way they are handled by the human small intestine (Figure 1.1). NDCs are those that enter the colon, where they become available as substrates for fermentation by bacteria in the large intestine; examples include non-digestible oligosaccharides (NDOs), resistant starch (RS) and dietary fibre (DF) (Table 1.1) (Grabitske and Slavin, 2009, Englyst et al., 2007). Epidemiological, systematic reviews and meta-analysis studies have reported that long term consumption of diets rich in NDCs, such as cereals, fruit and vegetables, may protect against several chronic diseases including cardiovascular disease (CVD) (Threapleton et al., 2013a, Dauchet et al., 2006), colorectal cancer (CRC) (Aune et al., 2011, Park et al., 2005, Wiseman, 2008), diabetes mellitus (Carter et al., 2010), obesity (Lindström et al., 2006) and irritable bowel syndrome (Ford et al., 2008). Similarly, the World Cancer Research Fund (WCRF) concluded that NDCs ‘probable’ provide protective effects against CRC incidence with high cereal, fruit and vegetable intake using their criteria based systematic review of the evidence (WCRF, 2007). The WCRF criteria of judgment

that support the probably decrease the risk of CRC was based on: 1) the evidence should be at least two independent cohort studies or at least five case-control studies. 2) the evidence should show no substantial unexplained heterogeneity between or within study types in the presence or absence of an association or direction of effect. 3) it should be based on good quality studies that exclude the detected relationship results from random or systematic error, including confounding, measurement error and selection bias (WCRF, 2007).

Moreover, several mechanisms have been proposed to explain the protective effects of NDCs; including increased stool bulk, dilution of carcinogens in the colonic lumen, reduced transit time, increased viscosity and water-binding, lowering colonic pH and bacterial fermentation of NDC to SCFA in the colon (den Besten et al., 2013). Only some of these physiological effects can be attributed to the fermentation of carbohydrates to SCFA by the colonic microbiota. The major products of bacterial fermentation of carbohydrates are acetate, propionate and butyrate, and the total concentration of SCFA in the colonic content may exceed 100 mmol/l (Cummings and Macfarlane, 1991). These SCFAs released in the intestinal lumen are readily absorbed and used as an energy source by colonocytes, and also by other tissues such as the liver and muscles (approximately 10 % of basal energy requirements) (den Besten et al., 2013). Before considering the role of fermentation in preventing disease, the definitions and properties of the different NDCs will be discussed.

Figure 1.1: Transit and fate of dietary carbohydrates through the human gastrointestinal tract and the digestion of nutrients in the small intestine and fermentation in the colon, ↑ = increase, ↓ = decrease (adapted from Eswaran et al. 2013; Cummings, 1997).

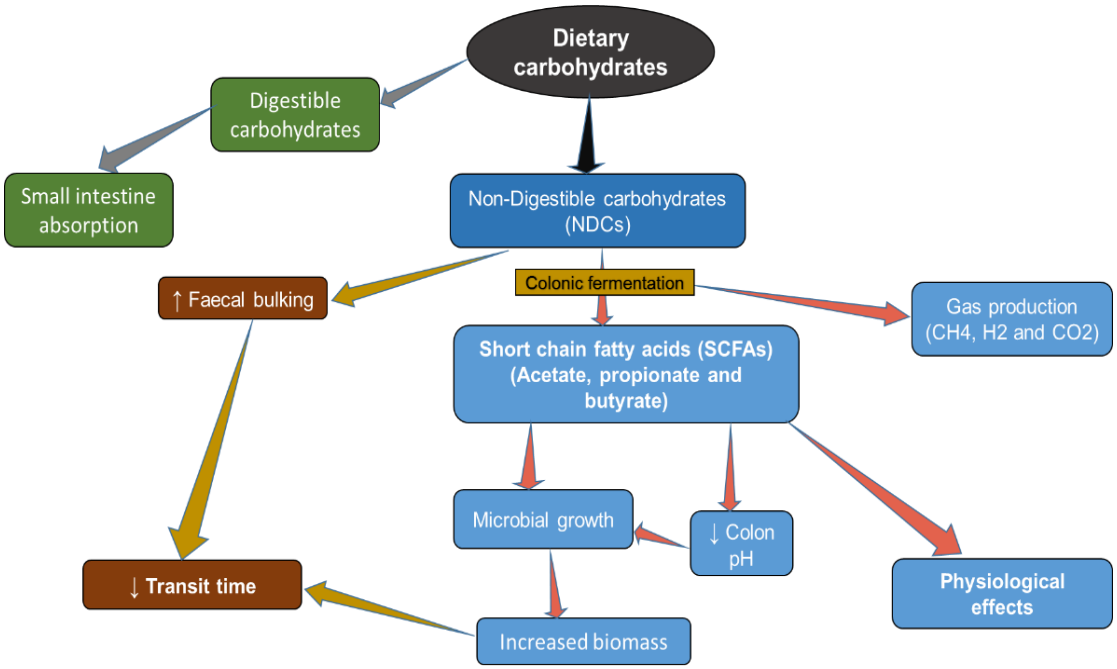


Table 1.1: Nutritional classification of dietary carbohydrates (adapted from Englyst et al., 2007a).

Main categories	Chemical components	Nutritional grouping	Physiology and health
Digestible carbohydrate	Sugars	Lactose	Malabsorbed by those with lactase deficiency.
		Fructose (including from sucrose)	Largely metabolized by liver. Possible detrimental effect on lipid metabolism.
	Starch	Rapidly and slowly digestible starch maltodextrins	RAG and SAG reflect the rate of glucose release from food, which is a main determinant of the GI. Evidence suggests that the metabolic response associated with slow release carbohydrates are most conducive to optimal health.
Non-digestible carbohydrate	Resistant starch	RS	Varied rate and extent of fermentation. Insufficient knowledge of effect on health.
	Non-Starch Polysaccharides (NSP)	Dietary fibre (intrinsic plant cell wall polysaccharides)	Marker for minimally refined plant foods that are rich in micronutrients and shown to be beneficial to health.
		Added NSP	Varied rate and extent of fermentation. Some have specific functional properties.
	Oligosaccharides	Present naturally and added	Varied rate and extent of fermentation. Some have specific functional properties.
	Sugar alcohols	Present naturally and added	Partly absorbed and metabolized, and partly fermented.

Abbreviations: GI, glycaemic index; NSP, non-starch polysaccharides; RAG, rapidly available glucose; RS, resistant starch; SAG, slowly available glucose.

1.2 Dietary fibre (DF) definitions

In the 1970s Burkitt and Trowell hypothesised that DF intake was a protective factor against a number of Western diseases (Trowell, 1976, Burkitt, 1971). They observed that diseases common in the West were rare in rural Africa, possibly because of the unrefined carbohydrates consumed by Africans. Trowell (1972) defined DF as “The residue derived from plant cell walls that are resistant to hydrolysis by human alimentary enzymes”. DF is a general term that includes a number of substances which escape digestion and absorption in the small intestine. All types of DF are fermented to some extent in the colon by microbiota and produce SCFA, but this varies considerably between carbohydrates and their sources (Bach Knudsen, 2001).

Since the 1970s, there has been no international agreement to produce a DF definition that is recognised worldwide, and the wording of the definition has changed over time after much debate (de Menezes et al., 2013). There has been disagreement over which carbohydrate substances should be included in the definition, and which analytical method should be used to derive fibre values (Buttriss and Stokes, 2008). Several definitions have been published based on different analytical methodologies and physiological properties (e.g. digestion, absorption and effects on health). These methods include the enzymic gravimetric method approved by the Association of Official Analytical Chemists (AOAC method 985.29), which is used globally, and the Englyst method which was used mostly in the UK (Agostoni et al., 2010). The AOAC method aims to isolate gravimetrically the part of the diet which resists breakdown by digestive enzymes; this method includes some, but not all, starch made resistant to amylase hydrolysis by food processing. The Englyst method measures non-starch polysaccharide (NSP) using gas liquid chromatography (GLC) of aldol esters, which provides values for the individual monosaccharides and does not include any starch, even that made resistant by processing as this is removed by dimethyl sulfoxide (DMSO) (Englyst, 1989).

In the UK 2015, Scientific Advisory Committee on Nutrition (SACN) recommended that dietary fibre should be defined as all carbohydrates that are neither digested nor absorbed in the small intestine and have a degree of polymerisation of three or more monomeric units, plus lignin. The regular authorities agreed to determine DF chemically using AOAC method (SACN, 2015). Several different definitions for DF

have been used around the world (Table 1.2). Almost all these definitions considered that some carbohydrates escapes digestion and absorption in the small intestine and enters the colon, but differ in their acceptance of which carbohydrates should be included and whether physiological effects should be required (Jones, 2014a). For example, the American Association of Cereal Chemists (AACC 2001) defined DF as consisting of NDCs, which include polysaccharides, oligosaccharides and lignin, that provide beneficial physiological effects including laxation, reduced blood cholesterol attenuation and reduced blood glucose (Buttriss and Stokes, 2008, Committee, 2001).

Table 1.2: Current definitions for dietary fibre (DF) from around the world (adapted from Jones, 2014)

Organisation	Definition
Non-Starch Polysaccharides (NSP) (UK Department of Health 1991)	The skeletal remains of plant cells that are resistant to digestion by enzymes of man, measured as non α-glucan polymers by the Englyst method. It includes NSP, which is comprised of cellulose, hemicelluloses, pectin, arabinoxlyans, beta-glucan, glucomannans, plant gums and mucilages and hydrocolloids, all of which are principally found in the plant cell wall. *Does not include oligosaccharides, resistant starch and resistant maltodextrins*.
American Association of Cereal Chemists (AACC) 2001	The edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and or blood cholesterol attenuation, and/or blood glucose attenuation. *Includes resistant oligosaccharides, resistant starch and resistant maltodextrins.*
Institute of Medicine (IOM) 2001	Dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fibre consists of isolated, non-digestible carbohydrates with physiological effects in humans. Total fibre is the sum of dietary fibre and functional fibre. *Includes resistant oligosaccharides, resistant starch and resistant maltodextrins.*
Food Standards Australia and New Zealand (FSANZ) 2001	Dietary fibre means that fraction of the edible part of plants or their extracts, or synthetic analogues that: Are resistant to digestion and absorption, usually with complete or partial fermentation, in the large intestine; and promote one or more of the following beneficial physiological effects: • Laxation • Reduction in blood cholesterol • Modulation of blood glucose *Includes resistant polysaccharides, oligosaccharides (DP >2) and lignin and resistant starches.*
European Food Safety Authority (EFSA) 2009	Non-digestible carbohydrates plus lignin, including all carbohydrate components occurring in foods that are non-digestible in the human small intestine and pass into the large intestine Includes non-starch polysaccharides, resistant starch, resistant-oligosaccharides.
Health Canada (HC) 2010	Dietary fibre consists of naturally occurring edible carbohydrates (DP > 2) of plant origin that are not digested and absorbed by the small intestine and includes accepted novel dietary fibres. Novel dietary fibre is an ingredient manufactured to be a source of dietary fibre. It consists of carbohydrates (DP > 2) extracted from natural sources or

synthetically produced that are not digested by the small intestine. It has demonstrated beneficial physiological effects in humans and it belongs to the following categories:

- Has not traditionally been used for human consumption to any significant extent, or
- Has been processed so as to modify the properties of the fibre, or has been highly concentrated from a plant source. *Includes resistant oligosaccharides, resistant starch and resistant maltodextrins.*

In addition, a definition that could be applied worldwide has been debated for 15 years by the international CODEX Alimentarius Commission; eight stages were developed by CODEX to define DF. The CODEX and Foods for Special Dietary Uses (CCNFSDU) accepted the following definition in 2008 (Phillips and Cui, 2011) (Codex Alimentarius, 2010):

Dietary fibre means carbohydrate polymers with ten or more monomeric units which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- *Edible carbohydrate polymers naturally occurring in the food as consumed,*
- *carbohydrate polymers, which have been obtained from food raw material by physiological, enzymic or chemical means, and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,*
- *Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.*

Footnote 1 states, "when derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fibre.
Footnote 2 states that, "Decision on whether to include carbohydrates of 3 to 9 monomeric units should be left up to national authorities."

National authorities are allowed to decide themselves whether to include NDCs with a degree of polymerization (DP), between 3 - 9 according to footnote 2. However, this potential variability will prevent international coordination on food labelling, food composition tables, and interpretation of research. For example, findings of studies including DP 3 - 9 might differ from those excluding them. DF values for foods, such as wheat and onions, that contain NDCs with DP 3 - 9 or RS might differ on food labels and in food composition tables (Jones, 2014b). Therefore, epidemiological and interventional studies using food composition tables might also differ if some countries include these materials and others do not. NDCs with DP 3 - 9 should be DF because they fit with the definition for many reasons: 1) they are neither digested nor absorbed by the enzymes in the small intestine, 2) they are fermentable in the large intestine, 3) they aid laxation in the large bowel, and 4) they may increase mineral absorption (de Menezes et al., 2013).

In 2007, in light of the Codex Alimentarius definition, the European Food Safety Agency (EFSA) Panel agreed that DF should be defined based on digestibility in the small intestine of carbohydrates with 3 to 9 monomeric residues, and the physiological effects of NDCs in human health, such as promoting the growth of colonic microflora and SCFA production in the colon (Wong and Jenkins, 2007). Therefore, EFSA defines DF as NDCs plus lignin that consists of:

- NSP – cellulose, hemicelluloses, pectins, hydrocolloids (gums, mucilages, beta-glucans)
- NDOs, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other oligosaccharides that resist digestion (with three or more monomeric units)
- RS – physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches
- Lignin naturally associated with dietary fibre polysaccharides (Agostoni et al., 2010).

Therefore, in the absence of a single analytical method the global confusion has continued. Different analytical methods may mislead consumers, for example, the food table values that are derive from the Englyst method (used in the UK) may be different from those calculated by the AOAC method (used globally), especially for foods rich in starch such as, potato, bread, beans and breakfast cereals such as cornflakes (Buttriss and Stokes, 2008).

1.2.1 Considering colonic fermentation in definitions

In healthy humans, the fermentation processes are primarily controlled by the amount and type of substrates available to bacteria in the colonic ecosystem (Floch, 2011). The fate of carbohydrates in the colon largely depends on the colonic microbiota and the physiochemical properties of the carbohydrate itself (Lupton, 2004). Oat bran, pectin, and guar are highly fermented; while cellulose and wheat bran may be poorly fermented (Muir et al., 2004). On the other hand, the type of carbohydrate can also affect the colon microbiota composition. for example, inulin, a polymer of fructose monomers present in onions, garlic and asparagus (Bosscher et al., 2009) stimulates the growth of specific bacteria. Forty healthy volunteers (18 males, 22 females) were involved in a study carried out by Bouhnik et al. (2006) to evaluate the dose-response effect of short chain fructo-oligosaccharide

supplementation in stimulating *bifidobacteria* growth in the human colon. Participants were divided into five groups of 8 and each received a dose of fructo-oligosaccharide of 2.5, 5.0, 7.5, 10 g/d or a placebo for 7 days, added to their habitual diet. The researchers observed that the *bifidobacteria* count was significantly increased with a dose of 2.5 g/d (9.15 ± 0.59 to 9.39 ± 0.70; p = 0.02), 5 g/d (10.21 ± 0.21 to 10.67 ± 0.22; p = 0.03), 7.5 g/d (9.28 ± 0.49 to 9.85 ± 0.35; p = 0.01) and 10 g/d (9.00 ± 0.81 to 10.18 ± 0.60; p = 0.003), when compared to placebo (Bouhnik et al., 2006).

Measuring SCFA production in humans *in vivo* has become very important because of the increasing interest in the link between fermentable carbohydrates (FC), specific SCFA, and diseases (den Besten et al., 2013). There is also significant interest in the relation between carbohydrate fermentation and SCFA production in relation to lipid and cholesterol metabolism, and their potential impact on CVD and colon cancer. Threapleton et al. (2013b) conducted a systematic review and dose response meta-analysis of 22 cohort studies to examine the relationship between DF intake and any potential dose response correlation with coronary heart disease (CHD) and CVD. They found that dietary fibre intake was inversely correlated with the risk of CVD, with a risk ratio (RR) of 0.91 per 7 g/day (95 % confidence intervals (CI): 0.88-0.94).

Recently, SCFAs have been identified as signals for specific orphaned G-coupled protein receptors (GPRs), which might be involved in the regulation of lipid and glucose metabolism (Frost et al., 2014a, Kimura et al., 2014). These receptors, for example GPR41 and GPR43, also known as free fatty acid receptors (FFAR3 and FFAR2 respectively), and have now been identified as SCFA receptors (Brown et al., 2003, Tazoe et al., 2008). Several studies have suggested that FFA2 and FFA3 mediate the beneficial effects associated with a high FC diet, and might be of interest as targets for the treatment of inflammatory and metabolic diseases (Lin et al., 2012, Thangaraju et al., 2009). FFA2 is also highly recognized in immune cells which might play a role in a dysfunctional neutrophil response, such as in inflammatory bowel diseases (IBD) (Kovatcheva-Datchary and Arora, 2013).

However, the impact of FC on colonic health has been rather neglected because of the paucity of methods available to study fermentation in humans *in vivo*. Currently there is no validated method to measure the extent of colonic fermentation in

humans *in vivo,* and in addition, 95 % of the SCFA produced is rapidly absorbed by the colonocytes, while only 5% is excreted in the faeces (Topping and Clifton, 2001), making the often measured faecal SCFA a poor index of colonic production of SCFA. Colonic fermentation is an important process, not only for the health of the colon, but the metabolites produced may have a beneficial impact on systemic metabolism (den Besten et al., 2013). The relationship between FC intake and colonic fermentation on health and disease can only be investigated if there is a straightforward way to quantify colonic fermentation in both dietary intervention and epidemiological studies.

Most studies use DF or NSP intake but not all fibres or NSP are equally fermentable. Another major obstacle to epidemiological studies on diet and the risk of chronic disease, is the relative inaccuracy of methods with which individuals' habitual long term intake of food or nutrients can be measured by structured dietary questionnaires (Westenbrink et al., 2013b). These methods could cause estimates of relative risk or other measures of diet disease associations to be weakened (an effect usually referred to as attenuation bias), and reduce the statistical power of epidemiologic studies (Kaaks, 1997). The diet disease association may be over- or underestimated due to the effect of relatively large measurement errors inherent in the traditional dietary assessment methods used in epidemiological studies (Marklund et al., 2010). Moreover, dietary intake tools often fail to accurately characterize habitual diets or food preparation effects (Westenbrink et al., 2013a), which will be particularly important for the estimation of RS as the content will vary due to the cooking method and whether the food is eaten hot or cold (Sharma et al., 2008). The traditional analysis method of epidemiological data depends on food composition databases, which might not have been updated to include oligosaccharide and RS, or to have accurate data on NDCs added to food (Westenbrink et al., 2013a, Hollmann et al., 2013). Moreover, it is quite likely that each of us would handle the food differently in our gut, meaning that the actual amount of FC entering the large intestine is impossible to determine.

Before developing an index of FC that can be determined from food diaries, it is important to consider the types of carbohydrate that escape digestion and absorption and enter the colon where they are available for fermentation.

1.3 Composition and types of dietary fibre

The main component of DF is the plant cell wall (Table 1.3), a major structure that is comprised of cellulose, hemicellulose and pectin. In addition fibre includes other polysaccharides of plant or algal origin, such as gums and mucilage, and these are included as NSP (Theuwissen and Mensink, 2008a). DF pass through the small intestine unchanged but are fermented in the large intestine.

Table 1.3: Description and sources of the main dietary fibre fractions captured by current definitions (adapted from Buttriss and Stokes, 2008; Paeschke, T. M. & Aimutis, W. R. 2011).

Fermentable carbohydrate	Structure	Classification	Food sources
Cellulose	β-(1→4)-D-Gl*cp*	Insoluble, poorly fermentable	Whole grains Fruits, vegetables
Hemicelluloses	β-(1→4)-linked backbones	Insoluble, soluble, fermentable	vegetables, fruits, cereals and legumes and nuts
β-Glucan	β-(1→3 and 1→4)-D-Gl*cp* (backbone); β-(1→6)-D-Gl*cp* (branches; only on some types)	Soluble, viscous, highly fermentable	Oat bran, barley flour
Chitin	β-(1→4)-D-GlcNAc	Insoluble, poorly fermentable	Shellfish
Chitosan	β-(1→4)-D-GlcNAc and GLcN	Soluble, viscous/gel-forming in acidic solution; insoluble in neutral pH	Alkali-treated chitin
Arabinoxylan	β-(1→3 or 1→4)-D-Xy*lp* (backbone); complex branches of Area, Xyl, GlcA and Gal; branches can be esterified with phenolic compounds; backbone can be acetylated; polymers often cross-linked through oxidative di- and trimerisation of phenolic compounds	Insoluble or soluble and poorly to highly fermentable depending on fine structure and processing condition; soluble forms can be (cereal arabinoxylan) or high viscosity/gel-forming (psyllium)	Whole grains, cereal bran, psyllium seed and husk
Arabinogalactan	β-(1→3 and 1→4)-D-Gla*p* (backbone); side groups of Ara, Gal, GlcA, Rha	Soluble, fermentable	Fruits and vegetables *Acacia Senegal* (gum arabic)
Pectin	α-(1→4)-D-GalA with varying degree of methyl esterification (backbone) and side groups containing Rha, Gal	Soluble, viscous, highly fermentable	Fruits and vegetables
Guar gum	β-(1→4)-D-Man*p* (backbone); side groups of Gal	Soluble, viscous, highly fermentable	*Cyamopsis tetragonolobus* seeds
Xanthan gum	β-(1→4)-D-Glcp (backbone); side chain of Man, GlcA, pyruvate	Soluble, viscous	*Xanthamanas campetris* cultures
Gellan	Tetrasaccharide repeat of 3 β-(1→4)-D-Gl*c*p interrupted with 1 α-(1→3)-L-Rha*f*; some acetylation	Soluble, viscous, gel forming	*Pseudomonas elodea* cultures
Alginate	Backbone of β-D-ManA and α-L-GulA	Soluble at neutral pH; insoluble at acidic pH; viscous; gel forming	Algae
Carrageenan	α (1→3 and 1→4)-D-Gla*p* with varying degrees of sulphate esters and presence of 3,6-anhydro-Gal*p*	Soluble, viscous, gel forming	Seaweed

Polydextrose	Random polymer of Glc, sorbitol and citric acid	Soluble, low viscosity, partially fermentable	Chemically synthesised
Inulin/fructooligosaccharides	Fructose oligo- or polymer linked β (2→1) with Glc at the non-reducing end	Inulin insoluble; FOS soluble and low viscosity, highly fermentable	Onion, Jerusalem artichoke, enzymatically synthesised
Galactooligosaccahrides	Oligomers of galactose	Soluble, low viscosity, high fermentable	β-galactosidase treatment of lactose
Lactulose	D-Gal*p*-β-(1→4)-D-Fru	Soluble, low viscosity, high fermentable	Glucose isomerase treatment of lactose

Gle, glucose; Gal, galactose; Xyl, xylose; Ara, arabinose; Rha, Rhamnose; GlcA, D-glucuronic acid; GlaA, galacturonic acid; GlcN, D-glucosamine; GleNAc, N-acetyl-glucosamine

1.3.1 Non starch polysaccharide (NSP)

NSPs are complex polysaccharides, other than starch, which are comprised of several hundred thousand monosaccharides units, joined through glycosidic linkages (Kumar et al., 2011). There are numerous types of NSP which vary according to the sequence and composition of monosaccharides, the types of linkages present, the number of monosaccharides with an acidic group (e.g., uronic acid), and the presence of branches from the polymer backbone joined predominantly by β-glycosidic bonds (Gurr and Asp, 1994). In plants, NSP are predominantly structural polysaccharides in the cell wall and other associated polysaccharides, proteins, and phenolic compounds (Cummings and Stephen, 2007). NSP comprises up to 90 % of the cell wall of plants, and is usually less than 10 % by weight of the grain. The most abundant forms of NSP include cellulose, hemicellulose, and pectin (Kumar et al., 2011). Fructans, glucomannan, and galactomannan are also included in NSP and they are storage polysaccharides. Other constituents of NSP are modified polysaccharides such as, mucilage, alginate, exudate gum and β-glucan (Asp and Björck, 1992, Cummings and Stephen, 2007). NSP can be classified into various groups based on their physicochemical properties, for example, viscosity, water-holding capacity, fermentation, and the capacity to bind organic and inorganic molecules. Moreover, NSP is classified as either soluble or insoluble based on its reaction with water; soluble NSPs are dispersed when mixed with water and have the ability to increase the viscosity of digesta, whereas insoluble NSP may not enhance digesta viscosity, but could be characterized by their faecal bulking capacity (Kumar et al., 2011).

1.3.1.1 Cellulose

Cellulose is a water insoluble fibre that is not digested and absorbed in the small intestine, and has a linear chain of β (1→4) linked glucose monomers (Mudgil and Barak, 2013). Cellulose chains are formed of long flat linear ribbons of glucose units with molecular weights of over 10,000, as the β-(1–4) linkage between the glucose units grips the chain in a flat conformation the cellulose chains may align next to each other and form numerous hydrogen bonds between the sugar hydroxyl groups. The long chains make cellulose highly insoluble in water, but could allow swelling in concentrated sodium hydroxide solutions (Mutwil et al., 2008). Moreover, cellulose could be used as a dietary fibre supplement; cellulose-rich maize bran could be

converted to a cellulosic gel through thermal and shear treatments, followed by alkaline peroxidation and shearing (Stone and Fincher, 2004). Cellulose quantity in whole grains may be different between species and is largely a consequence of the thickness of the husk and seed coat, higher concentrations of cellulose form a thicker and a stronger cell wall. Seed endosperm cells contain only thin cell walls, and in a well-filled grain the proportion of cellulose to starch, or other reserve polysaccharides, should be low. Cellulose is also the structural component of cell walls in green plants and vegetables and comprises of about 33 % of all vegetable materials. Cellulose can also found in whole grains, fruits and vegetables (Kumar et al., 2011).

Behaviour in the human gut

In the human large intestine, cellulose is fermented by microbiota in the colon to a certain degree, in turn producing SCFA (Wolin and Miller, 1983). In an *in vitro* study of fermentation of different types of DF, Barry et al. (1995) showed that only 6-7 % of Solka-floc cellulose was degraded after 24 h incubation.

1.3.1.2 Hemicelluloses

Hemicelluloses are a heterogeneous group of polysaccharides, and the term was coined by Schulze in 1891 to include substances extractable from agricultural biomass with aqueous alkaline solution. Hemicelluloses are polysaccharides in plant cell walls that have β-(1→4)-linked backbones with an equatorial configuration, and includes xyloglucans, xylans, mannans and glucomannans, and β-(1→3, 1→4)-glucans (Jensen et al., 2008). These types of hemicelluloses are present in the cell walls of all terrestrial plants, except for β-(1→3,1→4)-glucans, which are restricted to Poales and a few other groups. Hemicelluloses are a complexes of polysaccharides made up of a variety of pentose and hexoses, which include arabinose, fructose, galactose, glucose, mannose and uronic acides (Scheller and Ulvskov, 2010). The main two types of hemicelluloses that are usually found in the foods are xylans and galactans, and sometimes β-glucan and mannans are also included in this group. The structure of hemicelluloses and their abundance vary widely between different species and cell types, and the most important biological role of hemicelluloses is their contribution to strengthening the cell wall by the interaction with cellulose and, in some walls, with lignin (Ebringerová et al., 2005).

Behaviour in the human gut

Hemicelluloses can be either completely or poorly fermented based on their diverse structures; native hemicelluloses are an insoluble fibre and may contribute to the digestive process in the small intestine (Cummings, 1984). In the human colon, hemicelluloses which have been solubilized with alkaline are rapidly fermented by colonic bacteria (Scheller and Ulvskov, 2010).

1.3.1.2.1 Arabinoxylan

Arabinoxylan (AX) is a major component of hemicelluloses, and are comprised of a xylose backbone with arabinose side chains (Henry, 1985, Lu et al., 2000). In whole grains, AX is considered as the major component of dietary fibre, with considerable inclusions in both the endosperm and bran; in wheat it is around 64 to 69 % of the NSP in the bran, with ratio of soluble to insoluble NSP of (1:6), and AX is around 88 % of the endosperm (Ring and Selvendran, 1980, Lu et al., 2000). AX also occurs in the endosperm cell walls of barley at a concentration of 20 % (w/w) and rice at 40 % (w/w). The non-endospermic tissues of wheat (pericarp and testa) comprise a very high concentration of AX (64 % (w/w)) (Grootaert et al., 2007).

AXs are composed of β−(1,4)-linked D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains (Izydorczyk and Biliaderis, 1995). Some arabinoses can be substituted with ferulic acid, and the degree of substitution refers to the arabinose (A) moieties on the xylose (X) backbone and is further also described as A/X ratio (Kumar et al., 2011). The degree of substitution and distribution of the side chains are important factors in the physicochemical properties of AX. The degree of substitution of AX from wheat, rye, and barley is relatively low, they have higher proportions of unsubstituted xylose residues, and lower levels of mono-substituted xylose residues, when compared to the more highly branched AX from rice and sorghum. The highest levels of double substituted xyloses have been reported for wheat pericarp AX (Maes and Delcour, 2002).

Behaviour in the human gut

AX is a soluble fibre, as it is rapidly fermented by the microflora of the colon. Fermentation of AX by the human large intestinal (Amrein et al., 2003, Gråsten et

al., 2003) and in *in vivo* rats (Lopez et al., 1999) showed a greater impact on propionic acid production, which may result in a cholesterol lowering effect.

1.3.1.3 β-glucan

β-glucan is a water-soluble fibre and highly viscous at low concentrations, which contains linear polysaccharides of glucose monomers with β (1→4) and β (1→3) linkages, and is found in the endosperm of cereal grains, primarily barley and oats (Doublier and Wood, 1995). The β-glucan in barley contains approximately 70 % (1→4) linkages and 30 % (1→3) linkages, which correlates to segments of two or three (1→4) linkages separated by a single (1→3) linkage (Kumar et al., 2011). The β-glucans in cereal are made up of 58 %–72 % of β-(1→3) linked units and 20 %–34 % of β-(1→4) linkaged units (Brennan and Cleary, 2005). β-glucan is an important constituent of the walls of the starchy endosperm and aleurone cells of most cereal grains, and may account for up to 70 % by weight of the walls. β-glucan is found in barley, oat, and rye grains (Cui et al., 2000, Henry, 1987).

Behaviour in the human gut

In the human gut, β-glucan is extensively fermented by the microflora of the colon. It has a marked effect on increasing the viscosity of the digesta in the small intestine, affecting gastric emptying, absorption of nutrients and small intestinal motility (Wood, 2007).

1.3.1.4 Pectin

Pectin is a gum found in many plants but mainly in citrus fruits (oranges, lemons, grapefruits) and apples, and during grumbling, pectin may be changed from an insoluble substance to a much more water-soluble component. Pectin is a linear polysaccharide of about 300 to 1000 monosaccharide units, mainly D-galacturonic acid plus some polysaccharides, and the D-galacturonic acid molecules are joined together by α-(1→4) glycosidic bonds (Mohnen, 2008). Citrus fruit contain anywhere from 0.5 % to 3.5 % pectin, with a large concentration located in the peel. Commercially extracted pectin is widely used in food applications as gelling or thickening agent (Thakur et al., 1997).

Behaviour in the human gut

In the human small intestine, pectin had been shown to increase the viscosity of the digesta. It is a water soluble polysaccharide that bypasses enzymatic digestion of the small intestine, and is easily degraded by the microflora of the colon (Dongowski et al., 2002).

1.3.1.5 Psyllium

Psyllium is a mucilage from the Plantago ovata plant with high viscosity and water holding capacity. The term psyllium has been used interchangeably for the seed husk, the seed, and the entire plant (Fischer et al., 2004). It is also known as ispaghula. Psyllium has been cultivated because it has a rich source of water soluble fibre present in the seed husk, which can be known as psyllium hydrophilic mucilloid, psyllium hydrocolloid, and psyllium seed gum (Marlett and Fischer, 2003). The bioactive fraction in psyllium is a fibre which has been formed of a highly branched AX. The xylose units are the backbone of psyllium, whereas arabinose and xylose make up the side chains. The gel-forming of the husk represents 55-60 % of the dry weight, and the neutral AX comprises of β-(1 → 4)-linked d-xylopyranosyl residues, some carrying single xylopyranosyl side chains at position 2, others bearing, at position 3, trisaccharide branches having the sequence l-Araf-α-(1 → 3)-d-Xylp-β-(1 → 3)-l-Araf (Fischer et al., 2004). Psyllium can be added to foods, such as ready to eat cereals, due to its cholesterol lowering properties (Theuwissen and Mensink, 2008b).

Behaviour in the human gut

Psyllium is not digested and absorbed in the human small intestine, and it is fermented by microbiota in the colon (Marlett and Fischer, 2003). It is only partially fermented and retains its water-holding capacity sufficiently to be an effective stool bulker (Marlett et al., 2000, Ruepert et al., 2011).

1.4 Resistant starch (RS)

Until the early 1980's, it was believed that starches were completely digested and absorbed in the small intestine. In 1982, Englyst and colleagues, in the process of developing an *in vitro* assay to measure NSP, observed that some starch remained after enzymatic hydrolysis (Englyst et al., 1992). Based on this finding, Englyst and colleagues agreed to define all the starches that escape digestion in the small

intestine as resistant starch (RS). In 1992, a concerted action of European researchers defined RS as "the sum of starch and products of starch digestion not absorbed in the small intestine of healthy individuals" (Asp et al., 1996). Therefore, RS is the fraction of starch which is not degraded by digestive enzymes in the small intestine within 120 minutes of being consumed, and which then enters the colon for fermentation (Englyst et al., 1992).

There are four important mechanisms that may explain the non-digestibility of RS; first, a compact molecular structure limits the accessibility of digestive enzymes, and various amylases, in raw starch granules (Haralampu, 2000). This means the starch might not be physically bio-accessible to digestive enzymes, for example in grains, seeds or tubers. Second, the starch granules may have been formed in a way which prevents the digestive enzymes from breaking them down (e.g. raw potatoes, unripe bananas and high amylose maize starch) (Nugent, 2005). Third, although gelatinization of starch granules makes the molecules fully accessible to digestive enzymes, if these starch gels are then cooled, starch crystals are formed which are also resistant to the digestive enzymes. This form of ‘retrograded’ starch is found in small quantities (approximately 5 %) in foods such as ‘‘corn-flakes” or cooked and cooled potatoes, as used in a potato salad (Haralampu, 2000). Fourth, the chemical modified of starches by etherisation, esterisation or cross-bonding makes them resistant to digestive enzymes (Lunn and Buttriss, 2007).

1.4.1 Classification of resistant starch

RS has been classified into four types (Table 1.4); physically inaccessible starch (RS1), resistant starch granules (RS2), retrograded starch (RS3) and chemically modified starch (RS4).

1.4.1.1 Physically inaccessible starch (RS1)

This type of resistant starch is physically inaccessible and protected from the action of α-amylase in the human small intestine (Topping and Clifton, 2001). The presence of plant cell walls can entrap the starch to make the RS1 inaccessible for digestion in the small intestine, for example, in legume seeds, partially milled and whole grains (Champ et al., 2003). RS1 can also be found in highly compact processed food like pasta. The preparation and cooking processes are of great

importance for the RS1 content of food when consumed, as these procedures may contribute to a disruption of the cell walls (Champ et al., 2003, Englyst et al., 1992).

Table 1.4: Classification of types of resistant starch (RS), food sources and factors affecting their resistance to digestion in the colon (adapted from Nugent, 2005).

Type of RS	Description	Food sources	Resistance reduced by
RS1	Physically protected	Whole or partly milled grains and seeds, legumes, pasta	Milling, chewing
RS 2	Un-gelatinised B type resistant granules	Raw potatoes, green bananas, some legumes, high amylose starches	Food processing and cooking
RS 3	Retrograded starch produced during food processing	Cooked and cooled potatoes, bread, cornflakes, food products with prolonged and/or repeated moist heat treatment e.g. extrusion cooking	Processing conditions
RS 4	Chemically modified starches due to cross-bonding with chemical reagents, ethers, esters, etc.	Some fibre rich -drinks, foods in which modified starches have been used (e.g. certain breads and cakes)	Less susceptible to digestibility *in vitro*

RS, resistant starch

1.4.1.2 Resistant starch granules (RS2)

This type of RS refers to B type starches that are found in raw potato and unripe banana starch, and are known to be very resistant to enzymic hydrolysis when uncooked (Sharma et al., 2008). The B type refers to the X-ray diffraction pattern of the starch. Most cooking procedures produce gelatinization of the raw starches, which causes a reduction of RS2 in the food (Faisant et al., 1995, Cummings et al., 1996). The amount of RS2 in bananas depends on the degree of ripening, as during this process the banana's intrinsic enzymes convert the starch into simple sugars and sucrose (Champ et al., 2003).

1.4.1.3 Retrograded starch (RS3)

RS3 is the most abundant of the resistant starches present in foods which have been cooked, then cooled and stored for several hours, up to several months. Retrogradation is the recrystallization of starch chains, which happens after gelatinisation when the products have not been immediately dried, and single chains produce double helixes as mainly the linear fraction of the starch, amylose, is retrograded (Sharma et al., 2008). Amylopectin can also be retrograded, although a longer time of cooking is required. It has been observed that cooked and cooled potatoes contained RS3 in significant amounts (Englyst and Cummings, 1987). Reheating of this starch reduced the RS3 content of the potato, showing that the retrogradation was partly reversible, however several cycles of heating and cooling increased the RS3 levels in the food. RS1, RS2 and/or RS3 can co-exist in the same food, and retrograded starch can be found in bread, some brands of corn flakes, cooked-cooled potatoes and legumes (Faisant et al., 1995, Champ et al., 2003).

1.4.1.4 Chemically modified starch (RS4)

RS4 refers to the amylose-lipid complex and other modified starches which have been identified as sources of resistant starch (Sajilata et al., 2006). Amylose-lipid complexes occur when fatty acids (12 - 18 carbons) are held within the helical structure of amylose (Tufvesson et al., 2003). They are produced naturally during starch biosynthesis, and may also be formed during cooking. Lipids might also interfere with amylose retrogradation, impairing the formation of retrograded starch during processing, but overall they have lower digestibility properties compared with cooked starch. Amylase inhibitors occur in raw pulses and may lower the activity of

amylase in the human small intestine. However, most of these factors, especially enzyme inhibitors, become inactivated during food processing and cooking (Liener, 1996). Some chemical processes can also alter the bond structure in starches, as in pyrodextrinisation, and these would also be classified as RS4 (Higgins, 2004).

1.4.2 Fermentability of resistant starch

It has been suggested that around 80–90 % of the glucose produced by the enzymic hydrolysis of standard starch is metabolized in the human body (Sharma et al., 2008), which means 10 % escapes into the large intestine. Several studies have reported that 30–70 % of RS was degraded to SCFA in the colon by bacteria, while the balance of the RS escaped even colonic fermentation, and was excreted in the faeces (Cummings et al., 1996, Behall and Howe, 1996, Behall and Howe, 1995). The overall fermentability of RS is based on its physiological structure and the category of RS. Of the total amount of RS3 present in corn and wheat, approximately 84 % and 65 % are subjected to bacterial fermentation in the colon, respectively. Similarly, for RS2 present in raw potatoes and green bananas about 89 % and 96 %, respectively, are degraded by bacterial fermentation in the colon (Cummings et al., 1996). The degradation of RS is also influenced by the different food processing conditions under which the RS was produced. On a high RS diet, no more than 10 % of dietary RS is excreted in the faeces since most RS is fermented and absorbed (Cummings et al., 1996, Phillips et al., 1995).

Estimating daily RS intake at present is very difficult, because there are not enough data on the RS content of foods. Moreover, the values obtained from old dietary intake data might be misleading, as it may not have been updated to include RS that was added to the food (Westenbrink et al., 2013a). Estimates of RS consumption in Western developed countries varies in the literature with values ranging from 3-4 to 15-20 g/d; and for developing countries estimates vary from 9-10 to 30-40 g/d for countries with a high starch intake (Stephen et al., 1995). The average RS intake in the Chinese population has been estimated as high as 14.9 g/d based on a dietary survey (Chen et al., 2010). According to the EURESTA studies, the average daily RS intake in European countries ranged from 3·5-6.0 g/d (Dysseler and Hoffem, 1994). Baghurst et al. (1996) reported that the RS intake represents 5 % of the overall starch consumption. Generally it appears that the main categories of foods that determine the daily RS intake are cereal-based products (mainly bread, pasta

and rice), and vegetables (legumes and potatoes); for example in Italy Brighenti et al. (1998), reported that the average intake of RS was 8·5 g/d, with regional differences (from 7·2 in the North-West to 9·2 g/d in the South), mainly because of the different levels of consumption of some typical starchy foods (bread, pasta and legumes). In Australia, RS daily intake ranged from 5-7 g/d (Baghurst et al., 2001). The RS intake in Australia is higher than in Europe because most top-selling commercial breads, baked goods and cereal ingredients are fortified with RS. The recommended total daily intake of resistant starch set by Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) is 20 g/d based on a study by Baghurst et al. (2001) for good health. Therefore, this research suggests improving breads and other cereal products with ingredients high in RS could make a valuable contribution to dietary fibre intake, as slow fermentability of RS in the large bowel is tolerated better than other soluble fibres (Lunn and Buttriss, 2007).

1.4.3 Resistant starch as a component of dietary fibre

The awareness of consumers regarding the relationship between food, lifestyle and health has increased, and is one of the reasons for the popularity of food rich in fibre; therefore, the importance of RS has been put forward as a new source of dietary fibre (Sanz et al., 2008). Generally, the physiological behaviour of RS is similar to that of soluble fermentable fibre, such as guar gum, as increased faecal bulk and lower colonic pH were the most common findings of RS ingestion (Slavin et al., 2010). Overall, since RS acts physiologically as a fibre, it should be included in the total dietary fibre definition (Haralampu, 2000). RS is a substrate for the colonic microbiota, like soluble fibre, producing metabolites including SCFA (mainly acetate, propionate and butyrate) (Topping and Clifton, 2001). Postprandial glycaemic and insulinemic responses have been reduced after RS consumption, which shows it's consumption might have beneficial implications in the management of diabetes (Tharanathan and Mahadevamma, 2003). These findings provide solid justification that RS behaves physiologically like soluble fibre (Sajilata et al., 2006). Overall RS is nutritionally more similar to NSP than to digestible starch, but it is not a cell wall component. Recently there is evidence to suggest that RS has been treated as a new ingredient for creating fibre-rich foods (Sharma et al., 2008).

1.4.4 Sources of resistant starch

RS is naturally found in cereal grains, seeds and in heated starch or starch containing foods (Charalampopoulos et al., 2002). Potato starch has been considered to have the highest RS concentration and corn starch has the lowest, with raw potato starch being comprised of 75 % RS as a percentage of total starch (Bednar et al., 2001). Whole grains are rich sources of FCs including DF, RS and RO (Slavin, 2004). The RS concentration is generally low for the cereal flour, as it has an A-type crystalline pattern, unlike potato starch which is B type, and is more readily hydrolysed than raw cereals that are not as highly processed as flours. Therefore, cereal flours comprise more RDS and SDS than RS (Asp et al., 1996). Grain flour is made up primarily of two components, which are protein and starch. In contrast, cereal grains are made of the pericarp, aleurone layers and germ portions of the grain which provide lipid and fibre (Higgins, 2004). Cereal grain is processed and milled to flour, thereby altering the chemical composition of the flour compared with the cereal grain, meaning the RS concentration is five times higher in the cereal grain than in the flour (Bednar et al., 2001).

Legumes are considered to have a high content of both soluble and insoluble dietary fibre. Pulse grain has been shown to be high in RS and this retains its functionality even after cooking. Legume starches contain higher amylose levels than cereal and pseudo-cereal starches (Mikulíková et al., 2008), with the mean RS concentration in legumes being 24.7%. Generally, RS concentrations constituted the highest proportion of the starch fractions of legumes, and the digestibility of cereal starches is much higher than that of legume starches. Leguminous starches have a C-type pattern of crystallinity, which are more resistant to hydrolysis than A-type starches, which explains why legumes have high amounts of RS (Bednar et al., 2001). Cooked legumes are retrograded more quickly, thus lowering the process of digestion, and processed legumes contain a significant amount of RS3. The higher RS content could be due to the higher content of amylose in legumes, and might account for their low digestibility (Tharanathan and Mahadevamma, 2003). There is a very large variety in the content of RS in seeds of leguminous plants from 80 % to only a few percent (Fuentes-Zaragoza et al., 2010).

1.5 Oligosaccharides

Oligosaccharides are carbohydrate chains with 3 – 10 sugar units. NDOs include pyrodextrins produced by heating starch in acid conditions, fructooligosaccharide (FOS) from inulin, galactooligosaccharides (GOS) and xylooligosacharides (Cummings, 1997). Oligosaccharides are water soluble and typically they are 0.3–0.6 times as sweet as sucrose (Mussatto and Mancilha, 2007).

Other oligosaccharides which are not α-glucans include raffinose α-Gal (1- 6) α-Glc (1- 2) β-Fru), stachyose $(Gal)_2$ 1:6 Glu 1:2 Fru and verbascose $(Gal)_3$ 1:6 Glu 1:2 Fru) (Cummings et al., 2001, Cummings and Stephen, 2007).

1.5.1 Sources of oligosaccharide

Oligosaccharides are naturally found in milk, honey, fruits and vegetables such as, onions, Jerusalem artichokes, chicory, leeks, garlic, artichokes, asparagus, sugar beet, bananas, rye, barley, yacon and salsify, which are all considered special sources of FOS (Sangeetha et al., 2005, Mussatto and Mancilha, 2007). The concentration of oligosaccharides in most of these sources is quite low as it ranges between 0.3 % and 6 % of fresh weight. Chicory and salsify contain between 5 % and 10 % (respectively), while in Jerusalem artichokes and yacon the oligosaccharide concentration can reach up to 20 % showing they are much better sources of oligosaccharides (Van Loo et al., 1995). The galactosylsucroses raffinose and stachyose in soybean and other pulses and leguminous seeds, xylooligosaccharides in bamboo shoots and galactose-containing oligosaccharides in milk, particularly colostrum either in free form or as glycoconjugates, are other examples of naturally occurring oligosaccharides (Voragen, 1998, Vázquez et al., 2000). Isomaltulose are another example, they occur naturally in honey, sugar cane juice, and products derived from these, such as treacles or food-grade molasses (Lina et al., 2002). GOS appears naturally in human milk and to a smaller extent in cow's milk (Alander et al., 2001), and cyclodextrins are naturally occurring water-soluble glucans (Singh et al., 2002). Seeds of legumes, lentils, peas, beans, chickpeas, mallow, composite, and mustard are rich in raffinose oligosaccharides (Mussatto and Mancilha, 2007).

1.5.1.1 Prebiotics

Prebiotics are carbohydrates and may be oligosaccharides or polysaccharides. Gibson and Roberfroid (1995) defined prebiotics as *"non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health"* . This definition was later revised by Gibson et al. (2004) as *"a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health".*

All prebiotics are fibres, but not all fibres are prebiotics, therefore, for food ingredients to be classified as a prebiotic the following criteria should be fulfilled:

- Resists gastric acidity, hydrolysis by mammalian enzymes, and absorption in the upper gastrointestinal tract
- Is fermented by the intestinal microflora
- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being.

The usual targeted species of microflora for prebiotics are *lactobacilli and bifidobacteria*, and changes in *bifidobacteria* are more likely to be seen than *lactobacilli* (Gibson and Roberfroid, 1995). This is due to the fact that there are more *bifidobacteria* present in the human colon than *lactobacilli*, and they exhibit a preference for oligosaccharides (Miller et al., 1994). Many different carbohydrates are marketed worldwide as prebiotics, but only four have been well supported by good quality data from human trials, these are Fructans (inulin and FOS), GOS, and the synthetic disaccharide, lactulose (Boehm et al., 2005).

1.5.1.2 Fructans (Inulin and FOS)

Inulin and FOS are the two main prebiotics. They are comprised of fructose polymers which are generally linked to the moiety of a terminal glucose (Sangeetha et al., 2005). In the human small intestine, there is no enzyme able to digest fructans and so they pass through the upper gastric intestinal (GI) tract, reach the colon and are fermented by colonic microflora producing SCFA (Cummings et al., 2001).

Inulin and FOS are composed of a mixture of oligosaccharides (from 2 to more then 60), with a degree of polymerization (DP) from 2 to 20, and they primarily consist of glucose β (2→1) D-fructofuranosyl linkages (Gibson et al., 2004). Onions are a rich source of FOS with values in the range from 25 to 40 % of dry matter (Hogarth et al., 2000). FOS is produced commercially mainly based on two processing methods, which are either a continuous process using immobilized cells in calcium alginate gel or an enzyme on an insoluble carrier; or a batch conversion of sucrose by fungal fructosyltransferase (Sangeetha et al., 2005). In the human colon, FOS have been shown to be completely fermented mostly to lactate, SCFA and gas (Bornet et al., 2002).

Inulin is a naturally occurring storage carbohydrate commonly found in leeks, onions, wheat, asparagus, garlic, Jerusalem artichokes and chicory (Roberfroid, 2005). Most of the inulin commercially produced is from chicory roots (Van Loo et al., 1995). Native chicory root inulin is produced from fresh roots, which always contain glucose, fructose, sucrose and small oligosaccharides. The average daily consumption of inulin has been estimated to be between 3 and 11 g in Europe (Van Loo et al., 1995) and between 1 and 4 g/d in the USA (Moshfegh et al., 1999). In the UK, it was estimated that the mean intake of inulin and oligofructose to be between 4 and 3.8 g/d (Dunn et al., 2011). Inulin resists hydrolysis by the human small intestine enzymes due to the β-configuration of its fructose monomers, which is specific to glycosidic bonds (Roberfroid, 2005). Standard inulin is 10 % more as sweet as sucrose, while high performance (HP) inulin (from which the fraction with a degree of polymerisation lower than ten has been removed) is not sweet (Seifert and Watzl, 2007). Inulin is moderately soluble in water (maximum 10 % at room temperature) and has a low viscosity. Inulin is also used in synergy with most gelling agents, e.g. gelatine, alginate, gellan gum and maltodextrins, especially as the stability of food product foams and emulsions is improved by replacing fat with inulin, such as aerated desserts, ice creams, table spreads and sauces. Inulin is often used as a stabiliser in different food products (Macfarlane et al., 2008).

As well as their prebiotic effects, fructans also play a role in decreasing faecal pH, increasing SCFA production, decreasing faecal bacterial enzymatic activities, and modifying faecal neutral sterols (Boehm et al., 2005, Bornet et al., 2002).

1.5.2 Synthetic carbohydrate compounds

There are several NDCs which are made by the food industry add they have various properties which are useful in food production. These include the pyrodextrins mentioned previously (Anderson et al., 2009) and other synthetic carbohydrates, such as methylcellulose (MC) and hydroxy-propylmethylcellulose (HPMC) (Gallaher et al., 1993). Polydextrose is a non-digestible carbohydrate polymer, with an average degree of polymerization of 12. It is produced from glucose and sorbitol, using an organic acid (citric acid) as a catalyst. This results in a complex structure which is resistant to hydrolysis by human digestive enzymes, and polydextrose is partially fermented in the colon and has bulking and prebiotic properties (Craig et al., 2000).

1.6 Physicochemical properties of NDCs

The physiological effects of NDCs depend on their physicochemical properties, which include water holding capacity, viscosity, binding ability, bulking ability, and fermentability (Guillon and Champ, 2000a).

1.6.1 Solubility

Based on its solubility in water, DF is divided into soluble (pectin, gums) and insoluble (cellulose, lignin) types, and this nature of DF confers their technological functionality and also their physiological effects (Jiménez-Escrig and Sánchez-Muniz, 2000). Soluble fibre may increase luminal viscosity in the stomach and small intestine, and reduce postprandial glycaemia (McCarty, 2005) and plasma cholesterol (Brown et al., 1999). Insoluble fibres are associated with increased faecal bulk, and decreased intestinal transit (Dikeman and Fahey Jr, 2006). In food processing, soluble fibres in food products are considered more beneficial as they provide viscosity, the ability to form gels and act as emulsifiers, unlike insoluble fibres (Mudgil and Barak, 2013).

1.6.2 Water holding capacity (WHC)

The water holding capacity of a fibre is the amount of water retained by a known weight of dry fibre under specified conditions of temperature, time soaked, and duration and speed of centrifugation. However, WHC can be affected by the portion

of the soluble fibres lost during measurement, and the amount of water measured by centrifugation could be higher than the amount of water adsorbed (Dikeman and Fahey Jr, 2006). In general, the polysaccharide constituent of DF is strongly hydrophilic, therefore water is held on the hydrophilic sites of the fibre itself or within void spaces in the molecular structure (Mudgil and Barak, 2013). However, most of the WHC of soluble fibres is lost during colonic fermentation.

1.6.3 Viscosity or gel formation

Viscosity is a major physicochemical property associated with DF especially soluble forms such as gum, pectin, psyllium, and β-glucan (Dikeman and Fahey Jr, 2006), which may change the properties of gut content and affect digestion and absorption (Kendall et al., 2010). Viscosity is associated with fibre's ability to absorb water and produce a gelatinous mass. Viscosity makes soluble fibres more like solids than liquids in the gastrointestinal tract, and this phenomenon may explain the delayed gastric emptying often associated with the ingestion of fibre. Non fermented viscous fibres may also provide lubrication to faeces and make it easier to defecate (Gallaher et al., 1993).

1.6.4 Binding ability

Bile acids can be trapped by DF in the small intestine. The gel matrix produced by soluble fibres that are eventually excreted in the faeces might entrap some of the bile acids released from the gallbladder (Mudgil and Barak, 2013). This physical property seems to be more pronounced in the terminal ileum where bile acids are usually reabsorbed (Elleuch et al., 2011).

1.6.5 Bulking ability

Insoluble fibres, for example cellulose and lignin, are poorly fermented by colonic microflora and also increase faecal bulk by their particle formation and water holding capacity (Elleuch et al., 2011). Wheat bran is considered the best bulking agent, because it is poorly fermented (Lupton and Turner, 1999). Some fermentable hemicellulose fibres may increase faecal bulk by increasing faecal flora biomass, whereas highly fermentable fibres such as pectin have little effect on faecal bulk (Elleuch et al., 2011). However, when bran is fermented by about 50 % stool output is increased 2 fold (Chen et al., 1998).

1.6.6 Fermentability

The extent of DF fermentation is highly variable. It ranges from not at all with lignin to almost complete fermentation with pectin. and soluble fibre is almost completely fermented by colonic bacteria (Elleuch et al., 2011). Fermentation of soluble fibre may also play an important role in some of its physiologic effects. Plants are composed of varying proportions of rapidly fermented, slowly fermented, and non-fermentable dietary fibres. Fruits (such as apples and bananas) and vegetables (such as potatoes and beans) are believed to be fermented rapidly and may contribute less to faecal bulking than other fibres. RS is proposed to be fermented between 80 to 90 % and only 10% are excreted in the faeces (Nugent, 2005). On the other hand, psyllium and wheat bran are believed to be fermented slowly and help build up the faecal mass through fermentation, which takes place along the entire length of the colon (Mudgil and Barak, 2013).

1.7 Colonic fermentation of carbohydrate

The colonic fermentation of substrates, in particular RS, NSP and NDOs, is determined by several factors, including the chemistry and structure of the substrate, the presence of bacterial enzymes, environmental conditions such as pH and the transit time through the gut, all of which will be explained in the next section.

1.7.1 Colonic bacteria

The gut microbiota provides two major benefits; firstly, the microbiota influences the immune system and increases tolerance of microbial immune-determinants. Secondly, the microbiota works as a metabolic organ that can break down indigestible food components, degrade potentially toxic food compounds, like oxalate, and synthesize certain vitamins and amino acids (Xu and Gordon, 2003). The composition of microbiota varies between individuals and is influenced by the host genotype and physiology, colonization history, environmental factors, food, and drugs such as antibiotics (Zoetendal et al., 2001).

In humans, the large intestine (approx. 1.5 m long) is comprised of the proximal and distal colon, and the rectum (Cummings et al., 1990). Bacteria comprise 40-55 % of faecal solid matter (Stephen and Cummings, 1980) and approximately 15 g of faecal bacterial biomass is excreted daily in those consuming ‘’Western type’’ diets (Hill,

1995). It has been estimated that bacteria present in the colon consist of around 1000-1150 different species, which can belong to more than different 70 genera (Sekirov et al., 2010, Qin et al., 2010). Some studies have shown that the numerically predominant bacteria are anaerobes including *bacteroides, bifidobacteria, eubacteria, streptococci* and *lactobacilli*, with *bacteroides* constituting 30 % of the total bacteria in the gut (Gibson and Roberfroid, 1995). The proximal colon is the principle site of fermentation as most of the bacterial activities happen there where the substrate availability is greatest. Saccharolytic bacteria like *bacteroides* ferment FC specifically in the proximal colon (den Besten et al., 2013).

Within the colon NSP, RS (Englyst et al., 1992), oligosaccharides and mucins are all subject to fermentation processes (Edwards and Rowland, 1992), and it has been calculated that the daily amount of carbohydrate needed to sustain the colonic microflora is 60-70 g (Wolin, 1981, Cummings et al., 1978). A smaller amount than this has been reported to enter the colon for fermentation in studies of ileostomy patients (at 25-35 g) (Schweizer et al., 1990). This variation suggested that secretions are important substrates for bacterial fermentation in the colon.

1.7.2 Substrates

The degree and rate of SCFA formed during fermentation is dependent on the type and amount of FC substrate utilized, the types of bacteria present in the colon, and gut transit time (Cook and Sellin, 1998). Not all FC substrates produce an equal amount of and type of SCFA (Table 1.5). Pectin in particular is believed to be a good source of acetate (80 % of total SCFA), compared to arabinogalactan (54 % of total SCFA) and guar (59 % of total SCFA) which are the poorest sources. RS is considered a good source of butyrate (23 % of total SCFA), followed by oat and wheat bran (20 % of total SCFA), whereas pectin is the poorest source of butyrate followed by other non-digestible carbohydrates in corn, soya, sugar beet and peas (Cummings and Macfarlane, 1991).

Table 1.5: Bacterial fermentation products (molar ratios) formed from different carbohydrates (Cummings and Macfarlane, 1997).

Substrate	Acetate	Propionate	Butyrate
RS	62	15	23
Pectin	80	12	8
Bran	64	16	20
Other NSP	63	22	8
Mixed diets	63	22	8
Oligofructose	78	14	8

Cummings and Macfarlane (1991) estimated that in humans approximately 10-60 g of NDCs enter the colon daily for fermentation. Of this, it has been estimated that 8-40 g/d is RS which is the main fermentable substrate, followed by 8-18 g/d of NSP, 2-10 g/d non-digestible sugar and 2-8 g/d NDOs. It has been estimated that in Europe 4 g/d of RS is ingested (Elmstahl, 2002), and 3-8 g/d of RS in America (Murphy et al., 2008). There are other substrates that might produce toxic metabolites in the colon derived from microflora fermentation which are harmful to health, such as a fraction of dietary protein which includes branched chain fatty acids (BCFA), amines, phenols, indoles and reduced sulphurous compounds. These products can be irritants to the mucosa (Evenepoel et al., 1999).

1.7.3 Transit time

The transit time of NDCs through the colon is strongly influenced by the activities of the gut microflora, as whole gut transit times range from 20 -140 h with a median of 60 h in normal healthy individuals in the UK (Cummings and Macfarlane, 1991). Kaur and Gupta (2002) studied the relationship between transit time and faecal SCFA output in humans, and reported that the mean transit time was significantly related to the proportion of butyrate in faeces. They showed that rapid transit (30 – 40 hours) was significantly correlated with higher faecal butyrate concentration than a slow transit times (60 – 80 hours). Greater stool weights and excretion of bacterial dry matter were correlated with fast transit times, whereas the reverse is true with slow gut transits, which are categorized by low stool weights and outputs of bacteria (Stephen et al., 1987). The physicochemical properties of NDCs are correlated with physiologic effects in both the small and the large intestine, such as solubility,

viscosity, and fermentability (Mudgil and Barak, 2013). Several well fermented fibres produce viscous solutions in the gut (guar gum and pectin), but lose their WHC on fermentation, whereas others which are poorly fermented maintain their moderate water-holding capacity (cellulose) and increase stool output. Studies have shown that soluble and viscous fibres were correlated with delayed gastric emptying and increased small bowel transit time; whereas high-water-holding fibres, which escaped fermentation, influenced the volume and the bulk of intestinal contents (Tungland and Meyer, 2002). Faecal ammonia concentrations were also affected by gut transit time, with higher concentrations found in subjects with slow transit times (Cummings et al., 1979). Macfarlane *et al*. (1989) showed that increasing the retention time from 24 to 69 h in an *in vitro* fermentation system increased the amount of protein degraded and consequently, the levels of ammonia and phenols generated.

1.7.4 Colonic pH

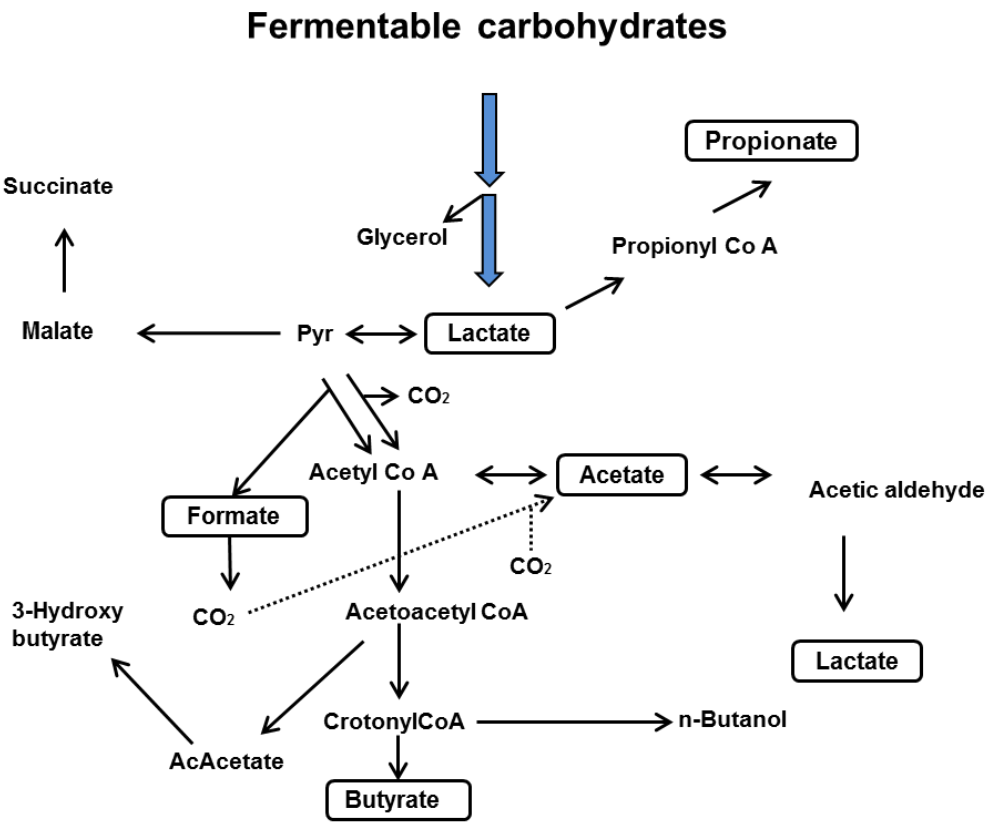
The diet and the intestinal environment interact in a complex way with the bacterial populations in the gut; FC leads to high concentrations of SCFA, and lowers the pH in the colon, which in turn influences the composition of the colonic microbiota and thereby SCFA production (Duncan et al., 2009). Several studies have observed that the concentration of SCFA declined from the proximal to the distal colon, whereas pH increased from the cecum to rectum (Cummings et al., 1987, den Besten et al., 2013). This shows the higher SCFA concentrations lead to a drop in pH from the ileum to the cecum, which might cause beneficial effects, such as altered gut microbiota composition, and prevention of overgrowth of pH sensitive pathogenic bacteria like *enterobacteriaceae* and *clostridia spp* (Cherrington et al., 1991, Duncan et al., 2009). Studies of human faecal microbial communities observed that at pH 5.5 the butyrate producing bacteria such as *Roseburia spp.* and *Faecalibacterium prausnitzii*, both belonging to the *Firmicutes* phylum, comprised 20 % of the total population. In the more distal parts of the large intestine, it has been observed that the luminal pH increases to 6.5, and the butyrate producing bacteria almost completely disappeared in the absence of FC, and the acetate and propionate producing *Bacteroides* related bacteria became dominant (Walker et al., 2005, Duncan et al., 2009). Similarly, Poulsen et al. (2012) examined the relationship in a rumen fluid-based *in vitro* fermentation system between FC (pectin, wheat and corn starch and inulin) and pH (ranging from 5.5 to 7.0). At pH 6.0 and 6.5, higher acetate

production was observed for pectin, and the lowest for inulin ($p < 0.001$), and vice versa for butyric acid production for pectin and inulin ($p < 0.001$). High propionate production was observed when the pH increased from 5.5 to 6.0, whereas a higher butyrate production has been observed when the pH decreased from 7.0 to 5.5 ($p < 0.001$) (Poulsen et al., 2012).

1.8 Colonic fermentation products

Carbohydrate fermentation starts with hydrolysis of NDCs to their basic sugars, which are then fermented and produce SCFA (Savage, 1986). NDCs are the main substrate for fermentation; plant cell-wall polysaccharides consist of hexose (glucose and galactose), pentose (xylose and arabinose) and uronic acid monomers that are fermented by saccarolytic bacteria, primarily in the proximal colon (Miller and Wolin, 1979, Cummings, 1981). Hexose is broken down through the Embden-Myerhoff-Parnas glycolytic pathway to pyruvate, and occasionally, hexose can be converted to 6-phosphate-glyconate, and then metabolised by the pentose phosphate cycle (Duncan et al., 2004a). Pyruvate is the principle metabolic fermentation reaction, hence very little is found in the human gut as it is converted into a series of end products, and these are used as energy for microbial growth and maintenance and other metabolic products are used by the host (Hamer et al., 2012). The main end products are SCFAs (acetate > propionate > butyrate) in a molar ratio of approximately 60:20:20, together with gases (CO2, CH4 and H2) and heat (Figure 1.2). Acetate is formed by the oxidation and decarboxylation of pyruvate, whereas butyrate is formed by the reaction of acetoacetate that forms from acetate. Propionate is produced by two main pathways; firstly, by fixation of CO_2 to form succinate, that is subsequently decarboxylated; secondly, through lactate and acrylate pathways (Cummings, 1981, Wong et al., 2006).

Figure 1.2: Overview of anaerobic bacteria metabolic pathways involved in fermentable carbohydrates. Boxes show SCFA products (adapted from Paeschke and Aimutis, 2011).



SCFA produced from NDCs fermentation supplies the human body with approximately 10 % of its daily caloric requirements (Musso et al., 2011), with more than 95 % of the SCFA formed in the caecum and large intestine being rapidly absorbed by the colonocytes, whereas the remaining 5 % is excreted in the faeces (Topping and Clifton, 2001). Several studies have reported that faecal excretion rates for SCFA were in the range of 10-30 mmol/d for a high fibre diet, compared with 5-15 mmol/d for control diets (Cummings et al., 1996, Holt et al., 1996). It is important to note that the concentration and production of SCFA in the large intestine were not predicted by faecal SCFA concentrations in these studies because most of the SCFA was taken up by the host. Therefore, faecal SCFA excretion might provide little information about actual levels of intestinal SCFA metabolism (den Besten et al., 2013).

Moreover, SCFA could also be produced by sources other than NDCs fermentation in the gut; several studies have reported that cellular metabolism, in particular fatty acid oxidation (but also amino acid and glucose metabolism), can also produce SCFA (Wolever et al., 1997, Freeland et al., 2010). The major sources of energy in metabolic states with decreased glucose metabolism are ketone bodies which are

generated via hepatic acetyl-CoA production during fatty acid oxidation (Pouteau et al., 2003). Hepatic acetate levels were also increased during fatty acid oxidation, and circulating acetate levels increased with the use of acetate as a fuel source in fasting states (Layden et al., 2013). A fasting state raises acetate production in the liver, but the impacts on other circulating SCFA are still not clear (Sakakibara et al., 2009). As these different sources can produce the same products, SCFA production happening in the gut is often referred to as the exogenous source of SCFA, and SCFA generated during cellular metabolism the endogenous source (Pouteau et al., 2003).

1.8.1 Acetate

Acetate is usually produced by hydrolysis of acetyl-CoA, or from CO_2 through the Wood-Ljungdahl pathway in which CO_2 is reduced to CO and converted with a methyl group and CoASH to acetyl-CoA (Ragsdale and Pierce, 2008). Acetate becomes the major SCFA produced (50-70 %) from the fermentation of all NDCs (Edwards and Rowland, 1992). Pouteau et al. (1998) reported in stable isotope study that acetate contributes to 6-10 % of energy expenditure in humans. Duncan *et al*. (2004a) investigated the contribution of exogenous acetate to butyrate formation, they found that the proportion of butyrate formed depends on the type of butyrate producing-acetate consumingers' bacteria in the gut and the type of NDCs available for fermentation. The important butyrate producing-acetate consuming bacteria found in the human colon are *Faecalibacterium prausnitzii, Roseburia intestinalis* and *Eubacterium rectale* (Pouteau et al., 1998, Duncan et al., 2002). The *Faecalibacterium prausnitzii and the Roseburia intestinalis* produced 85-90 % butyrate from exogenous acetate in the presence of 60 mM acetate and 10 mM glucose. Duncan et al. (2004a) reported that the amount of butyrate formed from exogenous acetate was ranged from 56 % in pectin to 90 % in xylan in continuous cultures, and from 72 % to 91 % in the batch cultures.

Only 70 % of the acetate is metabolised by the liver (Bloemen et al., 2009), where it is used as an energy source, a substrate for the synthesis of cholesterol and long-chain fatty acids, and as a co-substrate for glutamine and glutamate synthesis (Soergel, 1994, Wolever et al., 1995). The remaining acetate is used by other tissues such as the heart, adipose tissue, kidneys and muscle (Knowles et al., 1974).

1.8.2 Propionate

Propionate is the second most abundant SCFA product; its formation has been studied by Reichardt et al. (2014) using a combination of genomic analysis and microbial physiology to investigate three different pathways of microbial propionate formation in the colon. They reported that the succinate pathway was the most abundant route for propionate formation, followed by acrylate and propanediol. Propionate was formed by the conversion of hexose sugars to propionate in the presence of the abundant phylum *Bacteroidetes* and several *Negativicutes* bacteria, as specified by the expression of the *mmdA* gene encoding methylmalonyl-CoA decarboxylase. The acrylate pathway was restricted to only a few spieces of *Lachnospiraceae* and *Negativicutes* to produce propionate from lactate by *Coprococcus catus* (as specified by the *lcdA* gene encoding lactoyl-CoA dehydratase). The propanediol pathway was used by *Ruminococcus obeum* and *Roseburia inulinivorans* members of the *Lachnospiraceae* to convert deoxysugars, such as fucose and rhamnose, to propionate (as shown by the presence of the *pduP* gene encoding propionaldehyde dehydrogenase) (Reichardt et al., 2014).

Propionate is metabolised primarily by the liver, where it acts as a precursor for gluconeogenesis. In humans, there is a lack of data on the true production rates of propionate which means the extent to which propionate contributes to energy metabolism is unknown (Hosseini et al., 2011). Concentrations of propionate in portal blood and hepatic venous blood suggest that around 30 % of propionate produced is absorbed by the liver (Cummings et al., 1987). Studies have suggested that the peripheral tissues absorb the remainder of propionate as the peripheral venous blood levels were 23 % lower than the hepatic venous blood levels. Generally, it is thought that the liver clears a large fraction of propionate from the portal circulation but absolute values are still unknown (Cummings et al., 1987).

1.8.3 Butyrate

Butyrate is the main, and preferred, metabolic substrate for colonocytes, providing at least 60–70 % of the energy requirements they require for proliferation and differentiation (Suzuki et al., 2008). Butyrate is formed from the condensation of two molecules of acetyl-CoA, and subsequent reduction to butyryl-CoA, and lactate-utilizing bacteria might also produce butyrate by first producing acetyl-CoA from

lactate (Duncan et al., 2004b). In the so-called classical pathway, the enzymes phosphor-transbutyrylase and butyrate kinase convert butyryl-CoA to butyrate and CoASH, with the concomitant formation of adenosine triphosphate (ATP) (Louis and Flint, 2009). Duncan et al. (2002) showed an alternative pathway, in which butyryl-CoA could be converted by butyryl-CoA:acetate CoA transferase to butyrate, and this conversion utilizes exogenously derived acetate to generate butyrate and acetyl-CoA. This finding was confirmed by labelling studies, which observed that there was cross feeding between acetate-producing and butyrate-producing bacteria (Venema, 2010).

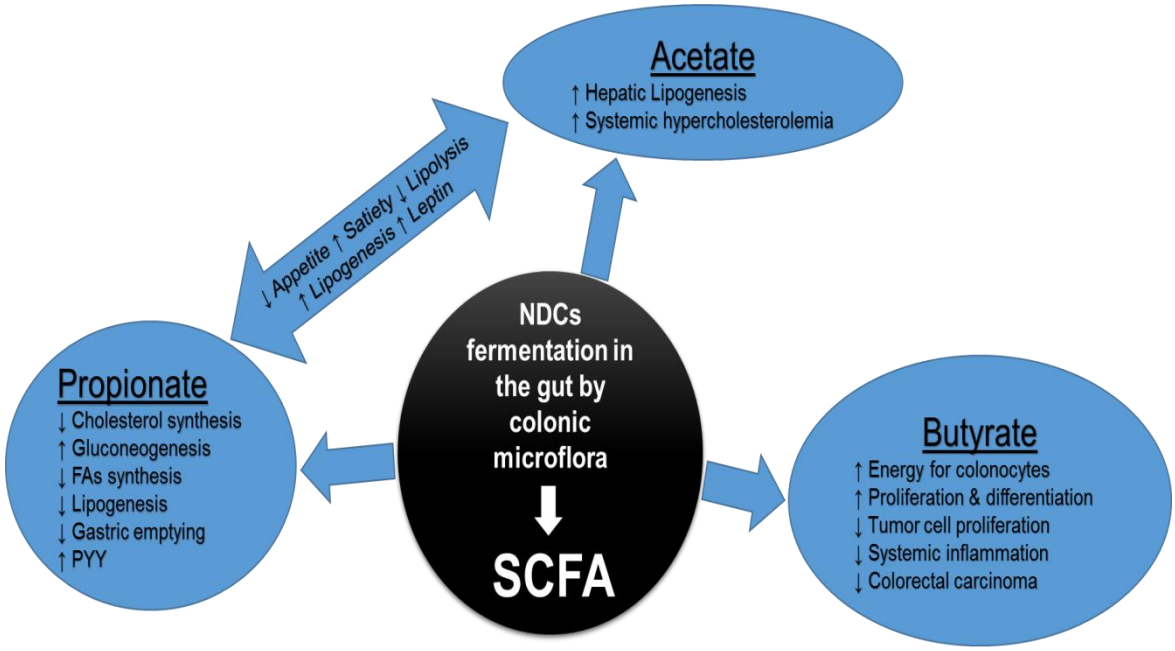
Like other SCFA, butyrate is absorbed from the colonic lumen. Butyrate that enters the portal circulation is metabolised in the liver to produce ketone bodies (Roediger, 1980). Approximately 95 % of butyrate produced by colonic bacteria is transported across the epithelium, but the concentration in portal blood is usually undetectable as a result of rapid utilisation (Csordas, 1996). The supply of butyrate to the colonic epithelium is dependent largely on the NDC components that escape digestion in the small intestine, and studies show that faecal concentrations do not reflect production rates of SCFA as a very high proportion of the SCFA is utilized by the colonic mucosa. However, there is substantial evidence that indicates that different dietary polysaccharides affect the amounts and relative molar proportions of SCFA in the gut of pigs, rodents and humans (Hamer et al., 2008). *In vitro* studies with mixed human faecal bacteria have proposed that RS and FOS fermentation yields a higher molar proportion of butyric acid amongst the SCFA products, when compared to pectin fermentation, therefore RS and FOS are considered butyrogenic (Topping and Clifton, 2001).

Ulcerative colitis has been linked with a lack of luminal SCFA and impaired mucosal metabolism of butyrate and healing of colitis has been observed after treatment with butyrate enemas (Scheppach et al., 1992). It is not clear which bacteria in the colon produce the butyrate however, cultural and molecular studies have reported that most of these bacteria are strict anaerobes, some of which are previously undescribed species related to *Eubacterium, Roseburia, Faecalibacterium* and *Coprococcus* (Pryde et al., 2002).

1.9 Physiological roles of SCFA in affecting host metabolism

The gut microbiota has become recognised as an important factor in the development of obesity and metabolic disorders, through its SCFA produced by anaerobic fermentation of NDCs and also genetic factors (Kasubuchi et al., 2015). Several human and animal studies have shown that alterations in intestinal microbiota composition, and shifts in the gut microbiome towards increased energy harvest, are associated with metabolic syndrome, bowel disorders, obesity and certain types of cancer (Kovatcheva-Datchary and Arora, 2013, Rahat-Rozenbloom et al., 2014, Schwiertz et al., 2010, Frost et al., 2014). Moreover, SCFA concentrations are recognized by GPRs, also known as FFAR, to potentially be involved in the regulation of lipids, obesity and glucose metabolism (Figure 1.3) (Brown et al., 2003, Tazoe et al., 2008, Kimura et al., 2014).

Figure 1.3: The physiological effects of the major SCFAs produced from NDCs fermentation by colonic microflora in the gut on energy metabolism, ↑ = increase, ↓ = decrease (adapted from Kimura et al., 2014, Hosseini et al., 2011, Kasubuchi et al., 2015).



1.9.1 The impact of SCFA production on obesity

The development of obesity is a multifactorial process involving genetic susceptibility and environmental factors, such as lifestyle and inappropriate diet, and the aetiological root of obesity is an imbalance between food intake and energy

expenditure (Seidell, 2005). Recently, the gut microbiota has been suggested as a dynamic force in the pathogenesis of obesity (Kovatcheva-Datchary and Arora, 2013). Schwiertz et al. (2010) examined 98 volunteers to evaluate differences in intestinal microbiota and faecal SCFA concentrations between lean and obese subjects. This study showed that the total amount of SCFA was higher in the obese subject group ($p = 0.02$) than in the lean subject group, and the proportion of individual SCFAs changed in favour of propionate in overweight ($p = 0.01$) and obese subjects ($p = 0.02$). Thus the authors observed that SCFA metabolism might play an important role in the development of obesity. These results have been supported by a study involving eleven lean (LN) and eleven overweight and obese (OWO) subjects carried out by Rahat-Rozenbloom et al. (2014), who showed that OWO had higher faecal SCFA than LN subjects. The differences in SCFA may be related to OWO subjects having higher *Firmicutes abundance* (83.1 ± 4.1 vs $69.5 \pm 5.8\%$, respectively, $p = 0.008$) and a higher *Firmicutes:Bacteriodetes* ratio ($p = 0.023$) than LN subjects and not just because of difference in diet. Overall these studies supported the hypothesis that OWO individuals produce more colonic SCFA than LN individuals because of differences in colonic microbiota.

There are some conflicting results in this area as Layden et al. (2011) observed a negative association between serum acetate levels and visceral adipose tissue and insulin levels, in a cohort of young obese women (n =18). Moreover, Fernandes et al. (2013) assessed the relationship between body mass index (BMI), number of faecal Archaea anaerobic microbiota, and faecal SCFA among 95 healthy subjects, and their results showed no association between colonic Archaea and obesity in healthy humans. Conversely, a study by Frost et al. (2014b) proposed direct effect of acetate in appetite regulation. They observed that acetate produced from NDCs fermentation in the colon induced an anorectic signal in the hypothalamic arcuate nucleus by supporting the glutamate–glutamine transcellular cycle, and leading to an increase in lactate and GABA production, which lead to appetite suppression.

Lin et al. (2012) studied the effects of SCFA supplementation in wild type (WT) and FFAR3 knockout mice, and observed that butyrate, propionate, and acetate all protected against diet induced obesity and insulin resistance. They also found that butyrate and propionate, but not acetate, induced gut hormones and reduced food intake. This study also examined the relationship between obesity, insulin resistance and FFAR3, and showed that normal body weight and glucose homeostasis was

observed among FFAR3 deficient mice. In addition, FFAR2 deficient Knockout mice (FFAR2-KO) were fed a high fat diet (HFD) or chow diet, and the results showed FFAR2-KO mice had lower body fat mass and increased lean body mass with HFD (Bjursell et al., 2011).

Another possible method the SCFAs have an effect is through Peptide YY (PYY), which is a hormone released by the cells in the ileum and the colon and is known to decrease appetite, as studies suggest that high NDCs diets are associated with increased levels of PYY (Karra and Batterham, 2010). Further evidence comes from the fact that PYY (3-36) peripheral injection in rats inhibited food intake and reduces weight gain, and the normal postprandial concentrations of PYY (3-36) injection significantly decreased appetite and reduced food intake by 33 % over 24 h in humans (Batterham et al., 2002). Moreover, Chelikani et al. (2005) assessed the dose dependent effects of 3-h IV infusions of PYY (3–36) and PYY (1–36) (0.5-50 $pmol \cdot kg^{-1} \cdot min^{-1}$) in non-food deprived rats. They observed significant reductions in food intake and gastric emptying following peripheral administration of physiological levels of PYY (3-36) with minimal effective dose was 5 $pmol \cdot kg^{-1} \cdot min^{-1}$ and maximal effective was 15 $pmol \cdot kg^{-1} \cdot min^{-1}$. Overall these results suggest that SCFA production in the colon through microflora fermentation of NDCs may induce obesity, decrease appetite and insulin resistance.

1.9.2 The impact of SCFA production on plasma lipids

Primary observational studies of dietary modulation of hepatic lipid synthesis have shown a strong correlation with NDCs intake (Chen et al., 1984, Venter et al., 1990a). The SCFA end products of NDCs fermentation, in particular propionate, have been proposed to have anti-lipogenic and cholesterol-lowering effects in animals (Chen et al., 1984) as well in humans (Venter et al., 1990b, Wolever et al., 1991). This has been supported by work by Wolever et al. (1989) who showed that an infusion of 60 mmol of propionate with 180 mmol of acetate resulted in decreased free fatty acids by an additional 10 %, and negated the increase in the serum cholesterol observed when acetate was infused alone. In rats, Chen et al. (1984) observed that serum and liver cholesterol concentrations were both significantly lower in rats fed the cholesterol-propionate diet, than in rats fed the cholesterol diet without propionate. This has also been observed in human studies as Venter et al. (1990b) reported that a normal diet supplemented with propionate fed to healthy

subjects for a period of seven weeks showed a significant increase in high-density lipoprotein (HDL) cholesterol with no differences in the glucose tolerance test, insulin response or total cholesterol, compared to the control group. Lin et al. (1995) using rat hepatocytes and [^{14}C] acetate, showed that 50 % of cholesterol and triglyceride synthesis was inhibited in the presence of a propionate concentration of 0.1 mmol/l.

The role of GPR43 in cholesterol and lipid metabolism has been examined in adipose tissue; Ge et al. (2008) showed that activation of GPR43 by acetate lead to the reduction in plasma free fatty acid levels, without inducing the flushing side effect that has been observed after the activation of the nicotinic acid receptor GPR109A. This result might show a potential role for GPR43 in regulating plasma lipid profiles. Similar findings have been observed by Hong et al. (2005), and Bjursell et al. (2011) observed that the liver weight and content of triglycerides, as well as plasma levels of cholesterol, were lower in the FFAR2-KO mice fed a HFD compared with FFAR2 wild-type mice, which supports the role of GPR43 as a regulator of plasma lipid profiles.

1.9.3 The impact of SCFA production on cancer

Butyrate is thought to have many potential anticancer properties; it stimulates the growth of colonic mucosa and inhibits the growth of cancer cells and induces differentiation in colonic cancer lines (Scharlau et al., 2009, Fung et al., 2012). Butyrate has been shown to produce growth arrest, differentiation (Augeron and Laboisse, 1984, Gamet et al., 1992) and apoptosis (Hague et al., 1995) in cancer cells grown in cultures. This is also supported by the results that showed a lower ratio of butyrate and higher ratio of acetate observed in colon polyp and cancer patients (Weaver et al., 1988). Lower concentrations of faecal butyrate and propionate have also been observed in patients with rectal cancer compared to those with more proximal cancers (Pryde et al., 2002).

The effect of butyrate on cancer cells has been further studied by Kim et al. (2004) who examined the potential sensitizing properties of sodium butyrate to TRAIL-mediated apoptosis in human colon cancer cells. They observed that when HCT116 cells were incubated with sodium butyrate and TRAIL, TRAIL-mediated apoptosis was enhanced, which suggests TRAIL-induced apoptosis can be sensitized by

sodium butyrate. The effect has also been investigated by Singh and Lai (2005) who examined the effectiveness of a combined treatment of dihydroartemisinin (DHA) and butyric acid in killing Molt-4 cells (a human lymphoblastoid leukemia cell line). They observed that combination of 20 μM DHA and 1 mM sodium butyrate killed all Molt-4 cells at the 24 h time-point and did not significantly affect lymphocytes.

SCFAs, in particularly butyrate, have also been shown to have anti-tumor suppressive functions via the SCFAs receptor GPR43; Tang et al. (2011b) observed that GPR43 immunoreactivity was completely lost in most colorectal adenocarcinoma tissues (N = 70) and their corresponding lymph node metastatic adenocarcinomas (N = 38), compared with normal colon tissues (N = 31). A full length GPR43 mRNA was found in only one (HT-29) of nine established human colon cancer cell lines after RT-PCR analysis. They restored GPR43 expression in tumor cells using propionate/butyrate treatment, which induced G0/G1 cell cycle arrest, activated caspases, upregulated p21, decreased cyclin D3 and cyclin dependent kinases 1 and 2, and increased apoptotic cell death. This findings suggest that GPR43 can act as a tumor suppressor, and provides a possible mechanism for the putative connection between a NDCs diet and lower incidence of colon cancer.

1.10 Physiological effects of NSPs

Several epidemiological and clinical studies have suggested that consumption of NSP is inversely related to obesity (Tucker and Thomas, 2009), type two diabetes (Meyer et al., 2000), cancer (Park et al., 2009) and cardiovascular disease (Streppel et al., 2008).

1.10.1 Effect of NSPs on colorectal cancer (CRC)

Colorectal cancer has become the third most common type of cancer, with 1.2 million new cases diagnosed in 2008 worldwide, and accounting for about 9.7 % of all cases of cancer (Ferlay et al., 2010). Burkitt (1971) hypothesised that dietary fibre reduces the risk of colorectal cancer, based on the observation of low rates of such cancer among rural Africans who ate a diet with high fibre content. Several plausible mechanisms have been suggested to support the hypothesis, including increased

stool bulk, dilution of carcinogens in the colonic lumen, reduced transit time, and bacterial fermentation of fibre to SCFA (Lipkin et al., 1999).

Therefore, an electronic literature search (using PubMed and ISI Web of Science) for articles published from 1 January 1996 to 20th of April 2014 was conducted to retrieve relevant studies on dietary fibre intake and incidence of CRC using subject-specific keywords. Nine studies examining the relationship between total dietary fibre, soluble, insoluble, cereal, fruit and vegetable fibre and the CRC incidence and mortality were identified (Table 1.6). Dietary fibre intake was calculated in quintiles (the lowest median-the highest median), with intake ranging from 5.4-36.7 g/d. The study of Mai et al. (2003) from the United States had the lowest median intake of dietary fibre, with a range between 5.4-18.2 g/d, followed by Fuchs et al. (1999) from the United States (8.5-28.5 g/day). The study from the UK (Sanjoaquin et al., 2004) had the highest median intake of dietary fibre, with a range between 17.0-36.7 g/d and the EPIC study (Bingham et al., 2003) has fibre intake ranged between 15 – 34 g/d for participants across Europe. All of these studies provided sufficient information for a comparison between the levels of dietary fibre consumed and the incidence of CRC.

Table 1.6: Evidence table of epidemiological studies on dietary fibre intake and CRC.

Reference	Aim	Study population	Type of fibre	Dietary fibre intake (highest median vs. lowest median) g/day	Effect (RR 95 % CI)	Comments
Michels K B et al. (2005) (NHS) and (HPFS) USA	To investigate the association of fibre intake and incidence of CRC.	76,947 Female registered nurses aged 30-55 years and 47,279 Health Professional males aged 40-75 years; self-administered semi-quantitative FFQ included 130 food items. AOAC and Englyst (NSP)	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre	30 vs 12 11.4 vs 2.8 9.3 vs 1.4 12.2 vs 3.6	0.91 (0.87-0.95) 0.93 (0.84-1.03) 0.89 (0.80-1.00) 1.05 (0.95-1.16)	This study fairly consistently indicated no important association between fibre intake and risk of CRC, but revealed considerable confounding by other dietary and lifestyle factors.
Lin J et al. (2005) (WHS) USA	To examine the association between dietary intake of fruit, vegetables and fibre and CRC risk	39,876, 75 % nurses, 11 % physicians and other health professionals' resident in the USA; aged 52.2-55.7 years; FFQ includes 131 food items	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre Cruciferous fibre Legume fibre	26 vs 12 6.1 vs 3.1 6.0 vs 2.5 8.0 vs 5.9 1.6 vs 0.7 1.8 vs 0.4	0.75 (0.48-1.17) 1.00 (0.67-1.49) 1.00 (0.64-1.55) 0.97 (0.66-1.43) 0.74 (0.47-1.17) 0.60 (0.40-0.91)	This study could not confirm an inverse association of intake of fruit, vegetables and fibre. However, data suggest that high intake of legume fibre and/or other sources related to this intake may reduce the risk of CRC
Sanjoaquin M A et al. (2004) UK	To examine whether the risk of CRC is lower in vegetarians than in non-vegetarians, and low in those who ate relatively large amounts of fruit or vegetables and other foods high in fibre.	10,998 (42.3 % vegetarians and 57.7 % non-vegetarians) recruited in the UK; median age 33 years; simple FFQ	Total dietary fibre	36.7 vs 17.0	0.82 (0.43-1.56)	No association observed between fibre and CRC risk; however, information needed to estimate dietary fibre intake was unavailable for 32 cases.

Bingham S A et al. (2003) (EPIC) Multi-centre prospective	To study the relationship between dietary fibre and colorectal cancer	519,978 persons aged 25-70 years old; extensive self-administered quantitative dietary questionnaire, containing up to 260 food items, semi-quantitative FFQ; Combined methods were used in the UK and Malmo in Sweden (semi-quantitative food-frequency questionnaires and a 7-day record) and 24-hour dietary recall was used as a reference method.	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre Legume fibre	35 vs 15	0.75 (0.59-0.95)**	In a population with low average intake of dietary fibre, an approximate doubling of total fibre intake from foods could reduce the risk of CRC by 40 %.
Bingham S A et al. (2003) (EPIC) Multi-centre prospective	To study the relationship between dietary fibre and colorectal cancer	519,978 persons aged 25-70 years old; extensive self-administered quantitative dietary questionnaire, containing up to 260 food items, semi-quantitative FFQ; Combined methods were used in the UK and Malmo in Sweden (semi-quantitative food-frequency questionnaires and a 7-day record) and 24-hour dietary recall was used as a reference method.	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre Legume fibre	35 vs 15 12.05 vs 4.72 7.76 vs 2.21 6.48 vs 2.85 1.73 vs 0.45	0.75 (0.59-0.95)** 0.78 (0.62-0.98) 0.88 (0.70-1.11) 1.04 (0.84-1.30) 0.78 (0.64-0.97)	In a population with low average intake of dietary fibre, an approximate doubling of total fibre intake from foods could reduce the risk of CRC by 40 %.
			Total dietary fibre	18.2 vs 5.4	0.94 (0.71-1.23)	

Mai V et al. (2003) (BCDDP) USA	To evaluate the association between total dietary fibre intake and incidence of CRC	61, 429 women aged 50-65 years; FFQ includes 62 food items	Fruit fibre Vegetable fibre Beans fibre Grain fibre	5.4 vs 0.5 4.9 vs 1.0 2.5 vs 0.1 6.0 vs 1.3	1.10 (0.83-1.46) 0.92 (0.69-1.21) 0.84 (0.63-1.10) 1.02 (0.76-1.37)	There was little evidence that dietary fibre intake lowers the risk of CRC.
Pietinen P et al. (1999) (ATBC) Finland	To examine the association between dietary fibre and the CRC during an average of 8 years of follow-up	29,133 male smokers aged between 50-69 years; self-administered modified diet questionnaire included 276 food items	Total fibre Soluble fibre Insoluble fibre	34.0 vs 16.0 7.3 vs 3.7 27.1 vs 12.2	1.0 (0.6-1.5) 1.1 (0.7-1.6) 1.0 (0.6-1.5)	There was no association between the risk of CRC and intake of total dietary fibre. Since these analyses were conducted among smokers the results may not be applicable to non-smokers.
Terry P et al. (2001) Swedish	To assess the relationship between the risks of CRC in individuals who consumed the highest and the lowest amount of fruit, vegetable and cereal fibre.	66,651 women aged between 40-74 years; self-administered FFQ that included 67 food items commonly eaten in Sweden	Total fibre Cereal fibre Fruit fibre Vegetable fibre	21.8 vs 12.3 16.0 vs 4.3 5.2 vs 0.8 2.0 vs 0.6	0.96 (0.70-1.33) 0.91 (0.69-1.20) 0.97 (0.69-1.38) 1.17 (0.85-1.61)	Individuals who consume a very low amount of fruit and vegetables have the greatest risk of CRC. Relatively high consumption of cereal fibre does not appear to lower the risk of CRC. Hence, increasing fruit and vegetables may reduce the risk of CRC among those who consume less than 2 servings/ day of fruit and vegetables.
Fuch C S et al. (1999) (NHS) USA	To examine the relation between fibre intake and risk of CRC	121,700 women aged 34-59 years; semi-quantitative FFQ including 61 food items was used; in 1984 questionnaire was expanded to 121 and in 1986 included 136 items	Total fibre Cereal fibre Fruit fibre Vegetable fibre	28.5 vs 8.5 4.8 vs 1.0 7.2 vs 0.8 10.0 vs 2.7	0.95(0.73-1.25) 1.0 (0.79-1.27) 0.86 (0.67-1.10) 1.35 (1.05-1.72)**	The results do not support the existence of an important protective effect of dietary fibre against CRC.

Kato I et al. (1997) USA	To assess the association between diet and CRC	15,785 women aged 34-65 years; semi-quantitative questionnaire that included 70-items of typical American food.	Total dietary fibre	No specific information on dietary fibre classification. Dietary fibre was grouped into quintiles.	1.51 (0.85-2.68)	There was no relation between dietary fibre and incidence of CRC among women.

FFQ-Food Frequency Questionnaire; CRC-Colorectal Cancer; RR-Relative Risk; CI-Confidence Intervals; EPIC-European Prospective Investigation into Cancer and Nutrition; NHS-Nurses Health Study; HPFS-Health Professional' Follow-up Study; WHS-Women's Health Study; ATBC-Tocopherol Beta-Carotene Cancer Prevention Study; BCDDP-Breast Cancer Detection Demonstration Project; CVD-Cardiovascular Disease

The study by Fuchs et al. (1999) found that a greater consumption of vegetable fibre was associated with significant increases in the incidence of CRC among women in the highest intake group. Only one out of the nine studies investigating the relationship between different sources of fibre intake and CRC incidence showed protective effects (Bingham et al., 2003). In a population with low average intake of dietary fibre Bingham et al. (2003) reported that an approximate doubling of total fibre intake from food could reduce the risk of colorectal cancer by 40 %. The other results with strong evidence supporting the hypothesis that dietary fibre protects against the risk of CRC, were from a meta-analysis of case control studies (Friedenreich et al., 1994). However, the categorical evidence from the large pooled analysis of prospective cohort studies failed to show a protective effect against CRC (Park et al., 2005), but this study by Park et al. (2005) did not include the EPIC study in the pooled analysis project, if they had the findings might be changed to a significant reduction effect in the multivariate analysis.

1.10.1.1 Mechanism behind health effects of NSPs on CRC

The Food and Drug Administration (FDA) currently accepted health claims for DF; the first claim was by decreasing the daily fat intake (by < 30 % of calories), and increasing the consumption of DF from fruit, vegetables and whole grains, this might reduce some types of cancer (Lattimer and Haub, 2010). This is also supported by some recent studies as they have shown an inverse relationship between DF consumption and the development of several types of cancer, including colorectal and small intestine (Schatzkin et al., 2008, Nomura et al., 2007). Although most studies have agreed with these findings, the mechanisms responsible are still unclear. Several modes of action have been suggested; first, that the DF escaped digestion and absorption in the small intestine, thereby allowing it to enter the large intestine where it is fermented to produce SCFA, which might have anti-carcinogenic properties (Young et al., 2005). For example butyrate is produced by anaerobic fermentation of DF and other substrates in the colonic lumen., and this has been shown to inhibit cell proliferation, induce differentiation, and enhance apoptosis in human colonic tumour cell lines (Medina et al., 1997). In addition, several studies have observed that the inclusion of DF and other oligosaccharides, especially FOS, as a dietary constituent produces a significant increase in probiotic-like bacteria, and these health-promoting bacteria inhibit the growth of pathogenic bacteria, lower the activity levels of genotoxic agent-forming enzymes, lower tumour promoters and

consequently decrease the production of carcinogenic substances (Hughes and Rowland, 2001, Kaur and Gupta, 2002).

The second action that could explain the anti-cancer properties is related to DF increasing faecal bulking and viscosity, which allowed less contact time between potential carcinogens and mucosal cells. A third potential mechanism action suggests that DF increased the binding between bile acids and carcinogens, which would mean they would be degraded target than continue in the intestine. Increased intake of DF may also yield an increase in the levels of antioxidants, which would have an effect on cancer cells (Fung et al., 2012). DF might also increase the amount of oestrogen excreted in the faeces because it can cause inhibition of oestrogen absorption in the intestines (Adlercreutz et al., 1987).

1.10.2 Effect of NSPs on cardiovascular disease (CVD)

The incidence of CVD has declined both among some European countries and also in north America (Smolina et al., 2012), even though it is considered as the main cause of mortality and accounts for almost half (48 %) of all deaths in Europe and a third (32.8 %) of all deaths in the USA (Unal et al., 2004). Dietary factors play an important role in the development of CVD through their effect on body weight and lipid profile (Liu et al., 2000). In 2003 the World Health Organization (WHO) reported that unhealthy dietary practices, such as the high intake of saturated fatty acids, salt and refined carbohydrates, contributed to increase the incidence and risk of mortality from CVD.

Trowell (1976) hypothesised that high dietary fibre intake is a protective factor against a number of western diseases, such as cancer, CVD, diabetes and obesity. Epidemiological studies have reported that a high intake of dietary fibre may play a major role in reducing the risk of CVD incidence and mortality. In 2011, an initial electronic literature search (using PubMed and ISI Web of Science) for articles published from 1 January 1996 to 15th of February 2011 was conducted to retrieve relevant studies on the dietary fibre intake and incidence or mortality from CVD using subject-specific keywords. This search was updated in 2014 using the same key search terms. Twenty-one studies that covered the relationship between total dietary fibre, soluble and insoluble fibre, and CVD incidence and mortality were examined (Table 1.7). A key study was a systematic review and meta-analysis of

22 cohort studies on dietary fibre intake and CVD risk showed no relationship between dietary fibre intake and the risk of CVD. The studies had a minimum follow-up of three years and published between 1 January 1990 and 6 August 2013 (Threapleton et al., 2013b).

The alternative Healthy Eating Index (AHEI) study conducted by Akbaraly et al. (2011) was not included in this analysis because no estimate of dietary fibre intake was presented. Of the studies selected nineteen out of the 22 examined the relationship between total dietary fibre and CVD incidence and mortality (Table 1.7), four of the 22 studies examined the association between soluble fibre intake and CVD incidence and mortality (Pietinen et al., 1996, Bazzano et al., 2003, Estruch et al., 2009, Threapleton et al., 2013a), two studies investigated the relationship between insoluble fibre intake and CVD incidence and mortality (Estruch et al., 2009, Threapleton et al., 2013a), ten studies investigated the relationship between cereal fibre intake and CVD incidence and mortality (Rimm et al., 1996, Wolk et al., 1999, Mozaffarian et al., 2003, Estruch et al., 2009, Baer et al., 2011, Crowe et al., 2012, Bernstein et al., 2011, Threapleton et al., 2013a, Buyken et al., 2010, Kaushik et al., 2009). and seven studies investigated the relationship between fruit and vegetable fibre intake and CVD incidence and mortality (Rimm et al., 1996, Wolk et al., 1999, Mozaffarian et al., 2003, Estruch et al., 2009, Crowe et al., 2012, Threapleton et al., 2013a, Buyken et al., 2010). Based on these twenty-two studies, the overall total dietary fibre intake ranged from 5.8-63.0 g/d, soluble fibre intake was 0.9-18.9 g/d, the intake was 25.6-5.9 g/d for insoluble fibre, with 1.1-15.7 g/d of cereal fibre, 0.4-11.1 g/d of fruit fibre, and 2.0-9.7 g/d of vegetable fibre. There were protective effects of dietary fibre intake against the incidence and mortality from CVD reported for 84 % of total dietary fibre studies, 75 % of soluble fibre, 50% of insoluble fibre, 60 % of cereal fibre and 43 % of fruit and vegetables studies.

Table 1.7: Evidence table of epidemiological studies on dietary fibre intake and CVD.

Reference	Aim	Study population	Type of fibre	Dietary fibre intake (highest median vs. lowest median) g/day	Effect (RR 95%CI)	Conclusion
Pietinen et al. (1996) (Alpha-Tocopherol Beta-Carotene Study) Finland	To examine the relations between intake of dietary fibre and risk of CHD heart disease in a cohort of middle aged smoker Finnish men	21, 930 smoking men aged 50-69 years; validated FFQ (276) diet over previous year, quality check by nurse; Englyst	Total dietary fibre Soluble fibre	34.8 vs 16.1 18.9 vs 5.4	0.69 (0.54-0.88) *** 0.74 (0.66-0.94) **	Increasing daily intake by 10 g/d and 3 g/d of total fibre and soluble fibre (respectively) appeared to lower the risk of CVD death by 27 %
Rimm et al. (1996) (Health Professionals' Follow-up Study USA	To examine the relationship between dietary fibre and risk of CVD	43,757 males aged 40-70 years; validated FFQ diet (131) diet over previous year; AOAC	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre	28.9 vs 12.4 7.9 vs 1.2 11.1 vs 3.2 9.7 vs 2.2	0.64 (0.47-0.87) ** 0.71 (0.54-0.92) ** 0.81 (0.62-1.06) 0.83 (0.64-1.08) *	10 g increase of TDF provides protective effect against the risk CVD and cereal fibre was also strongly associated with reduced risk of CVD
Wolk et al. (1999) (Nurses' Health Study) cohort USA	To examine the association between long term intake of dietary fibre from different sources and risk of CVD in women	68,782 women aged 37-64 years; validated FFQ (116), diet assessed at least 3 times; AOAC	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre	22.9 vs 11.1 7.7 vs 2.2 9.5 vs 3.6 6.8 vs 1.3	0.76 (0.57-.99) * 0.63 (0.49-0.81) *** 0.93 (0.74-1.16) 1.06 (0.84-1.32)	Increment of 10 g/d and 5 g/d of dietary fibre and cereal (respectively) might be effective in reducing the incidence of CVD
Todd et al. (1999) (Scottish Heart Health Study) UK	To investigate the relation between fibre intake and the incidence of CVD in Scottish population	11,629 men and women aged 40-59; validated semi-quantitative FFQ (60); Englyst and Southgate	Total dietary fibre (M) Total dietary fibre (W)	11 vs 7 13.5 vs 8.3	0.48 (0.25-0.90) * 0.90 (0.82- 0.99) *	This result might provide evidence that intake of dietary fibre is protective against the risk of CVD incidence

Appleby et al. (1999) (Oxford Vegetarian Study) UK	To evaluate the relation between fibre intake and the mortality from CVD in the study subjects	11 140 UK, volunteer cohort (half vegetarian), both sexes, aged 16-79 years; Simple validated FFQ; Southgate	Total dietary fibre	< 18.0 vs ≥ 32.0	2.25 (0.92-5.53)	No relationship observed between fibre intake and CVD mortality
Liu et al. (2002) Women's Health Study (WHS) cohort USA	To assess the association between higher intake of dietary fibre and the risk of CVD in US women	39, 876 women aged ≥ 45 years; validated semi-quantitative FFQ (131); AOAC	Total dietary fibre	26.3 vs 12.5	0.65 (0.51-0.84) ***	Findings support recommendation of high intake of dietary fibre-rich whole grains and fruits and vegetables as a primary preventive measure against CVD
Bazzaro et al. (2003) (NHNESI) cohort study USA	To examine the relationship between total and soluble dietary fibre intake and the risk of CVD	9776 adults 25-74 years; 24 h recall including portion size estimates; unclear, likely to be AOAC	Total dietary fibre Soluble fibre	20.7 vs 5.9 5.9 vs 0.9	0.89 (0.80- 0.99) * 0.90 (0.82- 0.99) *	Dietary fibre and especially soluble showed a protective effect against development of CVD
Mozaffarian et al. (2003) Cardiovascular Health Study; USA	To determine whether fibre consumption from different sources is associated with incidence of CVD in elderly people	3,588 men and women aged ≥ 65 years; validated FFQ (99) diet over previous year; AOAC	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre	16.2 6.3 vs 1.7 7.5 vs 2.8 9.2 vs 4.2	0.84 (0.66-1.07) 0.79 (0.62-0.99) ** 0.99 (0.78-1.25) 1.08 (0.86-1.36)	Increasing cereal fibre intake late in life is invisibly correlated with incidence of CVD
Streppel et al. (2008) Zutphen 1960-2000 Cohort study Netherland	To study recent and long term intake of dietary fibre in relation to the risk of CVD	1,373 men mean age 77 years; diet history, several times, intake over previous 6-12 months; AOAC	Total dietary fibre	33 vs 21	0.83 (0.70-0.98) *	The incidence of CVD reduced with high intake of dietary fibre. However, the inverse effect of dietary fibre was seen with increased age for long term intake.

Kaushik et al. (2009) (Blue Mountains Eye Study) Australia	To assess the relationship between cereal fibre intake and mortality from CVD	3654 Australians, sampled to represent the region, both sexes; validated FFQ (145); AOAC	Cereal fibre	5.9 vs 8.5	1.22 (0.95-1.58)	Negative association between cereal fibre intake and death from CVD
Eshak et al. (2010b) Collaborative Cohort Study Japan	To investigate the association between dietary fibre of different types and the risk of CVD in Japanese population	23,119 men and 35, 611 women aged 40-79 years; validated FFQ (40); method similar to AOAC	Total dietary fibre (M) Insoluble fibre Soluble fibre Cereal fibre Fruit fibre Total dietary fibre (W) Insoluble fibre Soluble fibre Cereal fibre Fruit fibre	14 vs 6.8 9.2 vs 5.9 3.2 vs 1.3 2.1 vs 1.4 1.7 vs 0.4 13.8 vs 7.4 9.1 vs 6.2 2.4 vs 1.5 1.7 vs 1.1 2.2 vs 0.7	0.81 (0.61-1.09) * 0.48 (0.27-0.84) ** 0.71 (0.41-0.97) * 0.86 (0.64-0.99) * 0.56 (0.35-0.90) ** 0.80 (0.57-0.97) * 0.49 (0.27-0.84) ** 0.72 (0.43-0.99) * 0.77 (0.59-0.98) * 0.40 (0.28-0.78) **	Total dietary fibre, soluble, insoluble, fruit and cereal fibre proved the importance of dietary fibre intake from different food sources in terms of protection against the risk of mortality from CVD
Kokubo et al. (2011) Public Health Centre-Based Cohort Japan	To examine the association between dietary fibre and the risk of CVD in a Japanese population	40,046 men and 46,341 women aged 45-64 years; validated FFQ (138); method similar to AOAC	Total dietary fibre (M) Total dietary fibre (W)	19.9 vs 6.0 21.6 vs 7.8	0.94 (0.74-1.20) 0.65 (0.48-0.87) **	Based on Japan dietary fibre intake recommendation (20-30 g/d), this study gave evidence that high intake of dietary fibre intake might reduce the risk of CVD in the Japanese
Buyken et al. (2010) (Blue Mountains Eye Study) Australia	To examine whether dietary fibre is associated with CVD mortality in older Australian cohorts	3654 Australians, sampled to represent the region, both sexes validated FFQ (145); AOAC;	Total dietary fibre (M) Cereal fibre Fruit fibre Vegetable fibre Total dietary fibre (W) Cereal fibre Fruit fibre Vegetable fibre	36.4 vs 18.4 11.5 vs 3.0 11.1 vs 2.4 13.7 vs 6.0 36.2 vs 19.7 10.9 vs 2.9 11.7 vs 2.8 13.5 vs 6.5	0.88 (0.53-1.46) 0.87 (0.55-1.38) 1.03 (0.61-1.75) 1.01 (0.64-1.60) 0.84 (0.53-1.34) 1.04 (0.67-1.61) 0.61 (0.38-0.99) * 0.88 (0.60-1.30)	Increasing cereal fibre intake later in life among females is invisibly correlated with mortality from CVD

Baer et al. (2011) (Nurses' Health Study) USA	To assess the associations of cereal fibre intake with the risk of CVD	121 700 females, occupational cohort, Aged 30-55 years; validated FFQ (116) administered 3 times; AOAC	Cereal fibre	3.4 vs 5.8	0.84 (0.78, 0.91) *	Increase of 4 g/d of cereal fibre associated with lower risk of CVD
Park et al. (2011) (NIH-AARP Diet and Health Study) USA	To investigate dietary fibre intake in relation to the risk of death from CVD	3 881 22 participants represent general population, aged 50-71 yeas, FFQ (124), intake over previous year AOAC	Total dietary fibre (M) Total dietary fibre (W)	29.4 vs 12.6 25.8 vs 10.8	0.78 (0.73-0.82) *** 0.78 (0.73-0.85) ***	Every 10 g/d increase of dietary fibre intake appeared to lower risk of death from CVD by 24 % to 56 % in men and 34 % to 59 % in women
Chuang et al. (2012) (EPIC) Europe	To assess the relationship between fibre intake and the risk of CVD in a large European prospective study	518 408 Europeans, sampled to represent population, both sexes, aged 25-70 years; semi quantitative FFQ, diet history; AOAC and standardised values	Total dietary fibre	≥ 28.5 vs < 16.4	0.90 (0.88-0.92) *	Increasing daily intake by 10 g/d of total fibre appeared to lower the mortality from CVD
Crowe et al. (2012) (EPIC-Heart) Europe	To assess the associations of total and food sources of dietary fibre with the risk of CVD	306 331 Europeans; semi-quantitative FFQ, diet history; AOAC and standardised values	Total dietary fibre Cereal fibre Fruit fibre Vegetable fibre	< 17.5 vs ≥ 27.5 < 5.0 vs ≥ 11.0 < 2.0 vs ≥ 6.0 < 2.0 vs ≥ 6.0	0.85 (0.73-0.99) * 0.91 (0.82-1.01) 0.94 (0.88-1.01) 0.92 (0.78-1.09)	Each intake 10 g/d of total fibre associated with 15 % lower risk of CVD

Wallstrom et al. (2012) (Malmo Diet and Cancer Cohort) Sweden	To examine the association between intake of macronutrients and dietary fibre and incident of CVD in men and women	8,139 male and 12,535 female participants aged 44-73 years; Interview based diet history method; non-starch polysaccharide	Total Dietary Fibre (M) Total Dietary Fibre (W)	11.4 vs 5.8 (g/1000cal) 12.9 vs 6.5 (g/1000cal)	0.54 (0.42–0.68) **	High fibre intake was associated with lower incidence rates of CVD
Wallstrom et al. (2012) (Malmo Diet and Cancer Cohort) Sweden	To examine the association between intake of macronutrients and dietary fibre and incident of CVD in men and women	8,139 male and 12,535 female participants aged 44-73 years; Interview based diet history method; non-starch polysaccharide	Total Dietary Fibre (M) Total Dietary Fibre (W)	11.4 vs 5.8 (g/1000cal) 12.9 vs 6.5 (g/1000cal)	0.54 (0.42–0.68) ** 0.76 (0.59–0.97) *	High fibre intake was associated with lower incidence rates of CVD
Ward et al. (2012) (EPIC-Norfolk) UK	To compare FFQ and 7DD measurements of fibre intake with the incidence of CHD	25 639 participants represent UK regions of both sexes, aged 40-79 years FFQ and 7DD; Englyst	Total Dietary Fibre	7DD (M) 24.1 vs 9.8 7DD (W) 20.1 vs 8.4 FQQ (M)26.2 vs 11.0 FQQ (W) 27.8 vs 11.8	0.87 (0.80–0.95) ** 0.84 (0.73–0.97) * 0.99 (0.90–1.08) 0.90 (0.80–1.01)	Inconsistency in fibre intake and CHD relationship which could be related to dietary assessment methods. However, dietary fibre intake may be better measured by 7DD than FQQ.
Bernstein et al. (2011) (Nurses' Health Study) USA	To examine the association between intake of cereal fibre and incidence of CHD	72 266 females, aged 30-55 years; validated FFQ (116) administered multiple times; AOAC	Cereal fibre	8.3 vs 2.0	0.65 (0.55-0.76) ***	Increment of 5 g/d of cereal fibre intake was inversely associated with risk of CHD

Threapleton et al. (2013a) (UK Women's Cohort Study) UK	To investigate the association between dietary fibre intake and CVD mortality	35 691 UK volunteer females (one third vegetarian), mean age 51.8 years; Validated FFQ (217); Englyst and AOAC	NSP AOAC Soluble fibre Insoluble fibre Cereal fibre Fruit fibre Vegetable fibre	38.3 vs 14.1 63.0 vs 21.0 16.4 vs 6.4 25.6 vs 8.4 15.7 vs 2.8 9.5 vs 1.4 9.5 vs 2.3	0.92 (0.80-1.05) 0.91 (0.76-1.08) 0.89 (0.75-1.07) 0.95 (0.84-1.07) 0.99 (0.89-1.09) 1.00 (0.90-1.11) 0.93 (0.84-1.02)	Total dietary fibre (NSP/AOAC) and fibre from different food sources showed no relationship with the risk of CVD

M = Men; W = Women; EPIC=European Prospective Investigation into Cancer and Nutrition; Association of Official Analytical Chemist (AOAC); FFQ=food frequency questionnaire; 7DD = 7 days' diet diaries; NIH-AARP=National Institutes of Health-American Association for Retired Persons Diet and Health Study; * ($P < 0.05$); ** ($P < 0.01$), *** ($P < 0.001$).

These results showing a protective effect of dietary fibre were also supported by one of the most comprehensive studies on dietary fibre and CVD, which included a pooled analysis of cohort studies investigating 336,244 people and 2,506,581 follow-up years (Pereira et al., 2004). This study showed that, per 10 g/d increased intake, total dietary fibre was correlated with a 14 % (RR, 0.86; 95 % CI: 0.78-0.96; p for trend = 0.005) decrease in risk of all CVD events, and a 27 % (RR, 0.73; 95 % CI, 0.61-0.87; p for trend < 0.001) decrease in risk of CVD death. Fruit fibre intake, per 10 g/d increase, was correlated with a reduction in all CVD events (RR, 0.84; 95 % CI: 0.70-0.99; p for trend = 0.04). Per 10 g/d increase of cereal and fruit fibres there was a correlated with the reduced risk of CVD death (RR, 0.75; 95 % CI: 0.63-0.91; p for trend = 0.003, RR, 0.70; 95 % CI: 0.55-0.89; p for trend = 0.004, respectively), which shows overall how effective the increase in the different fibre types can be for reducing CVD risk.

Total serum cholesterol is composed of low density lipoprotein (LDL) cholesterol (60-70 %) and high density lipoprotein (HDL) cholesterol (20-30 %) (Antonopoulos, 2002). Prospective studies among Western countries have shown a significant association between triglyceride concentration and CVD risk (Sarwar et al., 2007). Iso et al. (2001) stated that a 1 mmol/l increase in plasma triglyceride was significantly associated with CVD risk in women, with a RR of 1.42 (95 % CI: 1.15-1.75; p = 0.001), and in men a RR of 1.29 (95 % CI: 1.09-1.53; p = 0.004) (Iso et al., 2001). The Atherosclerosis Risk in Communities (ARIC) Study among 12,339 participants free of CVD showed that concentrations of LDL-cholesterol > 100 mg/dl was a predictor of CVD incidence (Sharrett et al., 2001).

Increased consumption of viscous soluble fibre has been recommended a therapeutic lifestyle change to reduce the risk of CVD. In 2002 the National Cholesterol Education Program Adult Treatment Panel III (ATP III) reported that intake of 5-10 g/d of viscous fibre might reduce LDL-C by 5 % (Antonopoulos, 2002). Observational and clinical trial studies have also documented that different types (total, soluble and insoluble) and sources (cereal, fruit, vegetable, nuts and seeds) of dietary fibre are associated with reduction in the risk factors of CVD especially LDL, and nine studies have investigated the relationship between dietary fibre and changes in serum lipids (Table 1.8). Seven of these nine (78 %) studies showed a protective effect of dietary fibre intake against the risk factors of CVD by altering serum LDL. Similarly, Brown et al. (1999) conducted a meta-analysis of 67

controlled trials to quantify the cholesterol lowering effect of soluble fibre, and showed a significant relationship between soluble fibre and reduced cholesterol levels and their protective effect against CVD. Overall these results show positive relationship between dietary fibre intake and reduce the risk of CVD.

1.10.2.1 Mechanisms behind health effect of NSPs on CVD

The exact mechanism by which these NSP components, especially water soluble NSP, affect the risk of CVD has been the focus of many investigations and various hypotheses have been proposed to explain these effects (Eshak et al., 2010a, Behall et al., 2006a). Evidence suggests that water soluble NSP may initiate lipid and/or bile acid metabolism, Which would cause a reduction in serum lipid levels (Kritchevsky and Story, 1974, Eastwood and Hamilton, 1968). Other research has suggested mechanisms which include the inhibition of hepatic cholesterol synthesis by SCFA products, and the delayed absorption of macronutrients leading to increased insulin sensitivity (Naumann et al., 2006).

1.10.2.2 Effects from SCFA produced by fermentation in the colon

Water-soluble NSP escapes absorption in the small intestine and are fermented in the large bowel by colonic bacteria. This fermentation results in the production of SCFA, such as acetate, propionate, and butyrate, and it has been hypothesized that production of SCFA, and in particular changes in the propionate: acetate ratio, may influence lipid metabolism. Bridges et al. (1992) examined the effects of oat bran and wheat bran on serum SCFA concentrations in 20 hypercholesterolemic men, they found that oat bran decreased serum cholesterol concentrations by 12.8 %, whereas wheat bran had no effect. Serum acetate values were significantly higher in subjects fed the oat bran diet when compared with the wheat bran diets. This would suggest that increased colonic SCFA production lowers LDL cholesterol concentrations.

1.10.2.3 Interference with lipid and/or bile acid metabolism

The Food and Drug Administration (FDA) has accepted that there is sufficient evidence to show the rate of bile excretion was increased through soluble NSP intake, therefore, reducing serum total and LDL cholesterol (Story et al., 1997). Evidence suggested that some water soluble NSPs implement this effect by binding

bile acids during formation of micelles in the intestinal lumen (Eastwood and Hamilton, 1968, Kritchevsky and Story, 1974). Water soluble NSP may also increase the unstirred water layer in the intestinal lumen, which leads to a decrease in the absorption of fats, including cholesterol and bile acids, which may lead to an increased faecal output of these two components (Theuwissen and Mensink, 2008b). Following this the hepatic conversion of cholesterol into bile acids increases; the hepatic pool of free cholesterol decreases and reaches a new steady state endogenous with increased cholesterol synthesis. This leads to increased activity of 7-α-hydroxylase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase to compensate for the loss of bile acids and cholesterol from the liver stores; hepatic LDL cholesterol receptors become up-regulated to restore hepatic cholesterol stores, which will lead to a decreased serum LDL cholesterol concentration (Hosseini et al., 2011, Flint et al., 2012).

Human studies have examined the effects of water soluble fibres on bile acid excretion, for example Kirby et al. (1981) fed eight hypercholesterolemic subjects 100 g of oat bran in their test diet for 10 days. The results showed that the oat bran diets induced reductions in serum total cholesterol concentrations by 13 % ($p < 0.01$, $N = 8$) and plasma LDL cholesterol concentrations were 14 % lower ($p < 0.05$); while HDL cholesterol concentrations were not changed compared to the control diet. Faecal excretion of total bile acids was higher by 54 % ($p < 0.001$) on an oat-bran diet than on a control diet.

In another study to investigate the effect of soluble fibre on bile acids conducted by Lia et al. (1995), nine ileostomy subjects were provided with four diets in a random order, with each diet for 2 consecutive days. Four different kinds of bread, mainly prepared from oat bran (OB diet, 12.5 g β-glucan /d), oat bran with beta-glucanase (OBE diet, 3.8 g β-glucan /day), barley (B diet, 13.0 g β-glucan /d), or wheat flour (W diet, 1.2 g β-glucan /d) were added to a basal diet. It was observed that the 24 h excretion of bile acids was 53 % higher in the OB diet period than in the OBE diet period ($p < 0.05$), and also was significantly higher than in the B and W diet periods ($p < 0.05$). The excretion of cholesterol was significantly higher in the B diet period than in the OBE and W diet periods ($p < 0.05$).

In related research, Andersson et al. (2002) examined whether consumption of β-glucan from oat bran increased bile acid synthesis within hours of consumption, as

measured by the serum 7α-hydroxy-4-cholesten-3-one (α-HC) concentration, in response to the loss of bile acids from the liver. In a randomized single-blind, wheat bran controlled study with crossover design, 8 subjects were provided with a controlled diet during 2 periods of 3 days each, with an 11-day washout between the periods. Breakfast test diets were included with either 75 g of extruded oat bran, of which 11 g was β-glucan, or 75 g of wheat bran, of which 1 g was β-glucan. The serum α-HC concentrations after 8 and 12 h of the oat bran diet period was higher by 84 % ($p = 0.012$) and by 92 % ($p = 0.017$) (respectively), than that before breakfast. This showed that α-HC in serum could be used as a marker of increased bile acid excretion induced by the diet. Also, Naumann et al. (2006) observed decreased cholesterol absorption and increased cholesterol synthesis in hypercholesterolemic subjects, as measured by the same markers. In ileostomy subjects, Ellegård and Andersson (2007) reported that 75 g of oat bran with 11.6 g of native β-glucan increased bile acid excretion within 24 h of consumption, compared to 4.5 g of hydrolysed β-glucan, and this increase was accompanied by a rise in the serum concentrations of α-HC. Overall these results suggest that oat bran has an effect on bile acid excretion, and that a-HC is a suitable marker for detecting diet induced changes in cholesterol.

Table 1.8: Characteristics of interventional studies on Dietary fibre intake and risk of CVD.

Reference & study design	Study population	Dietary intervention	Observation and effect	Conclusion
Estruch et al. (2009) RCT PREDIMED study Spain	772 high risk subjects (348 men and 424 women) aged 55-80 years, followed for 3 months, fasting blood sample and urine obtained.	Food-frequency Questionnaires (FFQ) Include 137 food items. Subjects assigned to Mediterranean (Med) diet plus virgin oil, Med-diet and nuts or control (low fat) diet, and dietary advice given to subjects based-on diet model.	Total dietary fibre ranged from 16 g/d in the lowest quintile to 26 g/d in the highest. Inverse association in total cholesterol (P = 0.04) and positive association in HDL-C (P = 0.02) observed among those with 20% increment of dietary fibre intake.	Mediterranean diet with recommended amount of dietary fibre might be protective against the risk CVD through its inverse effect on the lipids profile.
King et al. (2007) Randomized cross-over trial USA	28 women and 7 men (18 lean normotensive and 17 obese hypertensive), fasting blood sample drowned before and during study also weekly urine collection.	Cross-over design 2×3 weeks' dietary intervention either high fibre (30 g/d) DASH diet or fibre supplemented diet (30 g/d of psyllium). Calories composition of both diets was 50 % CHO, 34 % fat and 16 % protein.	Significant reduction in Creative protein (CRP) level among both naturally high fibre diet (4.4-3.8 mg/dl; P = 0.046) and fibre supplemented diet (4.4-3.6 mg/dl; P = 0.003). Lean subjects had significant reduction in CRP level than obese.	This study confirmed the recommendation of dietary fibre intake (25-35 g/d) that might reduce the risk of CVD.
Sola et al. (2007b) Randomized cross-over controlled single blind trial Spain	28 participants with CVD aged < 75 years, fasting blood sample obtained before beginning of the study and at the end of each period.	Cross-over design 2×8 weeks' dietary interventional trial either 10.5 g/d of plantago ovata husk (soluble) or plantago ovata seeds (insoluble) fibre diets. The two diets were isocaloric (30 % of energy from fat).	Beneficial effect of P. ovata husk shown through a significant reduction of plasma triglyceride (TG) (6.7 %; p < 0.02), the ratio of apo B: apo A-I decreased (4.7 %; p < 0.02) and apo A-I increased (4.3 %; p < 0.01). HDL-C significantly increased by (6.7 %; p= 0.006), decrease in total cholesterol: HDL-C and LDL: HDL cholesterol in soluble fibre compared to insoluble one.	High intake of soluble fibre provided a favourable reduction effect on the lipoprotein profile among CVD patients.

Chen et al. (2005) RCT USA	110 subjects aged 30-65 years, 24-dietary recall and fasting blood sample obtained at baseline and at the 12-week follow-up visit.	54 subjects received 8 g/d of soluble fibre as a supplement and 56 subjects were assigned as a control group.	After one month of high soluble fibre diet, no significant change on serum lipids observed.	Intake of 8 g/d of soluble fibre for one month was not enough to provide induced effect on lipids profile.
Behall et al. (2006a) RCT USA	18 moderately hypercholesterolemic men aged 28-62 years, fasting blood sample obtained at baseline and weekly during each period of study.	Subjects consumed controlled diet Step 1 (30 % fat, 55 % CHO and 15% Pro), followed by one month of interventional diet, either low (< 0.4 g/d) or med (3g/d) or high (6 g/d) of soluble fibre. The three diets contained the same amount of fibre 30 g/d and calories 2800 cal/d.	Low, med and high soluble fibre significantly associated with reduction of total cholesterol by 20 % (225-186.2 mg/dl), LDL-C by 24 % (153.3-116.1 mg/dl) and increased HDL-C by 18% (38.2-41 mg/dl).	This study was able to provide evidence to support the hypothesis that water-soluble fibre is protective against the risk of CVD through lowering serum lipids.
Keogh et al. (2003) Randomized controlled cross-over trial New Zealand	18 mild hyperlipidaemic 18-65 years old, fasting blood and urine samples were routinely collected on day 0, 1, 7, 14, 21, 28, 29 in both groups.	Cross-over design 2×4 weeks' dietary interventional trial. Subjects were assigned to either β -glucan enriched diet (8.1-11.9 g/d) or control diet (isoenergetic dose of 6.5-9.5 g/d of glucose).	No significant association observed after four weeks of high soluble fibre diet.	High intake of soluble fibre for 4 weeks was not enough to show beneficial reduction effect against CVD risk factors.
Jenkins et al. (2002) Randomized controlled cross-over trial Canada	68 hyperlipidaemic (37 men and 31 postmenopausal women) aged 33-82 years; fasting blood sample collected at baseline and week 2 and 4 of each phase.	Cross-over design 2×4-week dietary interventional trial. Either 4 servings of high fibre diet (8 g/d) of psyllium or β -glucan as soluble fibre or control diet without fibre supplement. Both diets were isocaloric and almost consist of the same amount of total fibre.	Significant reduction observed in total cholesterol ($p = 0.003$), total: HDL-C ratio ($p = 0.001$) and LDL-C: HDL-C among high soluble fibre group.	This study supports the protective effect of soluble fibre against CVD risk factors.

Wu et al. (2007) EPIC Norfolk Cross-sectional Europe	22,915 men and women aged 45-75 years, FFQs Includes 131 food items Non-fasting blood sample	Average intake of dietary fibre is 18 g/d.	Dietary fibre intake inversely associated with total cholesterol regression coefficient (β) – 0.03 (95 % CI: -0.052--0.01; P = 0.001), LDL-cholesterol, β – 0.03 (95 % CI: -0.052- -0.01; P < 0.001), triglyceride, β – 0.04 (95 % CI: -0.059- -0.023; P < 0.001), and positively with HDL-cholesterol, β 0.01 (95 % CI: 0.012--0.025; P < 0.001).	Dietary fibre intake (18 g/d) might reduce the incidence of CVD through association with serum lipids.
Lairon et al. (2005) Cross-sectional France	2,532 men and 3429 women aged 35-60 years, 24-hour dietary recall, fasting-blood sample collected	For men TDF 16-27.3 Insoluble 12.8-22.1 Soluble 3.1-5.2 For women TDF 13.4-22 Insoluble 10.6-17.7 Soluble 2.7-4.4	In both sexes, total dietary fibre intake inversely associated with total cholesterol, OR 0.68 (95 % CI: 0.53-0.87; P for trend = 0.01) and triglyceride, OR 0.68 (95 % CI: 0.48-0.96; P for trend = 0.01). Insoluble fibre inversely associated with triglyceride, OR 0.65 (95 % CI: 0.46-0.92; P for trend = 0.01).	High intake of dietary fibre and insoluble fibre might be protective against development of CVD through lowering serum lipids.

1.10.2.4 Reduced insulin stimulation of hepatic lipogenesis

DF, especially water soluble fibres, may lower the rate of glucose absorption, because they cause an increase in intestinal viscosity in the intestines (Würsch and Pi-Sunyer, 1997). The lowered postprandial glucose concentrations lead to lowered postprandial insulin concentrations, and decreased insulin-stimulated hepatic HMG-CoA activity, and hence cholesterol synthesis (Kimura et al., 2014). Changes in other hormones may also contribute to the decreased insulin concentrations after water-soluble fibre consumption. Whether these hormonal changes result in lowered fasting LDL cholesterol concentrations has, however, never been demonstrated in humans (Theuwissen and Mensink, 2008b). Therefore, further study into the mechanisms involved is required

1.11 Physiological effect of RS

Recently, RS has received much attention for both its potential health benefits and functional properties (Fuentes-Zaragoza et al., 2010). RS is one of the most abundant dietary sources of FC, and could be as important as NSP in promoting large bowel health and preventing CRC, but with a smaller effect on lipid and glucose metabolism (Fuentes-Zaragoza et al., 2010). The protective mechanism of RS could be implemented through butyrate production as a result of colonic fermentation (Topping and Clifton, 2001).

1.11.1 Effect of RS fermentation on colon cancer diseases

The incidence of CRC and other large bowel diseases in South African black people is less than in white people (Walker et al., 1973). This is despite the fact that the DF intake of urban blacks was lower than that in whites and decreased from 25 – 30 g/day to 15 – 20 g/day. This paradox may be linked to the fact that the starch intake of urban black people was higher, 212 and 162 g/d compared with 172 and 118 g/d in black and white men and women, respectively, and, for rural black people, the intake of starch might be even higher (Ahmed et al., 2000). Many black households cook maize porridge, the main provider of starch in the diet, more than once per day, and this maize porridge is sometimes consumed reheated or cold, especially in rural areas. It is believed that cold maize porridge contains large amounts of RS, and this might explain the variation in colon cancer rates, suggesting RS might quantitatively be more important as a substrate of fermentation than NSP (Ahmed

et al., 2000). In recent studies RS was considered as the major substrate for colonic butyrate production, and butyrate also showed a protective effect against colorectal cancer (O'Keefe et al., 1999).

Moreover, in an international survey carried out by Cassidy et al. (1994) that compared the intake of starch, NSP, protein and fat with CRC incidence in 12 populations worldwide, an inverse association between starch consumption and large bowel cancer incidence was seen (large bowel r = - 0.70, colon r = - 0.76). The association was not significant for NSP alone, whereas the association with large bowel cancer incidence was still significant when NSP was combined with RS to give an estimate of fermentable carbohydrate (large bowel; r = - 0.52, colon r = - 0.60). Even after adjusting for confounding factors (fat and protein), the relationships between RS with NSP and cancer incidence remained statistically significant. In this study the authors assumed that this protective effect of starch may be the result of RS consisting of 5 % of all starch consumed. The strong inverse association observed suggests an important role for starch or RS in protecting against CRC, and corresponds with the hypothesis that fermentation of FC in the colon could be the mechanism for preventing CRC.

In addition, combining RS with an insoluble NSP such as wheat bran, resulted in much higher levels of SCFA, particularly butyrate, observed in the faeces (Leu et al., 2002). A study that combined RS with psyllium and fed it to rats showed that this resulted in the site of RS fermentation being pushed more distally, and as the distal colon is known to be the site at which most tumours arise, this may provide additional protection from cancer if RS fermentation is actually protective (Morita et al., 1999).

1.11.2 Effect of RS fermentation on glucose metabolism

One of the main benefits of RS is that it behaves like soluble fibre without sacrificing palatability and mouth feel; RS combines the health benefits of dietary fibre/whole grain with the sensory feeling of refined carbohydrates (Higgins, 2004). The glucose metabolism of starchy foods may depend upon different factors, such as the amylose/amylopectin ratio, the native environment of the starch granule, the gelatinization of starch, the water content and also baking temperature of the processed foods (Champ et al., 2003). Several human studies involving RS have

shown a decrease in postprandial blood glucose and insulin levels, but it is still difficult to completely understand these effects due to differences in study design and the type of RS used (Lattimer and Haub, 2010). Therefore, further research is needed to clarify the effects and their mechanisms of action.

One of these studies by Behall et al. (2006b) reported that women consuming 0.71 g, 2.57 g or 5.06 g of RS had significantly lower postprandial glucose and insulin levels when compared to the control. However, this study was not able to maintain an equal amount of available carbohydrate between the treatments and control, therefore it is difficult to determine whether the attenuation of glucose and insulin was due to the RS, or the fact that there was less available carbohydrate in the meal for the treatment group. Similarly, Reader et al. (2002) showed that 7.25 g of RS added to an energy bar lowered blood glucose and insulin levels in healthy adults, however the ingredient amounts and nutrient levels were different for each treatment, which makes the results difficult to compare. In contrast to this recently Al-Tamimi et al. (2009) removed non-starch ingredients and available carbohydrates in their study and reported that postprandial blood glucose and insulin levels were significantly decreased with the supplementation of 30 g of RS4.

Studies have suggested that consumption of food high in RS may moderate the rate of digestion and control glucose tolerance, as RS metabolism occurs 5 - 7 h after consumption, compared to normally cooked starch which is digested almost immediately (Reader et al., 1997). This extended period of digestion may reduce postprandial glycaemia, and may also have a beneficial effect on increasing the period of satiety (Raben et al., 1994, Reader et al., 1997). There have been a number of studies on the effects of different forms and doses of RS on glucose and insulin responses, but overall the data are contradictory (Sharma et al., 2008). A good example of a positive result come from a study of 10 healthy normal-weight males that showed consumption of a meal containing high RS (54 %) significantly reduced the postprandial concentration of blood glucose, insulin, and epinephrine, compared to a meal containing 50 g of starch free of RS (0 % RS) (Raben et al., 1994). Moreover, another study reported that intake of a meal containing a commercial RS3 ingredient reduced the peak glucose response significantly more than the other carbohydrates investigated (simple sugars, oligosaccharides, and common starch) (Reader et al., 1997). Overall more research needs to be conducted to ascertain the action effect of RS on glucose levels.

1.11.3 Effect of RS fermentation on lipid metabolism

RS has been observed to affect the metabolism of LDL, cholesterol, triglycerides, triglyceride-rich lipoproteins and total lipids, and RS intake has been reported to lower the serum cholesterol level in animals (Lopez et al., 2001). In rats, RS diets (25% raw potato) increased SCFA production, absorption and lowered plasma cholesterol and triglycerides (Sajilata et al., 2006). Another feeding trial on rats used RS from Adzuki bean starch (AS) and Tebou bean starch (TS) and the results suggested that AS and TS have a serum cholesterol-lowering function due to enhanced levels of hepatic SR-B1 (scavenger receptor class B1), and cholesterol 7alpha-hydroxylase mRNA (Han et al., 2003). Other studies have shown that RS was not able to lower fasting serum lipid concentrations in healthy subjects (Heijnen et al., 1996, Achour et al., 1997). Research has suggested that the effect of RS diets in lowering lipid levels in animals may not be applicable to humans (Asp et al., 1996). Overall studies suggest the beneficial effects of RS are more similar to those of insoluble dietary fibre than to the cholesterol-lowering action of soluble fibres (Kritchevsky and Story, 1986, Anderson et al., 1984).

Further evidence of the benefit of RS comes from several studies that showed the longer term consumption of RS might decrease fasting cholesterol and triglyceride levels, for example in a five week study, Behall et al. (1989) found that men consuming 34 % of their energy from high amylose maize, had significantly reduced fasting cholesterol and triglyceride levels, when compared to a high amylopectin carbohydrate. Resier et al. (1989) also stated similar findings in an iso-caloric and iso-nutrient diet with either high amylose maize or fructose. Porikos and Van Itallie (1983) have proposed that an interaction exists between sucrose, and also most likely fructose, and saturated fatty acids, which in turn alter serum triglyceride levels; but remarkably, the relationship does not seem to exist for polyunsaturated fatty acids. The possible mechanism behind the ability of RS to decrease cholesterol levels might be an increased intestinal viscosity. Some studies, such as Jenkins et al. (1998), have reported conflicting data with RS2 and RS3, showing no effect on serum lipid profiles, however, using the same type of RS, subjects were only tested for two weeks, and therefore it is possible that the RS required a longer period of time to produce an effect.

1.11.4 Effect of RS fermentation on faecal bulking and bowel transit time

Several studies have observed positive effects of RS supplementation on reducing transit time and increasing faecal bulk (Cummings et al., 1996, Phillips et al., 1995, Jenkins et al., 1998). The faecal bulk is increased in humans eating mixed diets, and is believed to be higher than predicted from their NSP content in a ‘’carbohydrate gap’’, and (Topping and Clifton, 2001).

Over a period of 15 days Cummings et al. (1996) fed controlled diets, which included glycaemic starch, RDS, wheat bran (NSP), RS2 (from native potato and green banana) and an RS3 (retrograded high amylose corn starch), to 12 healthy human subjects. It was observed that overall RS significantly increased faecal bulk and faecal excretion of SCFA, with RS2 (native potato and green banana) having increased faecal bulk by 1.6 and 1.7 g/d, respectively (Cummings et al., 1996). A similar study by Phillips et al. (1995) fed 11 healthy subjects mixed diets (unprocessed wheat seeds, high amylose corn starch and green banana) high in RS (5g/d) over a period of 4 days. They observed that the high RS diet increased faecal output, lowered pH, and increased the daily excretion of butyrate. Similar findings have been observed by Jenkins et al. (1998).; the authors reported that faecal output on the wheat bran supplementation (258 ± 22 g/d) was significantly higher than the three other substrates (RS2, 187 ± 24 g/d; RS3, 182 ± 23 g/d; low fibre control, 163 ± 23 g/d). The faecal output in RS2 (24 ± 9 % g/d) and RS3 (29 ± 14 % g/d) supplemented diets were also significantly increased compared with the low fibre control diet. Overall these results show that RS fermentation significantly increased faecal bulk and faecal excretion of SCFA.

Further evidence of the effect of RS comes from a study by Hylla et al. (1998) who fed 12 healthy volunteers over a period of 2 x 4 weeks, a controlled basal diet enriched with either amylomaize starch (55.2 ± 3.5 g RS/d; high RS diet) or available corn starch (7.7 ± 0.3 g RS/d; low RS diet). Almost 90 % of the RS eaten disappeared in the intestinal passage, and they reported that a high RS diet significantly increased faecal wet and dry weight by 49 % and 56 %, respectively ($p \leq 0.005$), while stool water content was not changed significantly (Hylla et al., 1998). The values for increased faecal bulk by RS were less than that after condition of wheat bran fibre (4.9 % g/g of fibre) and represented only 32 % - 55 % of faecal bulk

(Cummings et al., 1996). The amount of RS excreted might be greater when compared to excretion of standard dietary starch as less RS is metabolised within the body compared with digestible starch (Jenkins et al., 1998). In humans, an undigested high RS diet was observed to produce as much as 10 % of the faeces weight, while the excretion of digestible starch in the faeces is generally insignificant (Cummings et al., 1996).

1.11.5 Effect of RS fermentation on mineral absorption

The ileal absorption of a number of minerals in rats and humans has been shown to be influenced by RS; for example, Lopez et al. (2001) and Younes et al. (1995) have observed an increased absorption of calcium, magnesium, zinc, iron and copper when rats were fed RS-rich diets, whereas in humans, these effects might be limited to calcium (Trinidad et al., 1996, Coudray et al., 1997). In pigs, Morais et al. (1996) compared the apparent intestinal absorption of calcium, phosphorus, iron, and zinc in the presence of either resistant or digestible starch, and showed that a meal containing 16.4 % RS resulted in a greater apparent absorption of calcium and iron, when compared with completely digestible starch. This increase in absorption by RS can be offset by the fact that wholemeal bread and bran contain phytate, which is a known inhibitor of mineral absorption (calcium, magnesium, zinc and iron) in humans (McCance and Widdowson, 1949, Andersson et al., 1983). In the present of mucosal phytase and alakaline phosphatase in the human small intestine, almost no digestion of phytate happened, while the dietary phytase may be an important factor for phytate hydrolysis (Sandberg and Andersson, 1988).

Most mineral absorption arises in the small intestine and, unlike viscous fibre, RS would not be expected to affect small intestinal absorption; but a significant proportion of mineral absorption occurs in the large intestine, and fermentation of RS may influence mineral absorption here (Lopez et al., 2001). RS fermentation in the large intestine could affect mineral absorption through lowering the pH which would make the minerals soluble and more absorbable. SCFA production might also increase the size and mucosal surface area of the proximal colon resulting in increased absorption capacity (Sajilata et al., 2006).

1.12 Physiological effect of NDOs

As discussed in section (1.5), the NDOs have several important physiological effects including increasing satiety and modulating lipid metabolism, several of which are shared with any fermentable fibre. However they also act as prebiotics act as a fermentable substrates within the colon, stimulating the preferential growth and activity of a limited number of microbial species that confer health benefits on the host (Gibson et al., 2004). FC with established prebiotic properties include inulin-type fructans (inulin, oligofructose and FOS) and GOS, which are known to improve the proliferation of beneficial lactic acid-producing species such as *bifidobacteria* and *lactobacilli* (Roberfroid et al., 2010).

1.12.1 The effect of NDOs on satiety

Several studies support the relevance of prebiotic fermentation in appetite management in humans (Cani et al., 2009, Cani et al., 2005). In a single-blinded, crossover, placebo-controlled design study, Cani et al. (2005), examined the effects of oligofructose (OFS) on satiety and energy intake in five men and five women aged 21 – 39 years, ingesting either 16 g of OFS/d or 16 g of dextrin maltose (DM) as a placebo for 2 weeks. The authors observed that OFS increased satiety following breakfast ($p = 0.04$), dinner ($p = 0.04$), with a reduced hunger ($p = 0.04$) and prospective food consumption following dinner ($p = 0.05$). It was noted that total energy intake per day was 5 % lower during OFS treatment than in the DM period. These results were confirmed by the same researchers in another study, a randomized, double-blind, parallel, placebo-controlled trial in healthy volunteers (n=10) (Cani et al., 2009). Participants receiving 16 g of soluble fructan/d for 2 weeks showed a 3-fold increase in breath-hydrogen (a marker of gut microbiota fermentation) and a decrease in hunger rates. Furthermore, plasma glucagon-like peptide 1 and breath-hydrogen excretion were significantly correlated ($p = 0.007$) and glucose response was negatively correlated with the breath-hydrogen excretion ($p = 0.02$) (Cani et al., 2009).

1.12.2 The effect of NDOs on lipid metabolism

Some studied have suggested that NDOs can have an effect on lipid metabolism, for example the effect of inulin supplementation on lipid concentrations was examined, in a double-blind, randomized, placebo-controlled crossover design

study conducted by Letexier et al. (2003). Eight healthy subjects were examined twice, receiving a moderately high carbohydrate, low fat diet (55 % of total energy) plus an oral placebo or 10 g of high-performance inulin/d for 3 weeks. The authors found that plasma triacylglycerol concentrations and hepatic lipogenesis were significantly lowered after inulin treatment compared to placebo ingestion ($p < 0.05$), but cholesterol synthesis and plasma cholesterol concentrations were not significantly different between the two groups (Letexier et al., 2003).

In another randomized, double blind, crossover design, participants (n =12) were supplemented with either sucrose or inulin (20 g/d) in one pint of vanilla ice cream for 3 weeks (Causey et al., 2000). They observed that 20 g/d of inulin significantly reduced serum triglycerides by 40 mg/dL ($p=0.05$) and improved the SCFA profile compared to the control (Causey et al., 2000). In addition, a randomized double blind cross-over design was used on young healthy males (n = 22), consuming either 11 % inulin-enriched or control pasta over a period of 2 weeks. The researchers found that HDL-cholesterol concentrations increased by 35.9 %, and total cholesterol/HDL-cholesterol ratio, triglycerides, and Apo-lipoprotein concentrations decreased by 22.2, 23.4, and 16.5 % in the inulin-enriched group compared to control pasta, respectively (Russo et al., 2008). These findings show that inulin supplementation improve lipid profiles. Further evidence of the effect of this supplementation comes from the study by Vulevic et al. (2013) who conducted a double-blind, randomized, placebo (maltodextrin)-controlled, crossover study on overweight adults (n = 45) with ≥3 risk factors associated with metabolic syndrome. Participants received either 5.5 g/d of the placebo (maltodextrin) or GOS supplementation with a mixture of Bi2muno (B-GOS) powder for 12 weeks. They observed that B-GOS supplementation decreased total cholesterol, triglyceride and the total cholesterol/HDL-cholesterol ratio. Overall these results suggest that NDOs fermentation significantly impact on lipid metabolism.

As discussed in this chapter, to evaluate the health benefits of fermentation of NDCs in humans in intervention and epidemiological studies. A suitable method to determine the portion of NDCs that escape digestion and absorption in the small intestine and are fermented in the colon is needed. Determination of SCFA production in human *in vivo* is not feasible or possible directly, and most studies use faecal concentrations which do not represent the full production of SCFA, as most are absorbed higher up in the colon. Most studies measure DF or NSP intake to correlate with health effects but not all DF or NSP are equally fermentable. Epidemiological and intervention studies currently use traditional dietary analysis methods, which depend on food composition databases, that may not have been updated to include NDOs and RS, and so do not provide accurate data on NDCs intake whether or not it is fermented. Moreover, different types of NDCs are fermented to varying extents in the human gut, meaning that the actual amount of FC entering the large intestine is very difficult at present to predict.

1.13 Aims and objectives

The aim of this study was to develop an index of the amount of carbohydrate in diets from which bacteria in the colon produce SCFAs.

The objectives of this study were:

- To develop an index of FC that can be used to estimate, from food diaries, the carbohydrate sources that escape digestion and absorption and are fermented fully to SCFA by the colonic bacteria.
- To test our prediction equations for FC using dietary records from a human study in which urinary SCFA was measured. To test our prediction equations against the SCFA produced by colonic bacteria *in vitro* from a range of dietary fibres, NDCs mixtures and model diets containing different proportions of NDCs.
- To use an *in vitro* enzymatic digestion model for the removal of starch and other digestible macronutrients prior to the *in vitro* fermentation of FC samples
- To use an *in vitro* human colon fermentation model, to study the metabolic capacity of the colonic bacteria when single fibre, mixtures of isolated fermentable carbohydrate diets and different amounts of FC in food mixtures

based on the habitual diets of subjects were fermented, and to test our equations against the SCFA amounts produced from each carbohydrate.

- To compare the SCFA concentration produced via *in vitro* fermentation of FC against the predictable amounts of SCFA using our prediction equations.

2 Chapter Two

General Materials and Methods

2.1 Introduction

This chapter describes the general materials and methods used throughout this thesis. The thesis comprises secondary analysis of a previous study conducted at the Universities of Glasgow and the Scottish Universities Environmental Research Centre (SUERC) which measured dietary intake and urinary SCFA (Chapter 4). This was used to help develop an index of dietary FC (Chapter 3); and the equations developed to predict fermentation in the colon were then further evaluated and validated using a series of *in vitro* studies (Chapter 5).

2.2 Secondary analysis data

2.2.1 Three day weighed record of habitual diet used in secondary analysis

This study used data obtained from a previous project "Validating *in vivo* biomarkers of colonic carbohydrate and protein fermentation" conducted in 2009 at the University of Glasgow and SUERC, which measured dietary intake and urinary SCFA. The study involved monitoring the habitual diet and corresponding urinary excretion of SCFA for three days in 122 participants from the general population, and the results of this were included in this study. Subjects had been asked to weigh and record their normal dietary intake for 3 days (one weekend and two week days), using the provided food scales and food diaries. The original dietary records were reviewed and inspected to ensure that the subjects' diaries were complete, and sufficient detail had been recorded. Initial dietary analysis was carried out using Windiets software version 2005 (Robert Gordon University, Aberdeen, UK). Diet diaries were analysed for daily energy and macronutrient intake in absolute amounts as well as the percentage of energy derived from the main macronutrients. For the work in this thesis these diets were reanalysed for fermentable carbohydrate content using data from a series of equations (Chapter 3). This also entailed a detailed literature review for oligosaccharide content and resistant starch in foods, as well as using websites and other data sources to calculate fibre content determined by different methods.

2.2.2 Database production

The database was developed using a Microsoft Excel spreadsheet; the subjects' diet analysis results were transferred from Windiets into Microsoft Excel. Dietary fibre AOAC (soluble and insoluble), NSP (soluble and insoluble), inulin and oligofructose of different fruits, vegetables and grains obtained from the literature search were entered into Microsoft Excel, along with other content information obtained from supermarket and manufacturers' websites. The amount of dietary fibre AOAC method (soluble and insoluble), NSP Englyst method (soluble and insoluble), inulin and oligofructose present in these products were entered as g/100 g wet weight against the products in the database. The amount of total starch was obtained from Windiets 2005 for each food item in the dietary record.

2.3 *In vitro* digestion model

An *in vitro* digestion model was used to mimic conditions and luminal reactions of the human gastrointestinal tract, which occur in the mouth, stomach, duodenum, ileum, according to the method of Aura et al. (1999). This was used to predigest all carbohydrate sources used for the *in vitro* validation of the fermentable carbohydrate concept (Chapter 5). The method of Aura et al. (1999) was chosen after consideration of the methods available in the published literature (**Table 2.1**); these methods were designed for the measurement of RS (Englyst et al., 1992, Berry, 1986, Muir and O'Dea, 1992, Champ, 1992, Åkerberg et al., 1998, Goñi et al., 1996, McCleary and Monaghan, 2002).

The studies in this thesis required the residue left after an *in vitro* digestion model to be tested in an *in vitro* fermentation model, therefore all digestion products need to be removed from the residue. Dialysis tubing (Biotech Cellulose Ester Membrane cut-off: 500-1000 MWCO; SPECTRUM LABS.COM) was used to remove digested products from the mixture. The retained materials (retentates) were subsequently freeze-dried prior to use in the *in vitro* fermentation model.

Table 2.1: Comparison between different methods to measure resistant starch (RS) in vitro adapted from Laurentin and Edwards (2005).

Method	Preparation	Pre-treatment	Sample Treatment	Post-treatment	Treatment incubation	Types of RS measured [a]
Berry (1986)	Milling	None	Pancreatic α-amylase and pullulanase	KOH [b] Amyloglucosidase	Shaking for 16 h at 37 °C, pH 5.2	Sum of RS2 and RS3
Faisant et al. (1995)	Milling	None	Pancreatic α-amylase	KOH [b] Amyloglucosidase	Shaking for 16 h at 37 °C, pH 6.9	Sum of RS2 and RS3
Goñi et al. (1996)	Milling	Pepsin	Pancreatic α-amylase	KOH Amyloglucosidase	Shaking for 16 h at 37 °C, pH 6.9	Sum of RS2 and RS3
Englyst et al. (1992)	Chewing	Pepsin	Pancreatic α-amylase, amyloglucosidase, and invertase	KOH [b] Amyloglucosidase	Shaking for 2 h at 37 °C, pH 5.2	RS1, RS2, RS3 and total RS
Muir & O'Dea (1992)	Chewing	Salivary α-amylase then pepsin	Pancreatic α-amylase, amyloglucosidase	Thermostable α-amylase Dimethyl sulfoxide[d] Amyloglucosidase and pancreatic α-amylase	Stirring for 15 h at 37 °C, pH 5.0	Total RS
Åkerberg et al. (1998)	Chewing	Salivary α-amylase then pepsin	Pancreatic α-amylase, amyloglucosidase	KOH [b] Thermostable α-amylase Amyloglucosidase	Stirring for 16 h at 40 °C, pH 5.0	Total RS
Aura et al. (1999)	Milling	Salivary α-amylase then pepsin	Bile acid, Pancreatin and mucin	Dialysis tubing	Shaking for 6 h at 37 °C, pH 6.5-7.5	Removing digested starch
McCleary and Monaghan (2002)	Milling	None	Pancreatic α-amylase, amyloglucosidase	KOH [b] Amyloglucosidase	Shaking for 16 h at 37 °C, pH 6.0	Sum of RS2 and RS3

a RS, resistant starch; RS1, physically inaccessible starch; RS2, resistant granules; RS3, retrograded starch. b, KOH and dimethyl sulfoxide are used as resistant starch solubilizing agents.

2.3.1 Mouth

All incubation steps were performed at 37 °C, with stirring and shaking in a water-bath under the same conditions. 1 g of each NDC was weighed into a 100 ml beaker containing mixture of 10 ml distilled water and 7 ml sodium chloride solution (0.85 wt %). In the *in vitro* digestion model used in this thesis, starch hydrolysis in the mouth was simulated in the *in vitro* model using commercial salivary α-amylase (Sigma A-3276) (50 U/50 µl), which was dissolved with sodium phosphate buffer (0.02 M, pH 6.9) and added to the sample in the presence of 10 ml of distilled water and 7 ml of sodium chloride solution (0.85 wt %) (Table 2.2). The samples were then incubated for 5 minutes.

2.3.2 Stomach

In the stomach, gastric glands secrete pepsinogen and hydrochloric acid (HCl) for protein digestion. The optimum pH for pepsin (which is activated pepsinogen) is between 1.8 and 3.5. In our *in vitro* digestion model the conditions of the stomach were simulated by adding HCl (0.15 M, 4 ml), which decreased the sample pH to 2.5. A 2 mg of Pepsin (Sigma P-7000) was dissolved in 1 ml of 0.02 M of HCl/ solution. Then, 1 ml of 0.02 M of HCl acid was added to the sample and blank and then incubated for 2 h. Residence time in the human stomach varies based on the particle size (Guyton and Hall, 1996b), but the incubation times used in the different *in vitro* batch models in the literature were fixed, ranging from 30 min to 2 hours depending on the method (Åkerberg et al., 1998, Muir and O'Dea, 1992, Lebet et al., 1998, Aura et al., 1999). Therefore, 2 h incubation time was selected to mimic stomach digestion condition.

Table 2.2: Composition and preparation of the solutions used for the in vitro digestion model

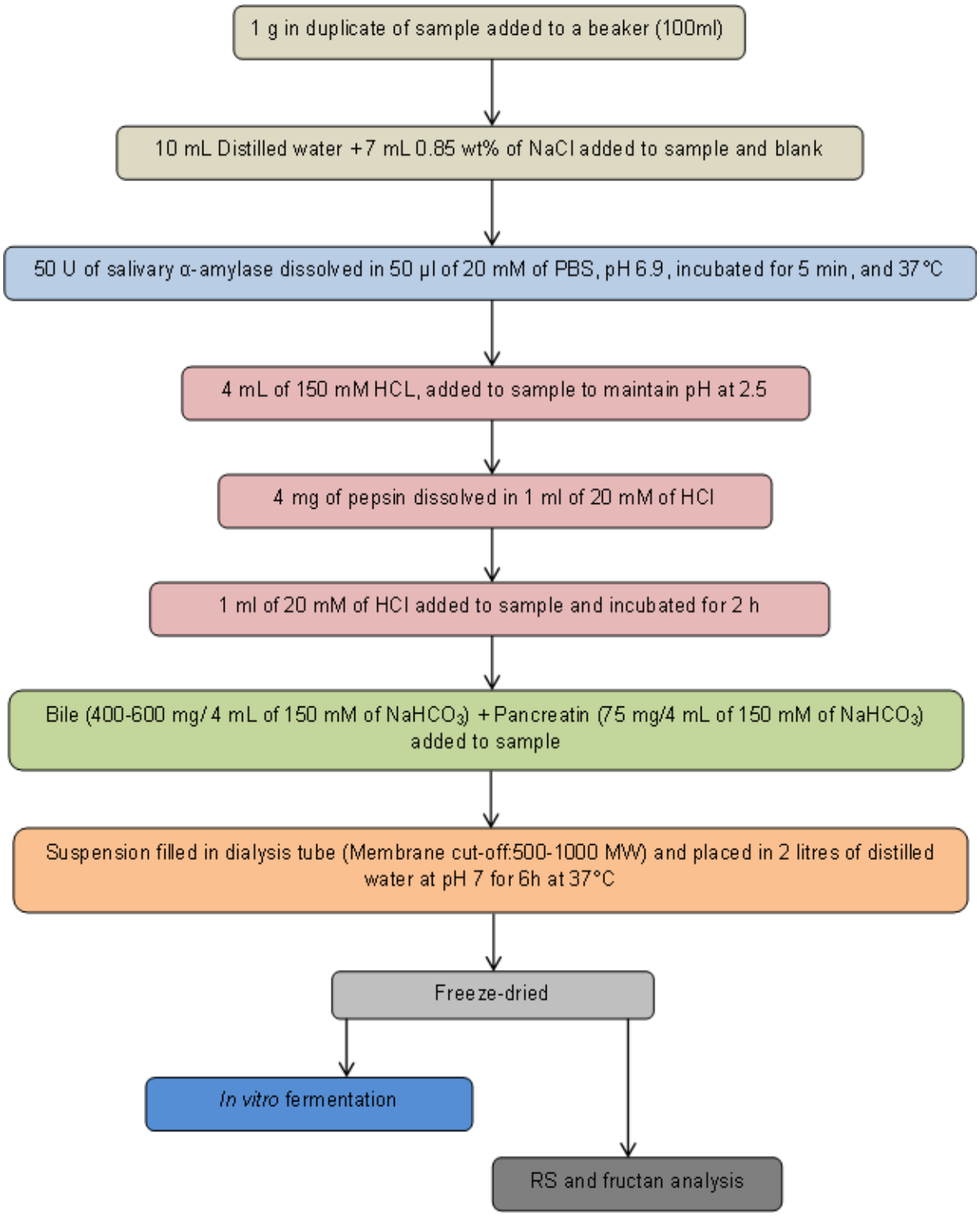
Name	Composition		Preparation
Sodium phosphate buffer (PBS)	0.02 M of KH_2HPO_4 0.02 M of Na_2HPO_4	0.272 g 0.283 g	Distilled water was added to make up 100 ml and stored at 4°C
0.15 M of Hydrochloric acid (HCl) solution	37% HCl	6 ml	Distilled water was added to make up 500 ml and stored at 37°C
0.02 M of Hydrochloric acid (HCl) solution	37% HCl	800 µl	Distilled water was added to make up 500 ml and stored at 37°C
0.150 M of Sodium bicarbonate solution	$NaHCO_3$ (MW 84.0)	2.25 g	Distilled water was added to make up 200 ml and stored at 4°C
0.85 wt % Sodium chloride solution (Normal saline)	Sodium chloride NaCl	4.25 g	Distilled water was added to make up 500 ml and stored at 37°C
Digestion Enzymes	Salivary α-amylase Pepsin Pancreatin Extract bile acid	50 U 2 mg 75 mg 0.4-0.6 g	Dissolved in 50µl of 0.02M of PBS Dissolved in 1 ml of 0.02M of HCL Dissolved in 4 ml of 0.15M of $NaHCO_3$ Dissolved in 4 ml of 0.15M of $NaHCO_3$

2.3.3 Duodenum and ileum

The pH between the duodenum and distal ileum changes to 6.2-7.9 (Gee et al., 1999). The conditions of the duodenum and ileum were simulated in the *in vitro* digestion model, the pH was adjusted to 6.5-7.0 using 6 M sodium hydroxide (NaOH). Pancreatin, a mix of amylase, proteases and lipase, was used mimic pancreatic enzymes released into the dueodenum. Pancreatin (Sigma P-7545) (75 mg) was dissolved in 4 ml of 0.15 M of sodium bicarbonate solution and then added to the sample (**Figure 2.1**). A porcine extract bile acid (Sigma B8631) (400-600 mg/sample) was then dissolved in 4 ml of 0.15 M sodium bicarbonate and added to the sample. The residence time of food in the duodenum and ileum is between 3 and 6 hours, during which time it is digested and absorbed (Guyton and Hall, 1996a). Absorption in the duodenum stage was simulated in the *in vitro* digestion model using a dialysis tube with small molecular weight cut off (35 cm with flat width 31mm, Dia 20 mm; cut-off 500-1000 MWCO; Biotech Cellulose Ester Membrane MWCO;

SPECTRUM LABS.COM) in a 2 L beaker and filled with distilled water at pH 6.5 - 7. The tubing was incubated for 6 h at 37 °C to remove only digested components from the mixture and to retain the non-digestible carbohydrates in the retentates, which was then freeze-dried prior to the *in vitro* fermentation. The dried digested samples were weighed and the sample was then subject to RS, fructan and dietary fibre measurement and/or the *in vitro* fermentation model.

Figure 2.1: In vitro enzymatic digestion model (Aura et al., 1999).



2.4 Measurement of FC fermentation

The freeze dried digested samples were then incubated in the *in vitro* fermentation model. *The in vitro* model of colonic fermentation used in this thesis was established in our laboratories (Jaganath et al. (2009).

2.4.1 Collection of faecal samples

Fresh faecal samples were obtained from volunteer healthy adults, who had not taken antibiotics for at least two months prior to each study. Volunteers were asked to pass an entire bowel movement and collect it using the kit provided (plastic bag inside a pot, using the support of a paper bedpan on the toilet seat). The pot was sealed in a bag with an anaerobic gas kit (Anaerocult® A Merck KGaA 62471 Darmstadt, Germany), to induce anaerobic conditions. An indicator strip (Anaerotest® A Merck KGaA 1151120001 Darmstadt, Germany) was included to ensure anaerobic conditions had been achieved. The stool sample was kept at 0 ^{o}C (in an insulated bag with frozen ice blocks) to slow on going bacterial metabolism, and was transferred to the laboratory within two hours of passage. Fresh faecal samples were used for *in vitro* faecal bath culture fermentation.

2.4.2 Preparation of fermentation medium

All chemicals and reagents used in the fermentation model including medium, buffers, macromineral, micromineral, and reducing solutions were supplied by Fisher Scientific (Leicestershire, UK), Sigma-Aldrich Company Ltd. (Dorset, UK), and BDH AnalaR Laboratory (Dorset, UK) (Table 2.3). Sodium phosphate buffer (SBP) (1/15 M KH2PO4 (9.078 g/L) and 1/15 M Na2HPO4 (11.876 g/L) mixed in a ratio of 1:4) was boiled for 5 minutes and cooled to 37 ^{o}C for 30 minutes under an oxygen-free nitrogen (OFN) atmosphere to reach an anaerobic environment. The basal fermentation medium was prepared according to the method of Jaganath et al. (2009) by mixing 2.25 g of tryptone in 450 ml of distilled water with 112.5 µl of micromineral solution (13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$, 8 g $FeCl_3.6H_2O$, and distilled water to 100 ml). After vortexing, 225 ml buffer solution (2 g NH_4HCO_3, 17.5 g $NaHCO_3$ in 500 ml of distilled water), 225 ml macromineral solution (2.85 g $Na_2HPO_4.H_2O$, 3.1 g $KH_2PO_4.H_2O$, 0.3 g $MgSO_4.7H_2O$ in 500 ml of distilled water) and 1.125 ml of 0.1% resazurin solution, as a redox indicator, were added and the medium was boiled for five minutes to remove oxygen and to sterilize

it. The pH of the medium was adjusted using a hand-held pH meter (Hanna pH instruments, USA) to 7.0 using 6 M HCl before boiling on the hot plate. The medium was then purged with oxygen-free nitrogen (OFN) to create an anaerobic condition, indicated by a colour change from indigo to colourless (**Figure 2.2**). At the same time, a reducing solution was prepared (312.5 mg of cysteine hydrochloride, 2 ml of 1 M NaOH, 312.5 mg Na_2S, and 50 ml of distilled water) and the pH was maintained at 7 by using 6 M HCl.

Each freshly voided human faecal sample was homogenised with sodium phosphate buffer (0.1 M, pH 7.0) in a blender (Braun Multi-quick 3 hand blender, MQ 325 Omelette BmbH; Germany) to make a 32 % (w/v) of faecal slurry (16 g faecal sample with 50 ml sodium phosphate buffer). The slurry was filtered using cotton or nylon mesh to remove large particles. 42 ml of the medium was transferred into 100 ml McCartney bottles and purged with OFN for 1 minute. The reducing solution was then added to the fermentation medium (0.5 ml/10 ml or 2 ml/42 ml of fermentation medium). 1 g of the retentates substrate was then added to the McCartney bottle along with 5 ml of fresh slurry. Each bottle was crimped with a self-sealing rubber cap, and was flushed with OFN for one minute. The samples were then incubated horizontally in a shaking water bath at 37 °C and 50 strokes /min for 24 h. All fermentations were carried out in duplicate apart from the blank, which had everything except the substrate. A 3 ml sample was transferred by syringe an into bijoux tube at 0 h to measure pH, which then was frozen at -20 °C for later SCFA analysis. At 4 h and 24 h, 3 ml of fermented samples were removed and analysed for pH and fermentation products.

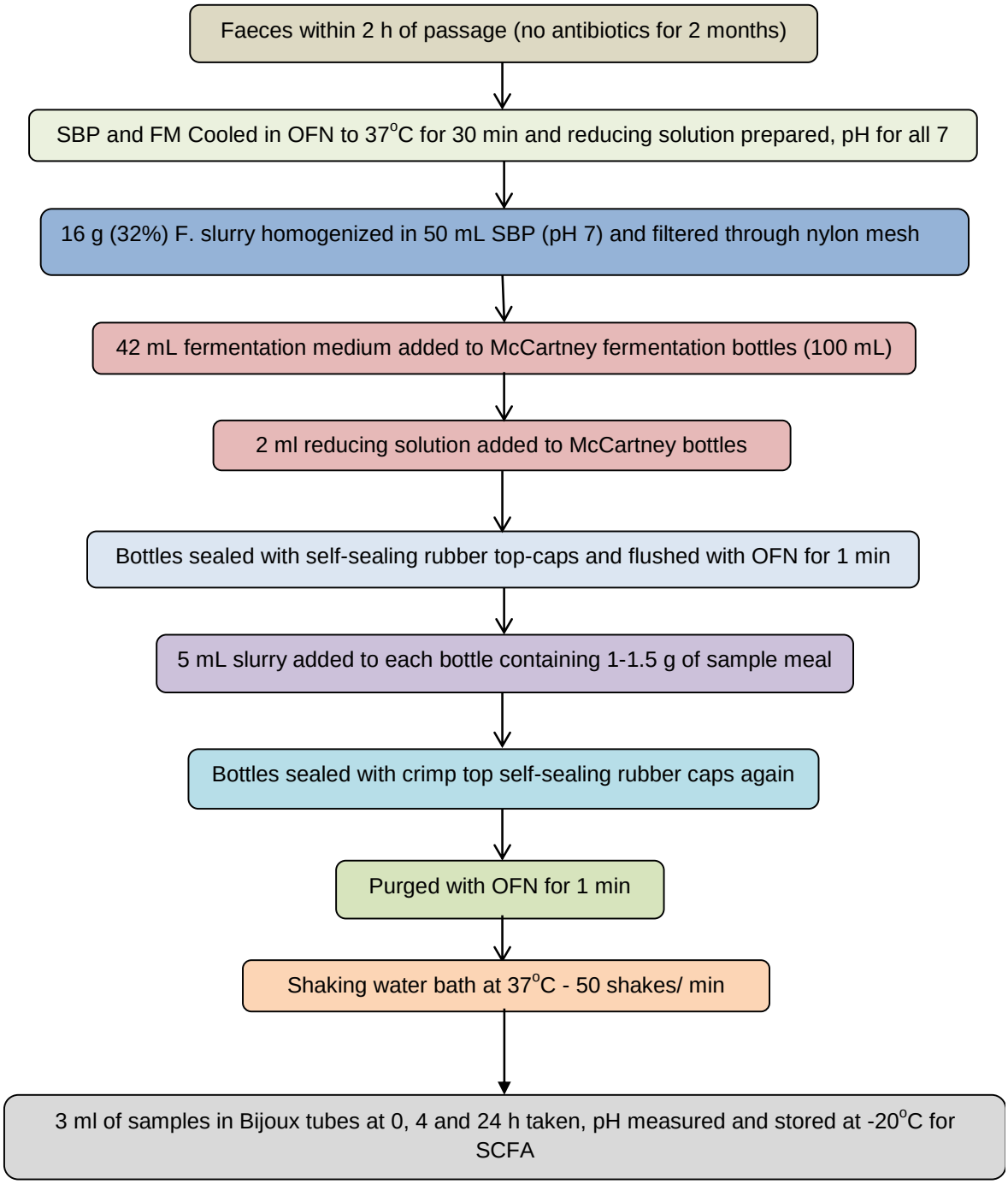
2.4.3 Determination of pH

pH was measured in the fermentation fluids using an auto calibrated portable digital pH meter (Hanna pH20instruments, USA). 3 ml of the fermented medium samples were transferred by syringe into bijoux tubes and the pH determined at different time points (0, 4, and 24 hours). The samples were then frozen at -20 °C for later SCFA analysis after the addition of 1 ml of 1 M NaOH to bring the pH to > 9.0.

Table 2.3: Composition and preparation of the solutions used for fermentation studies (Jaganath et al., 2009).

Solution	Volume	Composition	Preparation
Buffer Solution	500 ml	NH_4HCO_3 $NaHCO_3$ Distilled water Store at 4 ^oC	2 g 17.5 g up to 500 ml
Macro-mineral Solution	500 ml	Na_2HPO_4 or $Na_2HPO_4.2H_2O$ KH_2PO_4 or $KH_2PO_4.2H_2O$ $MgSO_4.7H_2O$ Distilled water Store at 4^oC	2.85 g or 3.57 g 3.1 g or 3.92 g 0.3 g up to 500 ml
Micro-mineral Solution	100 ml	$CaCl_2.2H_2O$ $MnCl_2.4H_2O$ $CoCl_2.6H_2O$ $FeCl_3.6H_2O$ Distilled water	13.2 g 10 g 1 g 8gm up to 100 ml
Fermentation Medium	1000 ml approx.	Tryptone in 450 ml of distilled water Micro-mineral solution Agitate solution to dissolve the chemicals Buffer solution Macro-mineral solution 0.1%resazurin solution Adjust the medium pH to 7, using 6M HCl Store at 4^oC	2.25 g 112.5 µl 225 ml 225 ml 1.125 ml
Sodium Phosphate Buffer	200 ml	KH_2PO_4 1/15 M (9.078 g/l): 1.82 g/200ml Na_2HPO_4 1/15 M (11.876 g/l): 2.38 g/200mL Maintain pH of 7 & at 20^oC by adding 1M NaOH Store at 4^oC	78 ml of (a) to 122 ml of (b)
Reducing Solution	50 ml	Cysteine hydrochloride NaOH 1M $Na_2S.9H_2O$ Distilled water	312.5 mg 2 mL 312.5 mg up to 50 ml

Figure 2.2: Flow chart of in vitro fermentation protocol.



2.5 Short chain fatty acid (SCFA) analysis

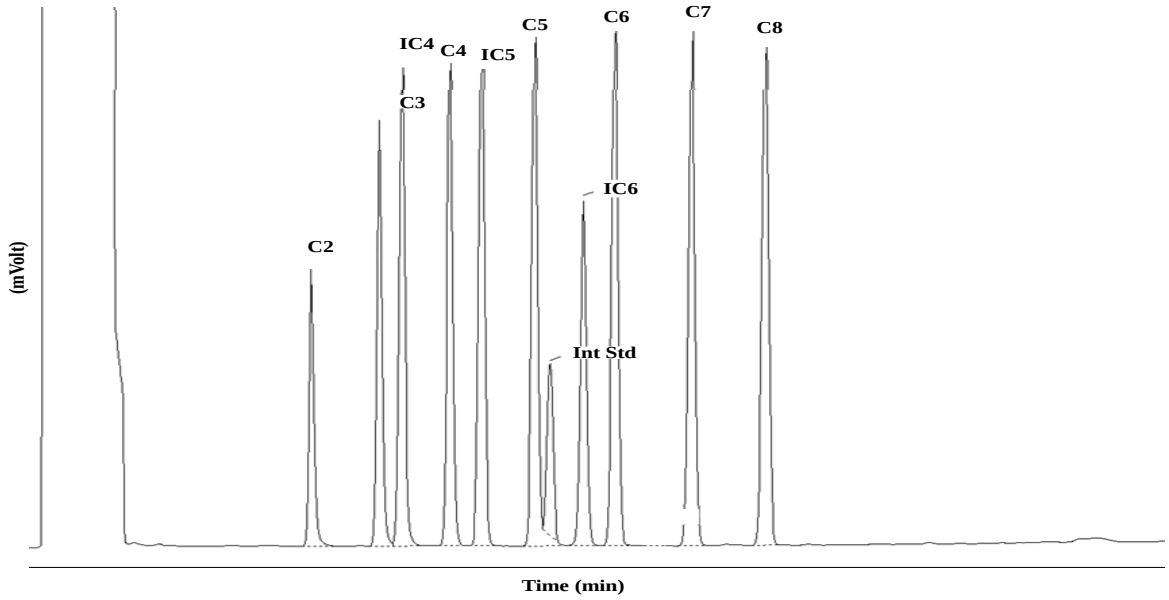
SCFA were measured using GC-FID using a TRACE™ 2000 gas chromatograph (Thermo Quest Ltd, Manchester, UK) (Laurentin and Edwards, 2004). All the chemicals used in the SCFA extraction (orthophosphoric acid, sodium hydroxide, diethyl ether, and external & internal standards) were obtained from Fisher Scientific (Leicestershire, UK), Sigma-Aldrich Company Ltd (Dorset, UK).

The GC has a flame ionisation detector (250 °C) and a Zebron ZB-Wax capillary column (15 m x 0.53 mm id x 1 µm film thickness), (catalogue No.7EK-G007 22, Phenomenex, Cheshire, UK). The carrier gas was nitrogen (30 ml/min). Samples (1 ul) were automatically injected (230 °C, splitless) onto the column. The column temperature was held at 80 °C for 1 min, increased at 15 °C/min until 210 °C and then held for 1 min. Analyses of GC-FID data were performed with Chrom-Card 32-bit software version 1.07β5 (2000) Thermo Quest (Milan, Italy). Identification of SCFAs was achieved by comparison with retention times of authentic standards. Quantification was based on the averaged area ratio with the internal standard of each external standard (concentrations based on expected values in healthy faecal samples) which was run batch-wise, with grouped analysis of samples from the same individuals to reduce inter-assay error. A set of five calibrated standards was extracted and analysed before and after the samples. A signal standard used as a calibrated quality control, was run every 10 samples. Samples were extracted in duplicate and their results were averaged, whereas the standards were extracted once and injected twice and each run lasted approx. 15 minutes (**Figure 2.3**).

2.5.1 Internal standard

2-Ethylbutyl acid was supplied from Sigma Chemicals Company Ltd., (Loughborough, UK). The internal standard was made up by adding 1.714 g of 2-Ethylbutyl acid to 200 ml of 2 M of NaOH. The 2 M of NaOH was prepared by adding 16 g of NaOH (Merck, Loughborough, UK) to 200 ml of distilled water.

Figure 2.3: Example of typical run on GC-FID.



2.5.2 External standard

The external standard was made up of a mixture of acids. These were placed in a volumetric flask with 2.9 g NaOH to adjust the pH to 7.0 before making the volume up to 100 ml with distilled water. These were used to obtain a standard curve, which allowed calculation of each of the SCFAs in the test samples (Table 2.4) and included a total of 11 short, medium and branched chain fatty acids, extracted with 6 dilutions (10 µl, 25 µl, 50 µl, 100 µl, 200 µl and 300 µl).

Table 2.4: Constituents of the external standard used for SCFA analysis.

No.	Acid Name	Molar Mass (g/L)	Calculated Molarity (mM)
1	Acetic acid	60.05	183.50
2	Propionic acid	74.08	134.52
3	Butyric acid	88.11	111.74
4	Iso-butyric acid	88.11	104.22
5	Iso-valeric acid	102.13	85.51
6	Iso-Caproic acid	116.16	52.41
7	Valeric acid	102.13	89.92
8	Caproic acid	116.16	80.12
9	Enanthic acid	130.18	68.53
10	Caprylic acid	144.21	57.59

2.5.3 SCFA extraction procedure

Distilled water was added to each external standard solution to make a final volume of 800 µl. 100 µl of internal standard (2-Ethylbuty) and 100 µl of Orthophosphoric acid were then added (Table 2.5). The faecal fermented samples were treated in the same way, except that 800 µl of liquid samples were used instead of distilled water and external standard. The mixture in all tubes was then extracted using 3 ml of diethyl ether. The tubes were mixed well for one minute and the top layer of the tubes was extracted. This step was carried out three times for each sample and standard. The extracted top layers were pooled and placed in clean tubes. The pooled samples were then placed in glass tubes with a crimp-top seal, which were placed in the GLC for SCFA analysis.

Table 2.5: Solutions used for SCFA extraction.

External solution extraction			
External solution	**Distilled water**	**Solution**	**Solution extraction**
10 µl 25 µl 50 µl 100 µl 200 µl 300 µl	790 µl 775 µl 750 µl 700 µl 600 µl 500 µl	+ 100 µl of internal standard + 100 µl of Orthophosphoric acid	3 ml of diethyl ether added, vortexed for one min × 3. The pooled top layer extracted and placed on clean tube. The pooled ether placed in crimped glass tubes and placed in GLC for SCFA analysis
Faecal sample			
800 µl	**Solution** + 100 µl of internal standard + 100 µl of Orthophosphoric acid		**Solution extraction** 3 ml of diethyl ether added, vortexed for one min × 3. The pooled top layer extracted and placed on a clean tube. The pooled ether placed in crimped glass tubes and placed in GLC for SCFA analysis

2.6 Determination of resistant starch (RS) in samples

The *in vitro* RS (McCleary and Monaghan, 2002) was quantified in this thesis using a RS kit (standard AOAC 2002.02 and AACC 32-40) after removing the digestible starch from the samples using an extensive *in vitro* enzymatic digestion (salivary α-amylase, pepsin, pancreatin and bile acid) as described by Aura et al. (1999). The RS kit was purchased from Megazyme International Ireland Ltd. All chemicals used in the RS determination including buffers and solutions (Table 2.6) were supplied by Sigma-Aldrich Company Ltd. (Dorset, UK).

100 ± 5 mg samples were weighed directly into each screw cap tube and gently tapped to ensure that the sample falls to the bottom (Figure 2.4). 4.0 ml of pancreatic α-amylase (10 mg/ml) containing Amyloglucosidase (AMG) (3 U/ml) was added to each tube. The tubes were then tightly capped, mixed on a vortex mixer and placed horizontally in a shaking water bath, aligned in the direction of motion. The tube samples were incubated at 37°C with continuous shaking 200 strokes /min for exactly 16 hours. The tubes were removed from the water bath and any excess surface water was removed with a paper towel. The tube contents were treated with 4.0 ml of ethanol (99% *v/v*) with vigorous stirring in a vortex mixer. The tube samples were then centrifuged at 1,500 g (approx. 3,000 rpm) for 10 min un-capped. The supernatants were carefully decanted and the pellets re-suspended in 2 m lof 50 % ethanol (*v/v*) with vigorous stirring in a vortex mixer. A further 6 ml of 50 % ethanol was added and the tubes were mixed and centrifuged again at 1,500 g for 10 min.

The supernatants were carefully decanted and the suspension and centrifugation steps repeated once more. The tubes were then inverted on absorbent paper to drain excess liquid.

A magnetic stirrer bar 5 x 15 mm and 2 ml of 2 M KOH were then added to each tube and the pellets re-suspended (dissolving the RS) by stirring for approx. 20 min in an ice/water bath over a magnetic stirrer. 8 ml of 1.2 M sodium acetate buffer pH 3.8 was added to each tube by stirring with the magnetic stirrer. Then, immediately 0.1 ml of AMG solution (1; 3300 U/ml) was added, mixed well and the tubes placed in a water bath at 50 °C. The tubes were incubated for 30 min with intermittent mixing in a vortex mixer.

Table 2.6: Solutions and enzymes used for RS determination (from manufacturer's leaflet).

Enzymes (Kit)	
Amyloglucosidase solution (AMG)	This solution is viscous and therefore should be dispensed with a positive displacement dispenser e.g. Eppendorf-Multipette. Dilute 2 ml of concentrated AMG solution to 22 ml with 0.1 M sodium maleate buffer. Divide into 5 ml aliquots and store frozen in polypropylene containers between uses. Stable for 5 years at -20 °C ***Immediately before use*** Suspend 1 g of the contents of **Pancreatic α-amylase** in 100 ml of sodium maleate buffer (100 mM, pH 6.0) and stir for 5 min. Add 1.0 ml of Dilute AMG (300 U/ml) and mix well. Centrifuge at > 1,500 g for 10 min, and carefully decant the supernatant solution. Use this solution *(Solution 2)* on the day of preparation
GOPOD Reagent Buffer	Dilute the contents in 1 L of distilled water. This is *solution 3* to be used immediately.
GOPOD Reagent Enzymes	Dissolve the contents in 20 ml of Solution 3 and quantitatively transfer this to the bottle containing the remainder of *Solution 3*. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is *Solution 4* Glucose Determination Reagent (GOPOD Reagent). Stable for 3 months at 2-5 °C or > 12 months at -20 °C.
D-Glucose standard solution and Resistant starch control	Use the contents as supplied. Stable for > 5 years at room temperature.
Buffers and solutions	
100 mM Sodium maleate buffer	Dissolve 23.2 g of maleic acid in 1600 ml of distilled water. Adjust the pH to 6.0 with 4 M (160 g/litre) sodium hydroxide. Add 0.74 g of calcium chloride dihydrate ($CaCl2.2H_2O$). Add 0.4 g of sodium azide. Adjust the volume to 2 L with distilled water. Stable for 12 months at 4 °C.
1.2 M Sodium acetate buffer (pH 3.8)	Add 69.6 ml of glacial acetic acid (1.05 g/ml) to 800 ml of distilled water. Adjust to pH 3.8 using 4 M sodium hydroxide. Adjust the volume to 1 litre with distilled water. Stable for 12 months at room temperature
100 mM Sodium acetate buffer (pH 4.5)	Add 5.8 ml of glacial acetic acid to 900 ml of distilled water. Adjust pH to 4.5 using 4 M sodium hydroxide. Adjust the volume to 1 litre with distilled water. Stable for 2 months at 4 °C
2 M Potassium hydroxide solution	Dissolve 112.2 g KOH in 900 ml of deionised water with stirring. Adjust volume to 1 litre. Store in a sealed container. Stable for 2 years at room temperature
Aqueous ethanol (approx. 50 % *v/v*)	Add 500 ml of ethanol (95 % *v/v* or 99% v/v) to 500 ml of H_2O. Store in a well-sealed bottle. Stable for > 2 years at room temperature
The reagent blank	Mix 0.1 ml of 100 mM of sodium acetate buffer (pH 4.5) and 3.0 ml of GOPOD reagent

For samples containing > 10 % RS content

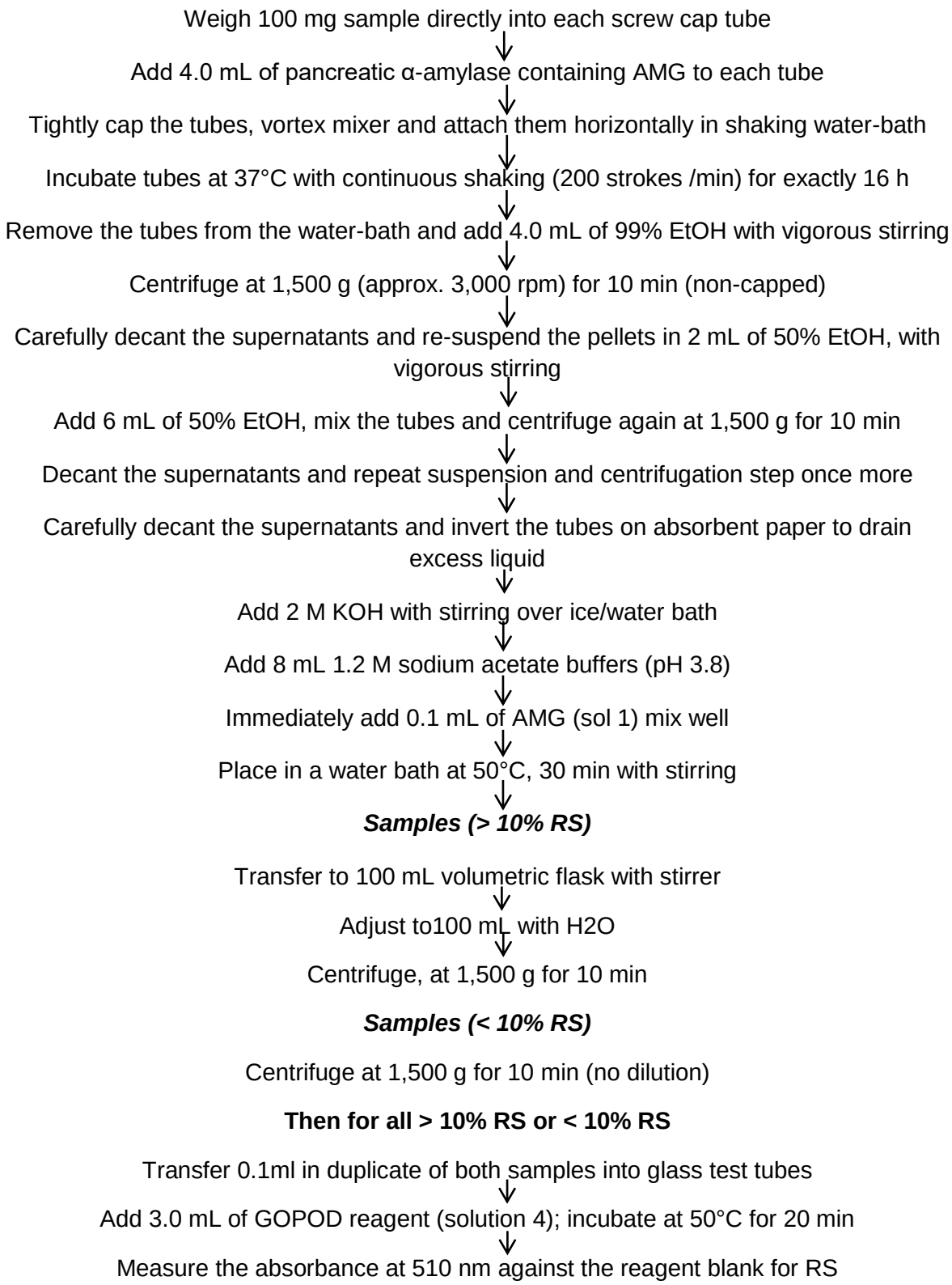
The contents of the tube were transferred to a 100 ml volumetric flask and washed (using a distilled water wash bottle). An external magnet was used to retain the stirrer bar in the tube during washing. The volume was adjusted to 100 ml with distilled water and mixed well. The mixed solution centrifuged at 1,500 g for 10 min.

For samples containing < 10 % RS content

The tubes were centrifuged at 1,500 g for 10 min (no dilution). The final volume in the tube was approximately 10.3 ml.

0.1 ml of aliquots (in duplicate) of the solution (either diluted or undiluted supernatants) were transferred into glass test tubes (16 x 100 mm). 3 ml of GOPOD reagent was added to each tube and incubated at 50 °C for 20 min. Finally, absorbance was measured at 510 nm against the reagent blank to determine the resistant starch concentration.

Figure 2.4: Flow chart of resistant starch extraction and measurement.



2.7 Determination of NDOs (inulin and oligofructose) concentration

NDOs (inulin and oligofructose) were quantified using a fructan kit (Megazyme International Ireland Ltd.) (McCleary et al., 2000), after removing the digestible starch from the samples using an extensive *in vitro* enzymatic digestion (salivary α-amylase, pepsin, pancreatin and bile acid) as described by Aura et al. (1999). All chemicals used in the fructan determination including buffers and solutions were supplied by Sigma-Aldrich Company Ltd. (Dorset, UK) (Table 2.7).

Sample containing (12-50 % of w/w fructan)

1000 ± 10 mg of each sample was weighed directly into a 100 ml beaker and 40 ml of hot distilled water added. Beakers were placed on a hot plate with magnetic stirring for 15 min until boiled. The beakers were cooled to room temperature, the content of each beaker transferred to a 50 ml volumetric flask, and the volume was adjusted to 50 ml with distilled water and mixed well (Figure 2.5).

Sample containing (0-12 % of w/w fructan)

1000 ± 10 mg of each sample was weighed accurately and directly into a 200 ml beaker and 80 ml of hot distilled water was added. The beakers were cooled to room temperature, then the content of each beaker was transferred into a 100 ml volumetric flask, the volume was adjusted to 100 ml with distilled water and mixed well.

Table 2.7: Solutions and enzymes used for fructan concentration determination (from manufacturer's leaflet).

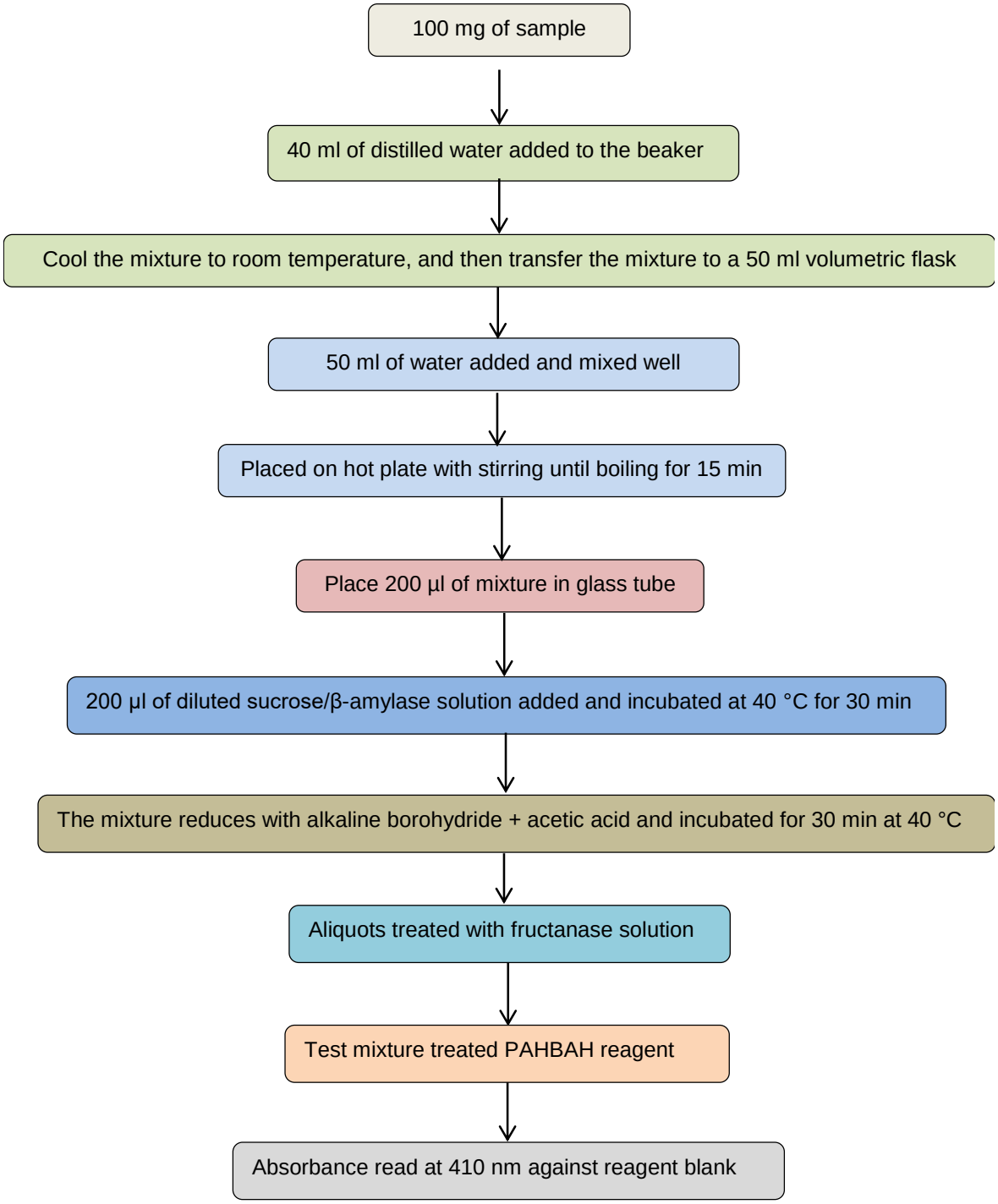
Enzymes (Kit)	
Sucrase enzyme bottle	Dissolve the contents of bottle in 22 ml of (0.1 M, pH6.5) of sodium maleate, store at -20 °C
Fructanase enzyme bottle	Dissolve the contents of bottle in 22 ml of (0.1 M, pH 4.5) sodium acetate, store at -20 °C
	PAHBAH Reducing Sugar Reagent
Sucrose/cellulose control powder	should be analysed with each new lot of reagents
D-fructose standard	0.2 ml of D-fructose standard solution (1.5 mg/ml) is added to 0.9 ml of Buffer 2; 100 mM sodium acetate (pH 4.5) and mixed thoroughly. Aliquots (0.2 ml) of this solution (containing 54.5 mg of D-fructose) are dispensed, in quadruplicate, into glass test tubes (16 x 100 mm). Buffer 2 (0.1 ml) is added to each tube plus 5.0 ml PAHBAH Working Reagent (immediately before incubation in the boiling water bath)
Buffers and solutions	
Sodium maleate buffer (100 mM, pH 6.5)	Dissolve maleic acid (11.6 g, Sigma cat. no. M-0375) in 900 ml of distilled water and adjust the pH to 6.5 with sodium hydroxide solution (2 M). Adjust volume to 1 litre. Stable for > 3 months at 4 °C
Sodium acetate buffer (100 mM, pH 4.5)	Add glacial acetic acid (5.8 ml) to 900 ml of distilled water. Adjust to pH 4.5 using 1 M sodium hydroxide. Adjust the volume to 1 litre. Stable for > 3 months at 4 °C
Solution (A)	10 g of p-hydroxybenzoic acid hydrazide dissolved in 60 ml of distilled water in a 250 ml beaker with stirring. During stirring add 10 ml of concentrated HCl solution. Adjust the volume to 200 ml using distilled water and store at room temperature
Solution (B)	24.9 g of tri-sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_20$) added to 500 ml of distilled water and stir to dissolve. 2.2 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) added to solution with stirring. 40.0 g of NaOH added to solution. Adjust the volume to 2 litres using distilled water
Important to remember: *PAHBAH reagent works by adding 20 ml of Solution A to 180 ml of Solution B and mixing thoroughly immediately before use. The mixed solution stored on ice is stable for 4 hours.*	
50 mM Sodium hydroxide	2.0 g of NaOH dissolved in 900 ml of distilled water. Adjust the volume to 1 litre and store at room temperature
Alkaline borohydride (NaBH) (10 mg/mL sodium borohydride in 50 mM of NaOH)	Weigh 50 mg of sodium borohydride into polypropylene containers (10 ml volume with screw cap). Record the exact weight on the tubes (approx. 10 for convenience), seal the tubes and store in a desiccator for future use. *Immediately before use*, dissolve the sodium borohydride (at 10 mg/ml) in 50 mM sodium hydroxide. This solution is stable for 4-5 hours at room temperature
0.2 M Acetic acid (C2H4O2)	11.6 ml of glacial acetic acid dissolved in 600 ml of distilled water. Adjust the volume to 1 litre and store at room temperature
The reagent blank	0.3 ml of 100 mM sodium acetate buffer (Buffer 2) + 5.0 ml of PAHBAH Working Reagent.

The mixture in the volumetric flask was filtered into a 200 ml beaker using Whatman paper No.1. 200 µl of the mixture solution was transferred to a glass tube, 200 µl of diluted sucrose/β-amylase solution was added and then incubated at 40 °C for 30 min. 200 µl of alkaline borohydride solution was then added to the tube stirring vigorously, which was then incubated at 40 °C for 30 min to convert the reducing sugar to sugar alcohol. 500 µl of 0.2 M of acetic acid was added to the tube stirring vigorously to adjust pH to 4.5. 200 µl of the mixture was transferred into three new glass tubes where 100 µl of fructanase solution was added to two tubes (sample) and 0.1 ml of 0.1 M of sodium acetate buffer was added to a third tube (sample blank). The tubes were then sealed with parafilm and incubated at 40 °C for 30 min. 500 ml of p-hydroxybenzoic acid hydrazide solution (PAHBAH) was added to all tubes and incubated in a boiling water bath for 6 min. Immediately after incubation, the tubes were placed in cooled water (18-20 °C) for 5 min. The absorbance of the tube content was then measured at 410 nm against the reagent blank. The fructan content (%, as-is basis) was measured using the reference equation by McCleary *et al*. (2000):

$$\boldsymbol{Total\ Fructan},\% = \boldsymbol{A} \times \boldsymbol{F} \times \mathbf{5} \times \boldsymbol{V} \times (\mathbf{1.1/0.2}) \times (\mathbf{100/}\boldsymbol{W}) \times \mathbf{1/1000} \times (\mathbf{162/180})$$

A = PAHBAH absorbance of reaction solutions (0.2 ml) read against reagent blank
F = factor to convert absorbance values to mg fructose (= 54.5 mg fructose/ absorbance value for 54.5 mg fructose)
5 = factor to convert from 0.2 ml as assayed to 1.0 ml
V = volume (ml) of extractant used (100 or 50 ml)
1.1/0.2 = 0.2 ml was taken from 1.1 ml enzyme digest for analysis
W = weight (mg) of sample extracted; 100/W = factor to express fructan as percentage of flour weight
1/1000 = factor to convert from mg to mg
162/180 = factor to convert from free fructose, as determined, to anhydrofructose (and anhydroglucose), as occurs in fructan

Figure 2.5: Flow chart of fructan measurement protocol.



2.8 Statistical analysis

Statistical analysis was performed for all data using Minitab statistical software version 16. Normality of the data was tested using the Anderson-Darling test. Descriptive statistics are presented as mean and standard deviation (SD), or medians and inter quartile range (IQR). Comparisons between groups were carried out using the Mann-Whitney test for non-normally distributed data, and a paired test and 2 sample t-tests for the normally distributed data. Spearmen correlation coefficients were calculated for the excretion of the urinary SCFA products with FC. Additionally, in the urinary analysis study (Chapter 4) a regression analysis was conducted on the preferred biomarkers to derive an index with confidence and prediction intervals to examine the power of a predictive marker of fermentable carbohydrate intake from urinary biomarkers. Other specific tests are described in each relevant chapter.

3 Chapter Three

Developing an index of dietary estimation of FC

3.1 Introduction

This chapter describes the development of the equations used to estimate the amount of FC that reaches the human colon. As it is the development of an index, the chapter does not follow a traditional layout of methods and results, but is more of a discussion of the evidence for the amount of each type of NDCs that reaches the colon and for the extent of its fermentation.

Different studies have shown that NDCs can have an impact on human health, and several physiochemical properties have been suggested to explain these impacts including regulation of intestinal transit time and digestive function through faecal bulking, dilution of carcinogens in the colonic lumen, lowering of colonic pH, and bacterial fermentation of NDCs to SCFAs in the colon (den Besten et al., 2013, Tan et al., 2013). Until now, there was no reliable estimate of SCFA production for individuals who consume mixed NDCs, as not all insoluble fibre is fermented. In the UK, wheat bran intake is > 10 times as much as white rice and oat (Stevenson et al., 2012), and wheat bran is considered to be a rich source of DF, and mainly (46 %) consists of NSPs, including AX, cellulose and β-glucan at 70 %, 24 % and 6 % respectively, whereas NSP in wheat endosperm are < 88% AX (Lu et al., 2000, Maes and Delcour, 2002). AX and β-glucan in wheat bran are fermented by colonic microflora to form SCFA, while cellulose increase faecal bulk and shorten the colonic transit time (Wood, 1997, Lu et al., 2000).

Several studies have reported the impact of wheat bran consumption on increased faecal bulk (Muir et al., 2004, Lupton and Turner, 1999), which have agreed with other studies that have shown wheat bran is not well fermented by colonic microflora, but it increases faecal weight and reduces colonic transit time (Elleuch et al., 2011, Stevenson et al., 2012). Chen et al. (1998) examined the impact of consuming (30 g/d) of wheat bran with oat bran as control diet in five men. They observed that wheat bran fibre mixed with the diet increased the wet stool weight by 52.4 g/d, which provided a 4.85 g/d increase in stool weight for each additional gram of fibre intake, which suggested the increased in faecal weight was due to the non-digestible components (50 - 60 %) of wheat bran. Similarly, Cummings (2001) reported that each gram of wheat bran consumed increased faecal weight by 5.4 g, which is similar to the results from EFSA and the UK Scientific Advisory Committee, who stated that each gram of wheat fibre consumed daily increases faecal weight

by 5 g, compared to other fibres for example, fruit and vegetables (4.1 g/g), gums (4 g/g), soya products (2.5 g/g) and pectin least of all (1.2 g/g) (Agostoni et al., 2010). This is probably due to the differences in bacterial fermentation of the fibres in the colon.

In Western diets the amount of NDCs that enter the colon may be in the range of 20 - 40 g/d, whereas in countries where starchy foods are the main part of the diet, NDC concentrations could reach 50 g/d (Cummings, 1997). However, it has been reported that an individual required approximately 80 g/d of NDCs to sustain the bacterial biomass and SCFA production (Stephen, 1991), and only 25 % might be provided by NSP (Hill, 1995).

Protein (amino acids) is also fermented by proteolytic bacteria in the proximal colon, and the main products of amino acid fermentation are SCFA, BCFA and ammonia (Hamer et al., 2012). Five volunteers were involved in a study to examine the impact of high protein diet on colonic metabolism products; after one week they had a significantly higher faecal ammonia ($p = 0.002$), faecal valeric acid ($p = 0.02$) and urinary p-cresol ($p = 0.04$), which shows the level of fermentation that was occurring (Geypens et al., 1997). BCFAs are often used as faecal and urinary biomarker for amino acid fermentation (Hamer et al., 2012).

An additional substrate for fermentation is mucus, which is made of glycoproteins in ileal effluent, and also formed by goblet cells in the colonic epithelium to form a protective gel that is spread over the mucosal surface (Cummings and Macfarlane, 1991). Studies have shown that it becomes a substrate for colonic microflora fermentation in the absence of NDCs and protein substrates (Miller et al., 1984). Stephen et al. (1983) used intubation studies to investigate mucus fermentation, and they estimated the amount of mucus that entered the colon to be 3 - 4 g/d, but much more may be made in the colon itself, therefore it is difficult to estimate this.

These results suggest adding RS and NDOs, protein and mucus might overcome the NDCs gap. Many of the health benefits of NDCs may be related to fermentation and SCFA production, therefore it is important to consider all of these substrates when looking at the role of fermentation in preventing disease. Not all of these can be measured, but we proposed to develop an index of FC that could be used to determine NDCs from food diaries, based on information and evidence in published

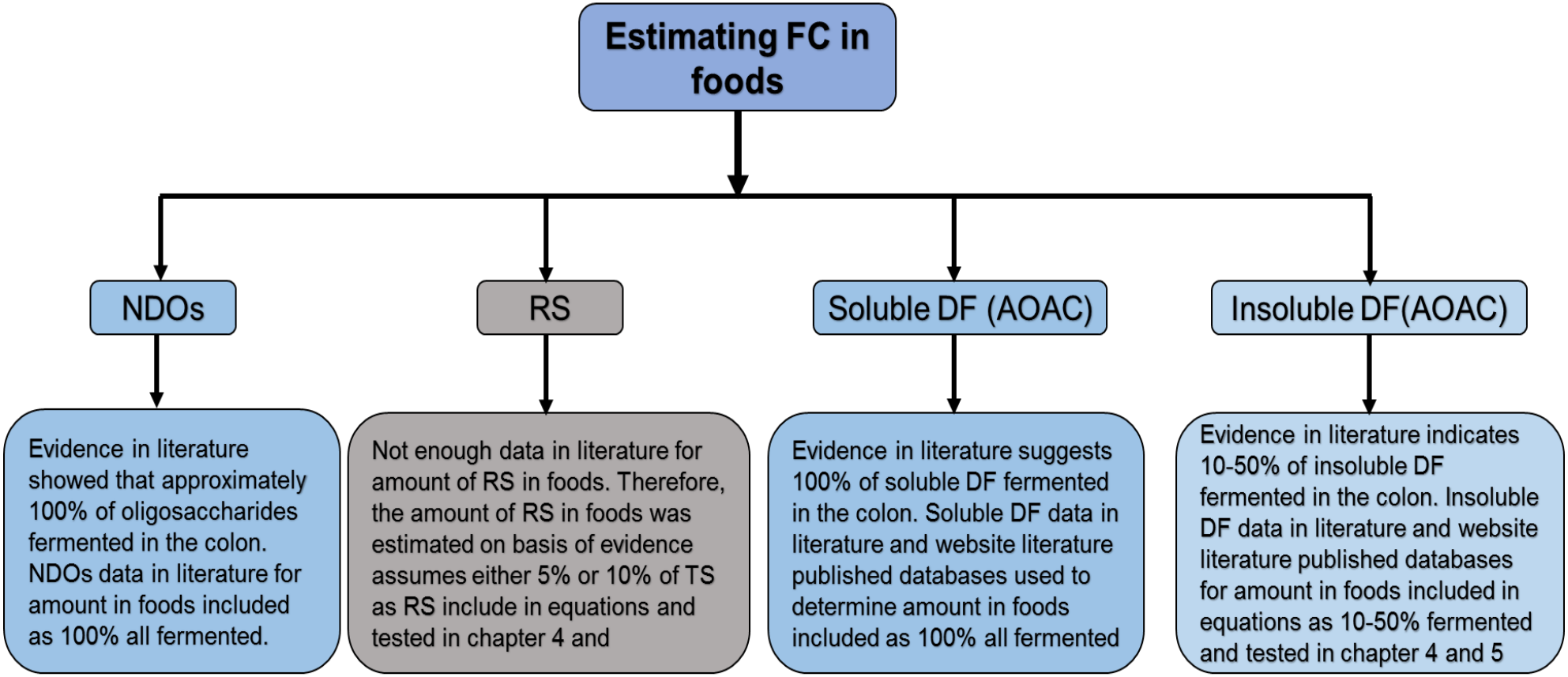
literatures. This index will consider all NDCs that escape digestion and absorption and enter the colon for fermentation by colonic microbiota.

One reason for the need for such index is that it is very difficult to measure fermentation *in vivo*. To develop such index, it is important to know how much NDCs enters the colon, and how much of this is fermented. In the next section different methods to estimate colonic fermentation, and also the amount of substrate entering the colon, will be discussed. These studies were examined for evidence to determine the different percentages of each type of NDCs that should be included in the final model, based on how much entered the colon intact, and also to what extent they were fermented to SCFA *in vivo*.

3.2 Studies which have estimated the amount of NDCs which reach the colon

This chapter discussed in detail the evidence for the digestibility of all ingested carbohydrates and for those that are not digested the likely extent of their fermentation by colonic bacteria. The search for evidence was carried out using PubMed, and the ISI Knowledge databases (Figure 3.1). The MeSH heading ‘starch’, ‘resistant starch’, ‘RS’, ‘fermentable’, ‘fermentation’, non-digestible’ and ‘digestible’ were applied either alone or in different combinations to search all databases to find literature regarding fermentable RS. The MeSH heading ‘dietary fibre’, ‘fibre’, ‘soluble fibre’ ‘insoluble fibre’, ‘cereals’, ‘whole grain’, ‘polysaccharides’, ‘fermentable’, ‘fermentation’, non-digestible’ and ‘digestible’ were applied either alone or in different combinations to search all databases to find literature regarding fermentable DF. The MeSH heading ‘prebiotics’, ‘inulin’, ‘oligofructose’, ‘non-digestible oligosaccharide’, ‘fructooligosaccharide’, ‘galactooligosaccharide’, ‘fermentable’, ‘fermentation’, non-digestible’ and ‘digestible’ were applied either alone or in different combinations to search all databases to find literature regarding fermentable NDOs. Most estimates of the amount of carbohydrate reaching the colon and their fermentation has been studied using indirect measurement of carbohydrate fermentation, for example, breath hydrogen (H_2), direct intubation of the ileum, and the ileostomy model and faeces. This is because quantifying the amount of NDCs that escapes digestion and absorption in the small intestine directly is difficult.

Figure 3.1: Flow chart of estimating fermentable carbohydrate in foods



3.2.1 RS measurement

RS has been reported as a major substrate for fermentation in the human colon (Englyst and Cummings, 1990, Stephen et al., 1983). Several models have been used in humans to estimate the amount of starch that resists pancreatic amylase, and escapes digestion in the small intestine. These models were direct intubation of the ileum, the ileostomy, and breath hydrogen (H_2) models (Cummings and Englyst, 1991).

3.2.1.1 Intubation model

This model measured starch malabsorption of normal foods through intubation of the ileum of healthy subjects. Stephen et al. (1983) intubated the terminal ileum of seven normal volunteers; they found that starch malabsorption from test meals containing either 20 g of starch from banana and rice was 6.0% or 60 g from banana, rice, and potato was 9.3%. Flourie et al. (1988) evaluated six subjects and fed them a diet containing 100 g or 300 g of starch from white bread, potato, and noodles for two periods eight days. The 24 hours aspiration test showed that 4.1 % and 3.2 % of starch were recovered in the terminal ileum for both low and high starch meals, respectively. These studies show that the % of malabsorption depends on the concentration of starch used.

3.2.1.2 Ileostomy model

This model is considered to be a direct and quantitative method to measure all intestine excretion. People with established Ileostomies who were involved in these experiments had had their large intestine completely removed, after colectomy surgery; and the terminal ileum brought out onto the anterior abdominal wall as a fistula. Ileal effluent is collected in a bag, which is changed every 2 h during the day. Most ileostomies have a normal life. This model was considered to be a reliable model for studying the small intestine digestion physiology for many reasons; the ileostomy effluents provide digestive enzymes, which resemble normal pancreatic performance, and the ileal excretion of substrates is believed to be comparable to that of a healthy subject. Studies have also shown that gut hormone responses to test meals were similar to that of those with an intact gut, and transit time of the stomach and small intestine is similar to that of intact gut subjects (Cummings and Englyst, 1991).

Table 3.1: Resistant starch measured in the ileostomy model adapted from Cummings and Englyst, (1991).

Food source	Starch fed (g/d)	Starch recovered (g/d)	% recovery	Reference
Rice, bread	98 – 127	0.5	< 1.0	(Sandberg et al., 1981)
Rice, bread	140	0.7	< 1.0	(Sandberg et al., 1983)
Bread	107	0.3	< 1.0	(Sandberg et al., 1986)
Wheat starch				
rice, bran	107	0.5	< 1.0	
Wheat, potato	157	1.9	2.4	(Chapman et al., 1985)
White bread	100	12.4	13	(Wolever et al., 1986)
Wholemeal bread	100	10	11	
Lentils	100	20	21	
White bread	61.9	1.3	2.5	(Englyst and Macfarlane, 1986)
Oats	57.8	0.4	2.2	
Cornflakes	74.2	3	5	
Banana	2-21	0.3	75	(Englyst and Cummings, 1985)
Potato				
Hot	45.4	1.1	3.3	(Englyst and Cummings, 1985)
Cold	47.2	3.1	12.2	
Wheat				
Fine	52.4	0.4	0.7	(O'Donnell et al., 1989)
Coarse	52.4	0.6	1.1	

Some studies have used this model to measure RS (Chapman et al., 1985, Sandberg et al., 1981). Chapman et al. (1985) recruited six ileostomies subjects and fed them three mixed meals in day containing approximately 157 g of starch (Table 3.1). Only 1.3 - 5.0 %, with mean 2.4 % of a 157 g load of starch appeared in ileal effluent of the six ileostomies. Sandberg et al. (1986) fed seven ileostomy subjects a low fibre diet supplemented with a mixture of bran gluten starch 54 g/d for (periods A), or the corresponding extruded product (period B) for 4 days each period. They found only 0.6 - 0.7 % of ingested starch was recovered in ileostomy contents in both periods, respectively, which shows that most of the starch was digested in this study. O'Donnell et al. (1989) fed nine non-obese ileostomies breakfast (containing 52.4 g starch) comprised of soda bread scones made from 76 g fine or coarsely milled whole meal flour. All subjects had the two breakfasts in a random order for seven days. They observed that 0.7-1.1 % of wheat flour escaped digestion in the small intestine, which almost was similar to the findings of Sandberg et al. (1986). Englyst and Cummings (1985) fed seven ileostomies subjects either 100 g oats, 100 g cornflakes, or 150 g white bread as breakfast. They reported that less than 0.6 %, 4 % and 2.5 % of oats, cornflakes and white bread (respectively) escaped digestion in the small intestine, which shows the different meals had different rates of

absorption. On the other hand, Wolever et al. (1986) gave three ileostomies subjects starchy meals (either 100 g white bread, 100 g whole meal bread, or 100 g red lentils). They observed that 10 % of white bread, 8 % of whole meal bread, and 22 % of the red lentils meals escaped digestion and absorption in the small intestine, which is much higher than the other studies. Englyst and Cummings (1987) showed that 3 % of hot potato starch, 12 % of cold potato starch and 75 % of banana starch escaped digestion in the small intestine of ileostomy patients. In contrast, the starch in oats is well digested, while that in lentils may well be partly resistant (Cummings and Englyst, 1991).

3.2.1.3 Breath hydrogen model

NDCs fermentation by colonic microflora produces hydrogen as one of its end products, and the hydrogen is excreted by the lungs with expired air. It has been reported that the hydrogen measured in the colon by a gas perfusion technique, correlated with the total hydrogen excreted by the lungs (Champ et al., 2003). Each subject was calibrated with a standard dose of fermentable substrate, which was lactulose, which was considered to be a good control for the model of NDC fermentation (Cummings and Englyst, 1991). Levitt et al. (1987) fed thirty healthy subjects with various complex carbohydrates. They reported that 2.8 % of white wheat, 13.6 % of whole wheat, 9.0 % of refined oats, 3.2 % of whole oats, 7.6 % of whole corn, 10.0 % of potatoes, 1.8 % of rice and 38.0 % of baked beans escaped digestion in the small intestine (Table 3.2). Wolever et al. (1986) fed subjects with 120 g of white bread; 120 g of wholemeal bread and 120 g of lentils. These foods produced 10.7 %, 8.3 % and 17.6 % of malabsorbed starch, respectively. Moreover, Scheppach et al. (1989) showed that 60 g of potatoes (cooked, cooled and reheated) produced 6.6 %, 22.8 % and 5.0 % of resistant starch, respectively. Overall, breath hydrogen studies measuring NDCs fermentation in human suffer from some problems in their interpretation. One of the problem, it could be that the hydrogen generated from NDCs fermentation, such as starch is the same on a milliliter of hydrogen formed from gram of other NDCs fermentation such as NSP (Cummings and Englyst, 1991). Levitt et al. (1987) stated that this is not so *in vitro* and *in vivo*. Secondly, it is also assumed that hydrogen gas is the only route for disposal of reducing equivalents in fermentation. Therefore, they are probably the least reliable way of obtaining quantitative data on starch malabsorption, although qualitatively they may confirm results obtained by other techniques. For example,

Stephen et al. (1983) and Flourie et al. (1988) examined breath hydrogen in their subjects in parallel with their intubation studies. They observed no correlation at all in starch malabsorption as determined by the two methods.

These variations in the digestion of starchy foods might be due to different of reasons (Silvester et al., 1995). Firstly, starch enclosed within plant structures may be physically inaccessible to pancreatic amylase, such as seeds or grains. Secondly, starch granules that categorized by X-ray diffraction which contain either a B or C type crystalline pattern for example, potatoes, bananas, and some begums are less digestible than those with an A type pattern, such as cereals. Thirdly, starch may retrograde on cooling into a less digestible B type crystalline pattern after gelatinization (Silvester et al., 1995, Nugent, 2005). Additionally, digestibility of starch may be affected be other factors for example intestinal transit time and interactions with other food components, such as NSP, lipids and protein (Nugent, 2005).

Table 3.2: Resistant starch measured by the hydrogen excretion in breath adopted from Cummings and Englyst, (1991),

Food source	starch fed (g/d)	Starch malabsorbed (g/d)	% malabsorbed	Reference
White bread	120	12.6	10.7	(Wolever et al., 1986)
Wholemeal bread	120	9.4	8.3	
Lentils	120	20.2	17.6	
White wheat	50	1.4	2.8	(Levitt et al., 1987)
Whole wheat	50	6.8	13.6	
Refined oats	50	4.5	9.0	
Whole oats	50	1.6	3.2	
Whole corn	50	3.8	7.6	
Potatoes	50	5	10.0	
Rice	100	0.9	1.8	
Baked beans	100	19	38.0	
Potato			2.2	(Scheppach et al., 1988a)
Cooked	60	5.7 and 2.3	6.7	
Cooled	60	13.0 and 14.4	22.8	
Reheated	60	3	5.0	

3.2.2 Validation of RS measurement *in vitro* and in *vivo*

There have been different studies that have combine measurements *in* vivo with results from *in* vitro models. Muir and O'Dea (1993) assessed the amount of total

starch (TS) that escaped digestion in the small intestine of eight ileostomy subjects and used this to validate an *in vitro* assay. Five different foods (baked beans 22.7 g, pearl barley 41.0 g, cornflakes 44.1 g, whole rice 48.9 g and ground rice 46.1 g) comprising different amount starch were provided to the subjects. The researchers found that the 0.7 % of TS in ground rice and 5.7 % of TS in baked beans was recovered in the ileostomy effluent which correlated ($r = 0.90$, $p < 0.05$) with NDCs of *in vitro* model after 6 h of incubation.

Englyst et al. (1996) used a more detailed analytical technique that developed to categorize and analyse RS in plant foods *in vitro*, which might reflect the rate and extent of starch digestion in the human small intestine. This technique gave values for those types of starchy foods that were rapidly digestible, slowly digestible and resistant to digestion. This method was validated against malabsorbed starch from these types of foods fed to healthy subjects. Nine subjects with an ileostomy were fed with 100 g of biscuits made from wheat, potato or banana flour, or moist-heat processed wheat or maize flours. On the day before the experimental day, each subject received a polysaccharide free diet, then subjects randomly received one of the five biscuits for two days (two subjects) or three days (seven subjects). In the *in vitro* study, TS and RS was determined using the Englyst method (1992). The starch that was recovered in the ileostomy subjects' effluent ranged from 12.5-15.5 g/d, and the starch that was recovered *in vitro* was in the ranged of 8.5-15.0 g/d. This finding confirmed that the values of RS obtained *in vitro* reflected the amount of malabsorbed starch in the human small intestine (Englyst et al., 1996). The proportion RS in a starchy food is usually less than 5 to10% but can be between 0% and 100% (Silvester et al., 1995). Chapman et al. (1985) and Englyst et al. (1996) reported that overall in the Western diet approximately 10 % of total starch might be RS, which would not be digested in the small intestine.

3.2.3 Measurement of NSPs and its fermentability

3.2.3.1 In vivo

The fermentability of NSP is highly influenced by a number of physiological and biochemical factors. The physiological factors include the extent of lignification of the cell wall polymers, water solubility of NSP, and the particle size of foodstuffs. The bran cell wall is the most lignified found in human food, and therefore the least digestible (Southgate et al., 1976). The more water soluble a material is the more

digestible, and the smaller its effect on faecal composition and bowel habit is (Stephen and Cummings, 1979). Similarly, breakdown of foodstuffs is affected by the particle size; foodstuffs with fine particles are less laxative and more readily digested than coarse ones (Brodribb and Groves, 1978, Heller et al., 1980).

In the early 19th century, a study carried out by Williams & Olmsted (1936), reported that at least 50 % of cellulose and 80 % of non-cellulosic polysaccharides (hemicellulose) from a variety of sources were fermented by microflora in the human gut. Similarly, *in vivo* in humans, Chen et al. (1998) have reported that the fermentability of oat bran was 96 %, compared 56 % of wheat bran, which correlates with the results of Cummings (1981) who reported that 40 % of bran fibre was fermented in the healthy human gut.

3.2.3.2 In vitro

An *in vitro* study carried out by Bourquin et al. (1992) examined the fermentability of three dietary fibre sources (corn wheat bran, corn fibre and oat bran). After 48 h of incubation with human colonic bacteria, they observed that 87.8 % of corn fibre, 73.5 % of wheat bran and 39.8 % of oat bran remained non-fermented. In the same manner, the same researcher conducted an *in vitro* experiment to assess the fermentability of 11 dietary fibres incubated with human faeces. This study showed that dietary fibre fermentability ranged from high fermentability (83.3 % of citrus, 76.6 % of gum Arabic, and 70.9 % of guar) to low fermentability (11.2 % of corn bran, and 5.8 % of oat fibre) (Bourquin et al., 1996). Moreover, fermentability of five dietary fibre sources using a simple *in vitro* batch system (human faeces and rats caecal) were estimated in five European laboratories in a studies carried out by Barry et al. (1995). After 24 h of incubation they observed that pectin (97.4 %) and soya bean (91.1 %) were highly fermented, compared with the lower fermentability of cellulose (7.2 %) and maize bran (6.2 %). An intermediate level of fermentability was observed with sugar beet fibre (59.5 %). The findings were similar from both in vitro models (inoculated with human faeces and rat caecal bacteria).

3.2.4 Measurement of NDOs and its fermentability

In the human small intestine, the recovery of inulin isolated from Jerusalem artichoke was measured in ileostomy subjects by Knudsen and Hessov (1995). The subjects were fed a low dietary fibre diet based on white wheat bread and virtually free of

inulin, and the same diet with the addition of 10 g and 30 g inulin product, respectively. Researchers observed that approximately 86 % and 87 % (respectively) of inulin was recovered in the ileal effluents at both ingestion levels. Two other studies conducted with ileostomy patients showed the mean excretion of inulin and oligofructose at the end of the ileum was about 88 %, and 90 % (respectively) of the ingested dose (Ellegård et al., 1997, Andersson et al., 1999). In another study, six healthy individuals were involved in a study over a period of eleven days to examine fermentability of FOS by the microflora in the human colon. Subjects received three doses of 20.1 g FOS, and the researchers evaluated FOS output using intestinal aspiration after each dose. They observed that 89 % of FOS entered the colon for fermentation (Molis et al., 1996). Overall these studies confirmed that 80 to 90 % of NDOs were not digested and absorbed in the small intestine, and entered the colon for fermentation.

3.3 Development of the models for calculation of FC

Our model equations for estimating fermentable carbohydrate were based on the above evidence, for example that hemicellulose fibre such as pectin is completely fermented (100 %) and cellulosic fibre such as wheat bran is less fermented (10-50 %). Similarly, NDOs were included as 100 % of NDOs is fermented. Finally, the amount of RS was estimated as 5 - 10 % of TS, but this is believed to be totally fermented. These data were considered in detail and several different equations proposed with varying amounts of insoluble fibre, and TS. These were modelled with real diets until the final equations to be tested in the *in vivo* and *in vitro* studies in the following chapters were agreed (Table 3.3; Figure 3.2).

Table 3.3: The FC equations were developed as follows:

Dietary fibre (AOAC) method	
FC-DF 1	100 % Soluble DF + 10 % insoluble DF + 100 % NDOs + 5 % TS
FC-DF 2	100 % Soluble DF + 50 % insoluble DF + 100 % NDOs + 5 % TS
FC-DF 3	100 % Soluble DF + 10 % insoluble DF + 100 % NDOs + 10 % TS
FC-DF 4	100 % Soluble DF + 50 % insoluble DF + 100 % NDOs + 10 % TS
Non-starch polysaccharide (NSP)	
FC-NSP 1	100 % Soluble NSP + 10 % insoluble NSP + 100 % NDOs + 5 % TS
FC-NSP 2	100 % Soluble NSP + 50 % insoluble NSP + 100 % NDOs + 5 % TS
FC-NSP 3	100 % Soluble NSP + 10 % insoluble NSP + 100 % NDOs + 10 % TS
FC-NSP 4	100 % Soluble NSP + 50 % insoluble NSP + 100 % NDOs + 10 % TS

3.3.1 Measurement of FC in habitual diet study

In this study, the aim was to further develop an index of FC, which required information from the literature on the degree of fermentation of NDCs. It also required information from the literature on the presence of dietary fibre (AOAC) method (soluble, insoluble), NSP (soluble, insoluble), NDOs (inulin and oligofructose) and TS in various foods. Information from websites of food manufactures, information from food products in the local markets and weight dietary records of study healthy volunteers, were also needed for the calculations. This information helped to create a database with various foods, and to estimate the amount of fermentable carbohydrate in the study volunteers' habitual diet.

3.3.1.1 NDOs in habitual diet

The search for evidence from literature on the presence of FOS in natural food was conducted using inulin and oligofructose, rather than looking for xylooligosaccahride, polydextrose and GOS, as inulin and oligofructose are naturally present in the foods and are also used for fortification in various processed foods (Van Loo et al., 1995). The search was carried out using PubMed, and the ISI Knowledge database. The MeSH heading 'prebiotics', 'inulin', 'oligofructose', 'fructooligosaccharide', 'content', 'concentration', 'plants', 'foods', 'feed', 'foodstuff', 'source', 'products', 'measurement', 'determinations' and 'methods' were applied either alone or in different combinations to search all databases to find literature regarding inulin and oligosaccharide content in the foods.11 studies in total were found from electronic databases searching on the amount of inulin and oligofructose in various food items using different determination methods. Of these 11 studies, only 3 studies were selected as they used approved determination methods by the AOAC, which included ion exchange chromatography, enzymatic method with high performance liquid chromatography (HPLC), and high performance anion exchange chromatography (HPAEC) (Van Loo et al., 1995, Hogarth et al., 2000, Campbell et al., 1997).

The principle of these methods to determine the amount of inulin and oligofructose in the foods were dependent on the enzymatic treatment of a food product with an inulinase enzyme, followed by determination of the released sugars. The NDOs were then extracted from the food products with boiling water. An aliquot of the

extract was treated with amyloglucosidase, prior to analysis using ion exchange chromatography (Prosky and Hoebregs, 1999).

The presence of inulin and oligofructose in processed foods that were not obtained from electronic search were assessed from local supermarkets such as, ASDA, Tesco and Sainsbury. The amount of inulin and oligofructose present in the food products were considered as DF. Food product manufacture websites for example, Kellogg's, DANONE and Muller, were also assessed for presence of inulin and oligofructose in food products, which were not obtained from the local supermarkets. Nineteen food items were obtained from websites and local supermarkets.

The inulin and oligofructose concentration of each food item containing oligosaccharide in the diet diary, was estimated by multiplying the amount of food intake in the diet (g) (obtained from Windiets, 2005) by the inulin and oligofructose of that food item (obtained from literature), which was then divided by 100 (Van Loo et al., 1995, Hogarth et al., 2000, Campbell et al., 1997). The inulin and oligofructose values were calculated in this way, for each food item containing oligosaccharide. The inulin and oligofructose was summed to give the inulin and oligofructose for three days, and this value was then divided by three to give the average daily inulin and oligofructose concentration.

3.3.1.2 Dietary fibre AOAC (soluble and insoluble) and NSP (soluble and insoluble) in habitual diet

The soluble DF content of each food item was obtained by using a DF medical calculator provided by the GLOBALRPH website, which calculated Insoluble DF as the difference between soluble and total DF. The presence of the soluble DF content in food items that were not obtained from the GLOBALRPH website DF calculator were assessed from literature searches. Soluble and insoluble DF fractions present in food items were determined using an enzymatic-gravimetric procedure method (991.43) approved by AOAC (Lee et al., 1992). The principle of this method to determine the amount of soluble and insoluble DF in the food sample, foods that contained a high level of sugar were extracted with 85 % ethanol before analysis to remove most of the sugars (Lee et al., 1992). The residue was suspended in MES-TRIS buffer and digested sequentially with: heat-stable alpha-amylase at 95 – 100 °C, protease at 60 °C, and Amyloglucosidase at 60 °C. The mixture then was filtered through glass crucible, and the crucible containing the insoluble DF was rinsed with

four volumes of 95 % ethanol followed by acetone, and dried overnight in at 105 °C in an oven. The filtrate plus washes were mixed with 4 volumes of 95 % ethanol to precipitate materials that were soluble DF. Total DF was calculated as the sum of soluble and insoluble DF.

The soluble NSP and insoluble NSP fractions present in food items were obtained from two studies by Englyst and colleagues using the enzymatic chemical method (Englyst et al., 1988, Englyst et al., 1989), which is a development of Southgate method. The aim is to determine the amount of cell wall polysaccharide as NSP in plant foods, and the principle of the method is to remove starch enzymatically after solubilisation. The total NSP is then measured as the sum of the individual constituent sugars that are released by acid hydrolysis. Total NSP is precipitated for one-hour extraction with phosphate buffer pH 7, at 100 °C instead of ethanol. The values that were obtained represent insoluble NSP, and soluble NSP is calculated as the difference between total NSP and the insoluble NSP. This method is suitable for individual NSP measurements by gas-liquid chromatography or colorimetric methods.

Within this study FC (AOAC) (soluble and insoluble), NSP (soluble and insoluble), inulin and oligofructose, and RS were estimated from the subjects' three day weighed intake diaries. Each food item that contained DF and NSP in the diet diary, had the soluble DF or NSP estimated by multiplying the amount of food intake in the diet (g) (obtained from Windiets, 2005) by the soluble DF or NSP content of that food item (obtained from GLOBALRPH website and literature), which was then divided by 100. The soluble fibre or NSP were summed to give the soluble (fibre or NSP) for three days, and this value was then divided by three to give the average daily soluble DF or NSP. The daily insoluble DF or NSP was then calculated as the difference between soluble fibre or NSP and total the DF or NSP intake (obtained from Windiets 2005).

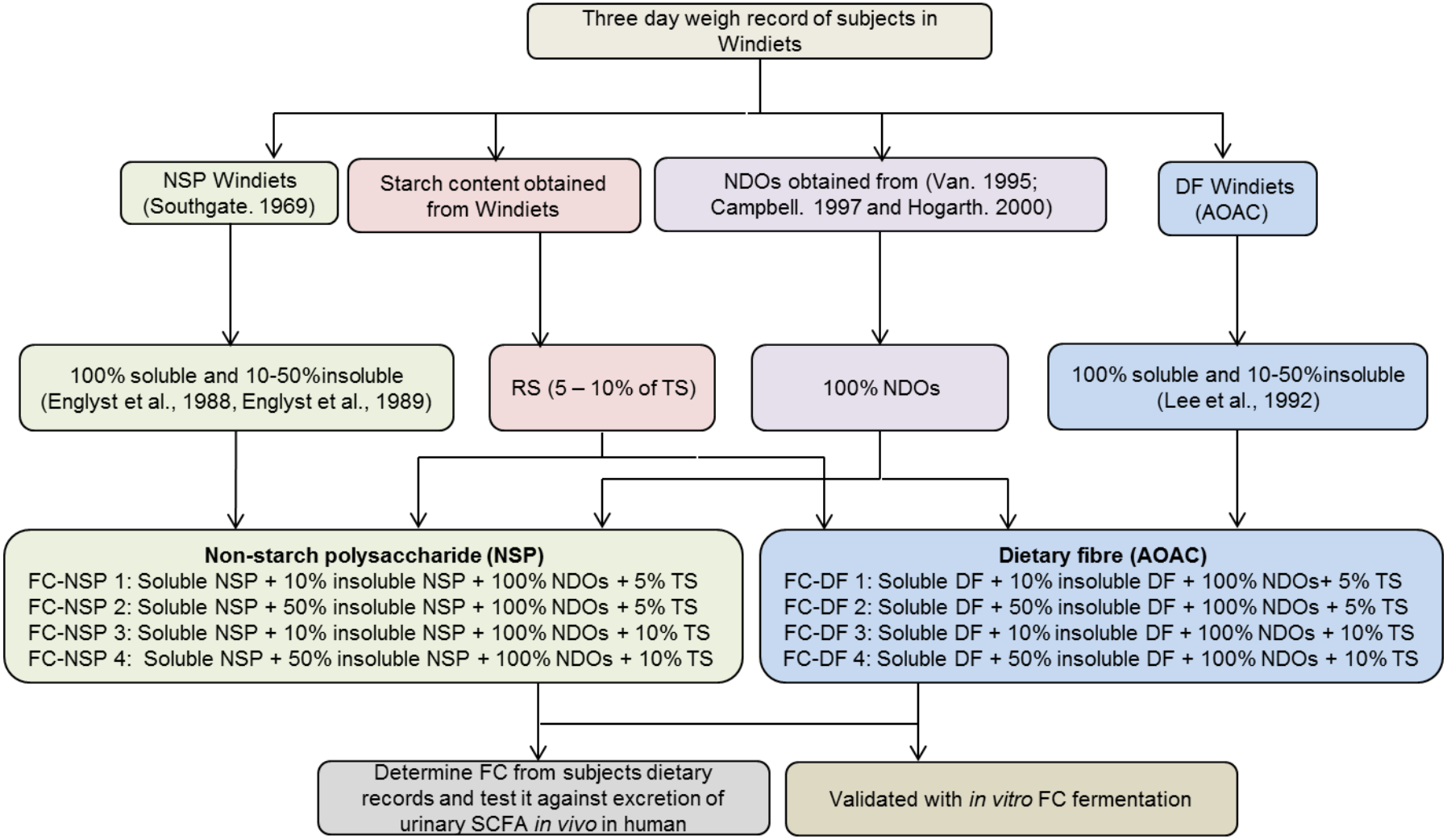
3.3.1.3 RS in habitual diet

Until recently, no *in vitro* method has been capable of quantifying all RS fractions likely to be present in food, even though RS has been reported as the major substrate for fermentation in the human colon (Cummings and Macfarlane, 1991). Several *in vivo* human studies have estimated RS from analysis of starch output in

ileostomy effluents (Englyst and Cummings, 1990, Stephen et al., 1983, Muir and O'Dea, 1992, Silvester et al., 1995, Chapman et al., 1985), and by ileal intubation of healthy subjects (Stephen et al., 1983, Faisant et al., 1995). The RS has also been measured indirectly by measurement of breath H_2 levels (Levitt et al., 1987, Wolever et al., 1986), or blood acetate concentrations (Cummings and Englyst, 1991, Muir et al., 1995). Several developed *in vitro* procedures which mimic the physiologic conditions in humans have simplified the determination of RS in food (McCleary et al., 2002, Åkerberg et al., 1998, Englyst et al., 1992, Goñi et al., 1996). However, the amount of RS that enters the colon each day is difficult measure due to lack of sufficient data on the RS content of foods. The average daily RS intake has been estimated as 4 g across Europe (Elmstahl, 2002) and 3 - 8 g in USA (Murphy et al., 2008).

Therefore, the RS amount of each food item containing starch in the diet diary that obtained from Windiets 2005 was estimated as 5 % and 10 % of TS, based on FC equations developed (Table 3.3). The RS values were calculated in this way, for each food item containing starch, the RS was summed to give the RS for three days, and this value was then divided by three to give the mean daily 5 % and 10 % of TS intake.

Figure 3.2: Flow chart of the fermentable carbohydrate calculation.



4 Chapter Four

Predicting SCFA production in the human colon *in vivo* derived from urinary output using developed index to estimate fermentable carbohydrate from dietary records

4.1 Introduction

This chapter describes a comparison between our predictive equations developed to estimate FC from dietary records for a secondary analysis of a previous study conducted at the SUERC, which measured urinary excretion of SCFA.

Epidemiological studies have frequently reported that diets rich in NDCs may reduce the risk of chronic diseases including CVD (Crowe et al., 2012), CRC (Aune et al., 2011b, Bingham et al., 2003), diabetes mellitus (Carter et al., 2010) and obesity (Bäckhed et al., 2007). Similar results were also found by the World Cancer Research Fund in their major systematic review, they reported that NDCs ‘‘probably’ protect against CRC incidence when there is a high cereals, fruits and vegetables intake (WCRF, 2007). Cereal fibre was the main component behind these protective effects, as NDCs are mainly found in whole grain cereals, pulses, fruit and vegetables (Guillon and Champ, 2000b). According to the European Food Safety Authority (EFSA) report, the average dietary fibre intake is 15 - 30 g per day with the lowest at 6 - 9 g/d and the highest at 39 - 51 g/d in adults across European countries (Agostoni et al., 2010). Therefore, this amount of variation allowed the differences in intake to be compared to investigate if there are any protective effects.

The main characteristics of NDCs are their relatively high content of RS, NDOs and NSPs; these components were shown in intervention studies to induce and sustain improvements to metabolic syndrome risk factors such as, CVD and diabetes mellitus (Steffen et al., 2003, Maki et al., 2012). Steffen et al. (2003) showed that adolescence who consumed of more than 1½ servings of whole grain foods per day were leaner and more insulin sensitive, when compared to those who consumed less than ½ serving per day. Other research shows a healthy diet supplemented with psyllium fibre at a concentration of 30 g/d improve insulin sensitivity, body weight and BMI, when compared with the control (Pal and Radavelli-Bagatini, 2012). There are many other studies that show the benefit of NDCs, for example Maki et al. (2012) observed that consumption of 15 - 30 g/d of high-amylose maize resistant starch (HAM-RS2) for 4 weeks, enhanced insulin sensitivity in overweight and obese men. Additionally, Lipoprotein profiles in patients with CVD improved after intake of 10.5 g/d of soluble fibre (psyllium ovata husk) for 8 weeks, more than a comparable amount of those of insoluble fibre (psyllium ovata seeds) (Sola et al., 2007a). Othman et al. (2011) conducted a systematic review to study the relationship

between oat β-glucan consumption and blood cholesterol level reduction. They found that a daily intake of at least 3 g of oat β-glucan may reduce plasma total and LDL cholesterol levels by 5 - 10 % in hypercholesterolemic subjects. In a randomized crossover study conducted by Willis et al. (2009) the results showed that 8 g of RS intake kept subjects significantly less hungry than the baseline reading for 120 minutes, and satisfied for the180 minute test period. This effect was also observed by Cani et al. (2006) who found that consumption of either 8 g of oligofructose or dextrin maltose twice a day for two weeks at breakfast, significantly reduced total energy 5 % per day and increased satiety. It is likely that the effects of NSP, NDOs and RS observed in these studies are brought about through the fermentation of NDCs in the colon, which results in the production of the SCFAs (Kimura et al., 2014).

The principal components of SCFAs are the end products of luminal microbial fermentation of predominantly NDCs (den Besten et al., 2013). SCFAs are produced in varying amounts depending on the diet and the composition of the intestinal microbiota, with different carbon chain lengths (acetate (C2), propionate (C3), butyrate (C4), valerate (C5) and caproate (C6)) creating different end products (van Nuenen et al., 2003). Several studies have shown that SCFAs not only exert effects in the colonic epithelial cells, but also enter the circulation and have an impact on metabolic processes in other tissues and organs, for example acetate and propionate have been shown to have an impact on glucose and lipid metabolism in the liver (Chen et al., 1984, Wolever et al., 1989, Venter et al., 1990a, Nishina and Freedland, 1990). Acetate is used in the liver as substrate for the synthesis of cholesterol and fatty acids, whereas it appears propionate inhibits these processes (Nishina and Freedland, 1990). The deorphanization of the free fatty acid (FFA) receptors FFA2 (GPR43) and FFA3 (GPR41) are activated by SCFAs and this effect of SCFA is likely to occur in adipose tissue metabolism due to the high expression of GPR43 in these tissues (Hong et al., 2005). Ge et al. (2008) observed that the anti-lipolytic effect is abolished in adipocytes isolated from GPR43 knockout animals, and this suggests there is a potential role for acetate and propionate in regulating plasma lipid profiles through activation of GPR43. In addition, some studies have suggested that FFA2 might play a role in regulation of appetite and metabolism (Sleeth et al., 2010, Psaltopoulou et al., 2010).

There is evidence to suggest a high fibre diet is associated with weight reduction and a lower incidence of diabetes (Psaltopoulou et al., 2010). Several studies have shown that SCFAs produced by colonic fermentation of fibre may be responsible for this through FFA2 activation (Sleeth et al., 2010, Zhou et al., 2008). Moreover, a high fibre diet has been linked with increased levels of PYY, a hormone known to decrease appetite (Karra and Batterham, 2010). Karaki et al. (2008) observed that FFA2 is responsible for PYY secretion in entero-endocrine L-cells and these L-cells are also responsible for GLP-1 secretion, an effective anorectic incretin hormone, which also regulates insulin secretion from pancreatic β-cells and can increase insulin sensitivity in target tissues (Tolhurst et al., 2012). Further evidence for the importance of these cells comes from the work by Tolhurst et al. (2012) who confirmed that FFA2 and FFA3 expression are enriched in L-cells, and FFA2 mediate SCFAs promoted GLP-1 discharge from mixed colonic cultures *in vitro*. These findings suggest that the FFA2 receptor might have potential to be used as a therapeutic for treatment of type 2 diabetes and related metabolic conditions.

Studies have shown that butyrate provides 70 % of the energy required by the colonocytes for cellular respiration, it influences a wide range of cellular functions affecting colonic health; it may also have an anti-carcinogenic and anti-inflammatory potential, and can affect the intestinal barrier and play a role in satiety (Hamer et al., 2008). Further evidence for its anti-cancer properties comes from studies that have reported that Butyrate has a protective effect against colon cancer and adenoma development (Bornet et al., 2002). Several studies have reported that butyrate inhibits proliferation, induces differentiation and apoptosis in CRC cells *in vitro*, at concentrations similar to those found in the large bowel *in vivo* (Fung et al., 2012, Thangaraju et al., 2009). Other studies have shown that high butyrate production reduces the incidence of carcinogen-induced colon tumours in rodent models, partly through induction of apoptosis (Clarke et al., 2008, Le Leu et al., 2009).

The primary mechanisms of butyrate anti-inflammatory action are through the suppression of nuclear factor κB (NFkB) activation, the inhibition of interferon γ production, the upregulation of peroxisome proliferator-activated receptor γ (PPARγ), and possibly through the inhibition of histone deacetylase (HDAC) (Hamer et al., 2008).

The effect of NDCs has been further investigated in intervention studies, for example in ulcerative colitis (UC) patients, which proposed that high intake of NDCs stimulate luminal butyrate production, and may results in an improvement of the inflammation and symptoms (Hallert et al., 2003, Vernia et al., 2003). The study involved twenty two quiescent UC patients being advised to add 20 g of NDCs to their daily diet, and the results showed a significant increase of faecal butyrate concentration, and improvement of abdominal symptoms, after four weeks of NDCs intake (Hallert et al., 2003). In a double-blind, placebo-controlled multicenter trial, Vernia et al. (2003) observed that patients with active distal UC who were treated with rectal enemas containing 5-ASA plus sodium butyrate (80 mM) twice a day, show a significant improvement of the disease activity score, compared with those treated with 5-aminosalicylic acid (5-ASA) alone. These studies suggest butyrate is a useful therapy for the treatment of this condition.

Studies have shown that butyrate and propionate favorably activate FFAR3 more than acetate, which leads to suppressing weight gain and stimulating gut hormones (Gao et al., 2009, Lin et al., 2012). This has been further studied by Lin et al. (2012) who fed FFAR3 knockout mice and wild-type littermates with standard chow diet, and for one week with high fat diet (HFD). They observed that the FFAR3 knockout mice showed no significant difference in body weight compared to their wild-type littermates on standard chow and HFD diets. Then the HFD was supplemented with butyrate and propionate and provided to the mice for eight days. The results showed that the butyrate and propionate inhibited weight gain and food intake in FFAR3 knockouts to the same extent as in wild-type mice. Moreover, Gao et al. (2009) reported that a HFD supplemented with butyrate (5 % wt/wt) reduced obesity and insulin resistance in obese mice; they also observed that the plasma butyrate concentration increased and blood lipids decreased, showing that butyrate has an effect on lipid profiles as well as suppressing weight gain. The results of these studies showed that butyrate and propionate have an impact on reducing body weight and lipids profiles.

Numerous studies have shown that urinary output can be used as functional markers of human vitamin C status for individual and population dietary intake assessments (Carr et al., 2012, Carr et al., 2013). In randomized cross-over study of fifteen male university students for 4 weeks, Carr et al. (2012) examined the effect of a high vitamin C containing fruit intake such as kiwifruit on blood plasma and urine

vitamin C levels and specifically to determine the dosage required to reach ‘healthy’ and ‘optimal’ levels. They observed that one kiwifruit per day was sufficient to achieve ‘healthy’ plasma and urine levels of vitamin C (>50 µmol/l) and with 2 or 3 per day, ‘optimal’ plasma and urine levels of the vitamin were reached. They have suggested that plasma and urinary vitamin C may reflects dietary intake. On the other hand, most of interventional studies in this area have demonstrated that consumption of diets rich in NDCs, such as cereals, fruit and vegetables might be protective against CVD, CRC, obesity, satiety and diabetes, and it is believed that some of the protective effects of NDCs may be attributed to their fermentation to SCFA in the colon by the colonic microbiota (Tan et al., 2013). However, until now these interventional studies have relied on many food composition tables’ old values, due to the lack of new values (Westenbrink et al., 2013b). Additionally, the amount of NDCs that enters the colon is difficult to estimate because we cannot measure SCFA production, not all non-digestible fibres are fully fermented, and some dietary components normally considered to be fully digested and absorbed in the small intestine may actually escape and enter the colon, for example fructose if eaten in large amounts (Cummings, 1997). RS intake is difficult to determine because any handling of starchy foods in the diet, for example mixing with water, heating, homogenization, freezing or cooling, may affect RS content (Sharma et al., 2008). Overall the importance of FC on colonic health remains unrecognized, mainly because there is no validated method to study SCFA production and colonic fermentation *in vivo*, the only way to quantify NDC intake is food tables; however, most FC may be hidden in food tables, not defined or unavailable (Westenbrink et al., 2013b). Therefore, an index of FC value needs to be developed to determine the bioactive fraction and to predict SCFA production in the colon.

This chapter describes the further development of the equations to predict FC in the diet, based on the content of NDCs in foods, their fermentability and an approximation of RS content. This was then compared with urinary SCFA concentrations measured in human volunteers who had kept 7 day dietary diaries.

4.2 Objectives

The objectives of the study were:

1. To develop an index to estimate FC in the diet from dietary records to predict SCFA production in the colon *in vivo* in humans
2. Assessment of the relationship between FC intake and urinary excretion of SCFA products for human volunteers based on their habitual diet

4.3 Methods

4.3.1 Study population

4.3.1.1 Habitual diet study

The data used in this study was obtained from a previous project "Validating *in vivo* biomarkers of colonic carbohydrate and protein fermentation" conducted in 2009 by the Human Nutrition Section, University of Glasgow, and was provided by Professor Christine Edwards, Professor Tom Preston, Dr Douglas Morrison, Dr Laura Hanske and Alexandra C Small. Of 241 healthy volunteers, 77 subjects withdrew their willingness to participate, and 25 subjects met the exclusion criteria, therefore only 122 subjects completed the study (52 males, 70 females, mean age 34.8 years (19 – 64 years), mean body mass index (BMI) 25.5 kg/m^2 (16.7 – 39.9 kg/m^2)) and provided urine collection and dietary records that had satisfactory urinary analysis conducted. The 122 subjects were also examined for under-reporting of dietary intake by using physical activity level (PAL) 1.2 × Basal Metabolic Rate (BMR) equation, calculated from weight according to Schofield (1984) as a cut-off to determine if calculated caloric intake fell below 1.2 × BMR. Of the 122 included in the study, only 78 subjects were considered as valid participants and included in the second group (42 females and 36 males).

4.3.2 Three day weighed record of habitual diet

The results from a three day weighed record of habitual diet, and corresponding urinary excretion of SCFA products, for 122 participants from the general population were included in this study. Diet diaries were analysed for daily energy and macronutrient intake in absolute amounts, as well as the percentage of energy derived from the main macronutrients. This entailed a literature review for contents

of oligosaccharides and resistant starch in foods on websites, as well as other data sources for fibre content. Habitual diet was analysed for FC using equations developed in chapter 3.

4.4 Statistical analysis

Statistical analysis was performed for all data using Minitab statistical software version 16.1.2 (Minitab Inc., State College, Pennsylvania, USA). The normality of the data was tested using the Anderson-Darling test, which showed the data were not-normally distributed. Descriptive statistics are presented as medians and inter quartile range (IQR). Mann-Whitney U test was applied to measure the differences in independent variables between genders and anthropometrics characteristics, genders and FC, genders and the excretion of the urinary SCFA products. Spearman Rank correlation was used to determine rank correlations between different continuous variables such as, DF (AOAC 991.43 method), FC-DF, NSP, FC-NSP, protein and alcohol intake (since alcohol is a potential confounding factor in acetate production) and colonic fermentation biomarkers. Additionally, in the habitual diet study a regression analysis was conducted on the preferred biomarkers to derive an index with confidence, and prediction intervals, to examine the power of predictive markers of FC intake from urinary biomarkers. Any p-values of less than 0.05 were considered significant.

4.5 Results

4.5.1 Anthropometric Characteristics

Dietary records and urine were collected from 241 healthy volunteers who responded to the recruitment advert expressing an interest in the study. Of 241 healthy volunteers, 77 subjects withdrew their willingness to participate and 25 subjects met the exclusion criteria. Of 139 volunteers who had not taken antibiotics for at least three months prior to the study, only 122 subjects completed the study. A total of 122 healthy volunteers (52 males, 70 females, mean age 34.8 years (19 – 64 years), mean BMI is 25.5 kg/m^2 (16.7 – 39.9 kg/m^2)) completed the 24 h urine collection (Table 4.1).

Table 4.1: Characteristics of all study participants (n = 122); values are the median and range.

	Female n = 70		Male n = 52	
	Median	**Range**	**Median**	**Range**
Age (years)	31	19 - 64	30	19 - 57
Height (cm)	165	153 - 181	176	154 – 194 *
Weight (kg)	65.8	46.5 - 109	79.1	58.6 – 112 *
BMI (Kgm^{-2})	24.4	16.7 - 39.9	25	20 - 33.4
Energy (kcal/day)	1770	1073 - 3163	2448	1271 – 4035 *
Protein (g/d)	70.5	29.8 – 119.1	102.4	48.1 – 230.1*
Alcohol (g/d)	0.0	0.0 – 29.2	0.0	0.0 – 61.3

* Mann-Whitney; Statistical significant value ($p < 0.05$)

As mentioned previously, the 122 subjects were also examined for under-reporting of dietary intake by using physical activity level (PAL) 1.2 × BMR equation from weight according to Schofield (1984). Of the 122 in the study, only 78 valid subjects were included (42 females and 36 males) (Table 4.2).

Table 4.2: Characteristics of the valid participants (n= 78); values are the median and range.

	Female n = 42		Male n = 36	
	Median	**Range**	**Median**	**Range**
Age (years)	31.5	19 - 64	31	19 - 57
Height (cm)	165	155 - 176	177	156 – 193 *
Weight (kg)	64.5	46.5 - 109	76.8	58.6 – 113 *
BMI (Kgm^{-2})	23.6	16.8 - 35.2	24	20 - 33.1
Energy (kcal/day)	2054	1542 - 3163	2579	1912 – 4035 *
Protein (g/d)	78.9	50.7 – 119.1	103.7	50.3 – 230.1*
Alcohol (g/d)	2.0	0.0 – 29.2	0.0	0.0 – 61.3

* Mann-Whitney; Statistical significant value ($p < 0.05$)

4.5.2 Characteristics of daily fermentable carbohydrate intake

The results (Table 4.3) for the daily estimated FC intake of all the healthy volunteers (n = 122) in absolute amounts after three days of habitual diet, showed how the different markers measured varied between participants. There was no significant difference in the daily NSP intake between females and males observed after three days of habitual diet intake, whereas DF intake was higher in males compared to females (IQR 9.9; p = 0.03). There was a significantly higher intake of estimated FC-DF 1 (IQR 6.1; p = 0.0001) and FC-NSP 1 (IQR 4.9; p = 0.0001) in males compared with females. After the addition of 50 % insoluble fibre to FC equations, males continued to have higher intake of estimated FC-DF2 (IQR 9.7; p = 0.0001) and FC-NSP 2 (IQR 8.1; p = 0.0001) compared to females. Moreover, the estimated FC-DF 3 (IQR 7.6; p = 0.0001) and FC-DF 4 (IQR 11.7; p = 0.0001) and FC-NSP 3 (IQR 7.0; p = 0.0001) and FC-NSP 4 (IQR 10.3; p = 0.0001) intakes were also higher in males than females. This suggests that the diets differed between the males and females in this study.

Table 4.3: Characteristics of all the study participants and predicted fermentable carbohydrate intake (n = 122); values are the median and range.

	Female n = 70		Male n = 52		
	Median	IQR	Median	IQR	p value*
DF (AOAC; g/d)	18.5	11.8	22.6	9.9	0.03
FC-DF 1æ (g/d)	12.4	5.5	16.6	6.1	0.0001
FC-DF 2Θ (g/d)	21.3	9.4	26.4	9.7	0.0001
FC-DF 3¥ (g/d)	18.7	8.5	25.2	7.6	0.0001
FC-DF 4§ (g/d)	27.9	12.2	34.5	11.7	0.0001
NSP (Southgate; g/d)	17.2	8.4	18.7	8.7	0.06
FC-NSP 1Æ (g/d)	12.6	4.4	16.8	4.9	0.0001
FC-NSP 2ф (g/d)	18.8	6.11	24.7	8.1	0.0001
FC-NSP 3Ж (g/d)	18.7	8.7	25.9	7.0	0.0001
FC-NSP 4Л (g/d)	25.5	8.9	33.3	10.3	0.0001

æ FC-DF1 = soluble DF + 10% insoluble DF + oligosaccharide + 5% TS; Æ FC-NSP1 = soluble NSP + 10% insoluble NSP + oligosaccharide + 5% TS; Θ FC-DF2 = soluble DF + 50% insoluble DF + oligosaccharide + 5% TS; ф FC-NSP2 = soluble NSP + 50% insoluble NSP + oligosaccharide + 5% TS; ¥ FC-DF3 = soluble DF + 10% insoluble DF + oligosaccharide + 10% TS; Ж FC-NSP3 = soluble NSP + 10% insoluble NSP + oligosaccharide + 10% TS; § FC-DF4 = soluble DF + 50% insoluble DF + oligosaccharide + 10% TS; Л FC-NSP4 = soluble NSP + 50% insoluble NSP + oligosaccharide + 10% TS; * Mann-Whitney; Statistical significant value ($p < 0.05$)

The daily estimated FC intake of the valid participants (n = 78), after three days of habitual diet showed no significant difference in the daily DF and NSP intake between females and males (Table 4.4). It was observed that there was a significant difference in the intakes of estimated FC-DF 1 (IQR 5.9; p = 0.0001) and FC-NSP 1 (IQR 4.3; p = 0.0001) between males and females. After the addition of 50 % insoluble fibre to FC equations, males continued to have a higher intake of estimated FC-DF2 (IQR 11.9; p = 0.0003) and FC-NSP 2 (IQR 7.1; p = 0.0001) compared to females. Similarly, the estimated FC-DF 3 (IQR 7.4; p = 0.0001) and FC-DF 4 (IQR 12.9; p = 0.0001) and FC-NSP 3 (IQR 5.7; p = 0.0001) and FC-NSP 4 (IQR 8.8; p = 0.0001), which contained 10 % of total starch, showed that male subjects have higher FC intakes than females.

Table 4.4: Characteristics of the valid participants and predicted fermentable carbohydrate intake (n = 78); values are median and range.

	Female n = 42		Male n = 36		
	Median	**IQR**	**Median**	**IQR**	**p value***
DF (AOAC; g/d)	22	8.2	22.9	11.3	0.11
FC-DF 1æ (g/d)	13.6	4.0	16.7	5.9	0.0001
FC-DF 2Ɵ (g/d)	21.7	9.0	26.7	11.8	0.0003
FC-DF 3¥ (g/d)	20.8	6.6	25.5	7.4	0.0001
FC-DF 4§ (g/d)	29.4	12.2	36.0	12.9	0.0001
NSP (Southgate; g/d)	18.6	7.4	19.9	11.2	0.12
FC-NSP 1Æ (g/d)	13.6	2.8	17.7	4.3	0.0001
FC-NSP 2ф (g/d)	20.1	6.6	24.9	7.1	0.0001
FC-NSP 3Ж (g/d)	20.8	4.4	26.4	5.7	0.0001
FC-NSP 4Л (g/d)	26.5	8.5	33.3	8.8	0.0001

æ FC-DF1 = soluble DF + 10% insoluble DF + oligosaccharide + 5% TS; Æ FC-NSP1 = soluble NSP + 10% insoluble NSP + oligosaccharide + 5% TS; Ɵ FC-DF2 = soluble DF + 50% insoluble DF + oligosaccharide + 5% TS; ф FC-NSP2 = soluble NSP + 50% insoluble NSP + oligosaccharide + 5% TS; ¥ FC-DF3 = soluble DF + 10% insoluble DF + oligosaccharide + 10% TS; Ж FC-NSP3 = soluble NSP + 10% insoluble NSP + oligosaccharide + 10% TS; § FC-DF4 = soluble DF + 50% insoluble DF + oligosaccharide + 10% TS; Л FC-NSP4 = soluble NSP + 50% insoluble NSP + oligosaccharide + 10% TS; * Mann-Whitney; Statistical significant value ($p < 0.05$)

4.5.3 Urinary excretion output of SCFAs

The 24 h urinary excretion output of SCFA products of all the participants in the study of healthy volunteers (n = 122) (Table 4.5), showed that the 24 h urinary excretion output acetate was higher (IQR 377.8; p = 0.06) in males. The 24 h urinary excretion output SCFA was also higher (IQR 379.1; p = 0.06) in males compared to females, but the difference did not reach statistical significance. The 24 h urinary excretion of propionate was higher (IQR 5.0; p = 0.008) in males compared to females. In addition, the 24 h urinary excretion output BCFA was also higher (IQR 20.9; p = 0.002) in males than females. On the other hand, the 24 h urinary excretion of butyrate did not show any difference between males and females (IQR 1.5; p = 0.92).

Table 4.5: Total daily excretion of 24 h urinary SCFA products (µmol/24 h) of all study participants (n=122); values are median and range.

	Female n = 70		Male n = 52		
	Median	**IQR**	**Median**	**IQR**	**p value***
Acetate 24h	445.4	292.3	511.9	377.8	0.06
Propionate 24h	6.4	3.9	8.2	5.0	0.008
Butyrate 24h	1.0	1.5	1.0	1.4	0.92
SCFA 24h	451.8	298.0	519.0	379.1	0.06
BCFA 24 h	21.5	21.5	28.6	20.9	0.002
SCFA: BCFA 24 h	20.17	20.2	18.6	10.8	0.08
Acetate: 2mB 24 h	46.0	30.5	41.5	24.5	0.24
Acetate: 3mB 24 h	82.8	42.0	73.5	34.8	0.12

* Mann-Whitney; Statistical significant value ($p < 0.05$)

However, the 24 h urinary excretion of acetate (Table 4.6) was higher (IQR 395.4; $p = 0.04$) and the 24 h urinary excretion of SCFA was also higher (IQR 397.2; $p = 0.04$) in males compared to females, for the valid subjects (n = 78). The 24 h urinary excretion of propionate was also higher (IQR 5.4; $p = 0.01$) in males compared to females. Moreover, the 24 h urinary excretion output BCFA was also higher (IQR 18.8; $p = 0.001$) in males than females. The 24 h urinary excretion of butyrate was higher in males compared to females, but still did not show any significant difference (IQR 1.6; $p = 0.66$).

Table 4.6: Total daily excretion of 24 h urinary SCFA products (µmol/24 h) of the valid participants' outputs (n = 78); values are median and range

	Female n = 42		Male n = 36		
	Median	**IQR**	**Median**	**IQR**	**p value***
Acetate 24 h	454.9	266.2	527.7	395.4	0.04
Propionate 24 h	6.4	3.5	8.2	5.4	0.01
Butyrate 24 h	1.0	1.4	1.0	1.6	0.66
SCFA 24 h	460.3	270.3	537.1	397.2	0.04
BCFA 24 h	23.7	20.6	28.5	18.8	0.001
SCFA: BCFA 24 h	21.5	11.8	18.7	11.4	0.07
Acetate: 2mB 24 h	46.9	32.3	42.0	24.1	0.18
Acetate: 3mB 24 h	82.8	46.2	69.9	44.5	0.09

* Mann-Whitney; Statistical significant value ($p < 0.05$)

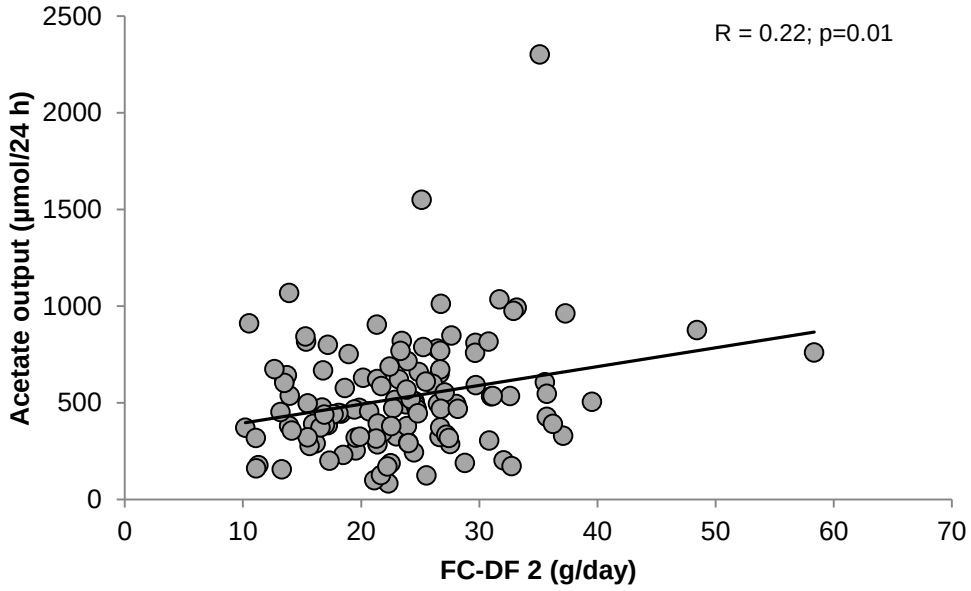
4.5.4 Relationships between fermentable carbohydrate and urinary excretion of SCFA products

The study data were analysed for all healthy volunteers (n = 122) and study valid participants (n = 78). The Spearman correlation coefficient (R) was used to determine the relationship between FC variables and urinary biomarkers of colonic fermentation.

4.5.4.1 Relationship between DF, FC-DF and urinary biomarkers of colonic fermentation (n = 122)

In all study participants (n = 122) the results (Table 4.7) showed that data analysis confirmed the predicted relationships between DF (AOAC 991.43 method), FC-DF, NSP, FC-NSP, protein and alcohol intake (since alcohol is a potential confounder in acetate production) and colonic fermentation biomarkers. The predicted FC-DF 1 showed no relationship with the 24 h urinary excretion of colonic fermentation biomarkers. In FC-DF 2, the addition of 50 % insoluble fibre improved the relationship between FC and the 24 h urinary excretion of acetate (R = 0.22; p = 0.01; Figure 4.1) and SCFA (R = 0.21; p = 0.01), whereas no relationship with other SCFA products was seen.

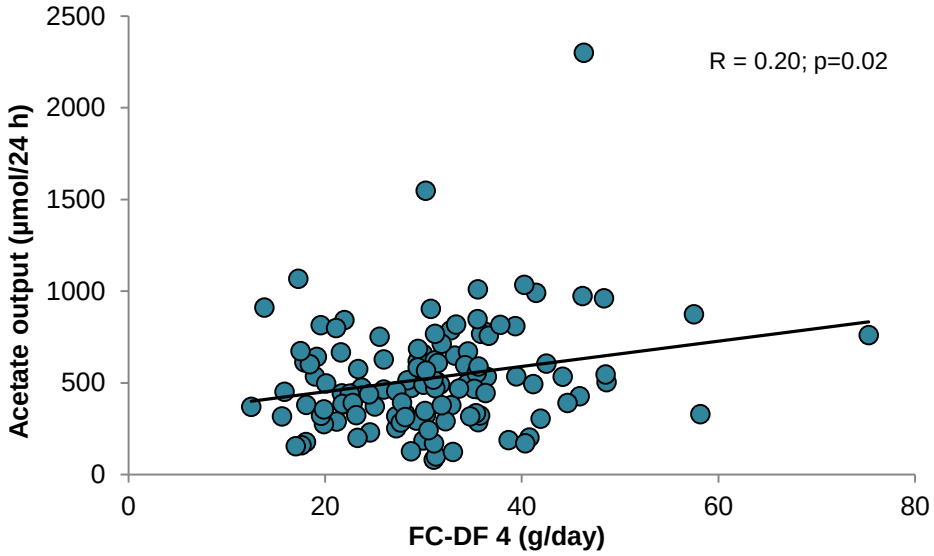
Figure 4.1: Scatter-plot showing correlation of FC-DF2 with urinary excretion of acetate (µmol/24 h) of all participants.



R; Spearman rank correlation, FC-DF2; fermentable carbohydrate-dietary fibre 2. P-value based on Spearman rank correlation.

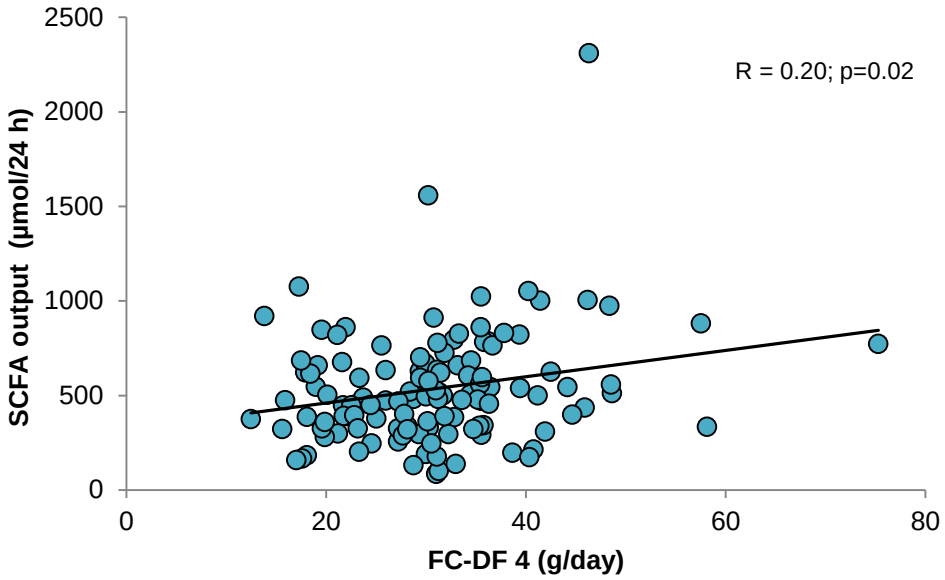
The relationship between the predicted FC-DF 3 and the 24 h urinary excretion of colonic fermentation biomarkers was similar to the relationship reported by FC-DF 1, which could be due to the presence of only 10 % of insoluble fibre in the FC-DF 3 equation. When 50 % insoluble fibre was combined with 10 % of TS in the predicted FC-DF 4 equation (Table 4.7), a significant positive relationship was observed between FC and the 24 h urinary excretion of acetate (R = 0.20; p = 0.02; Figure 4.2) and SCFA (R = 0.20; p = 0.02; Figure 4.3), whereas there was no relationship was observed with the other SCFA products. There was also a significant correlation between DF and the 24 h urinary acetate and SCFA.

Figure 4.2: Scatter-plot showing correlation of FC-DF 4 with urinary excretion of acetate (µmol/24 h) of all participants.



R; Spearman rank correlation, FC-DF4; fermentable carbohydrate-dietary fibre 4. P-value based on Spearman rank correlation.

Figure 4.3: Scatter-plot showing correlation of FC-DF 4 with urinary excretion of SCFA (µmol/24 h) of all participants.

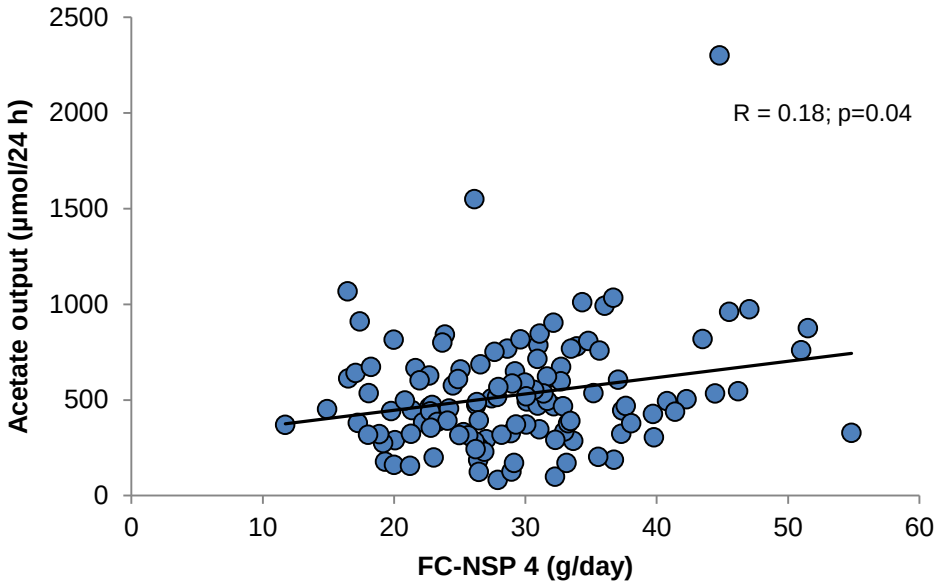


R; Spearman rank correlation, FC-DF4; fermentable carbohydrate-dietary fibre 4. SCFA; short chain fatty acid. P-value based on Spearman rank correlation.

4.5.4.1 Relationship between NSP and FC-NSP and urinary biomarkers of colonic fermentation (n = 122)

Data from all the study participants (n = 122) showed a higher correlation between the predicted FC-NSP and the urinary colonic fermentation biomarkers. There was a significant positive relationship between FC-NSP 1 and 24 h urinary excretion of acetate (R = 0.19; p = 0.03) and SCFA (R = 0.19; p = 0.03). The predicted FC-NSP 2 also showed a positive correlation with the 24 h urinary excretion of acetate (R = 0.22; p = 0.01) and SCFA (R = 0.22; p = 0.01); while there was no relationship observed between FC-NSP 3 and the 24 h urinary biomarkers when only 10 % of insoluble fibre was added to the predicted FC-DF3 equation (Table 4.7). The predicted FC-NSP 4 was positively correlated with the 24 h urinary excretion of acetate (R = 0.18; p = 0.04) and SCFA (R = 0.18; p = 0.04; Figure 4.4). There was a significant positive relationship between the protein intake and BCFA output in the urine (R = 0.45; p < 0.01). Moreover, a regression predictive model was developed to assess the association between predictive FC equations and urinary biomarker, this showed there was a positive significant relationship between FC-DF 4, FC-NSP 4 and 24 h urinary excretion of acetate.

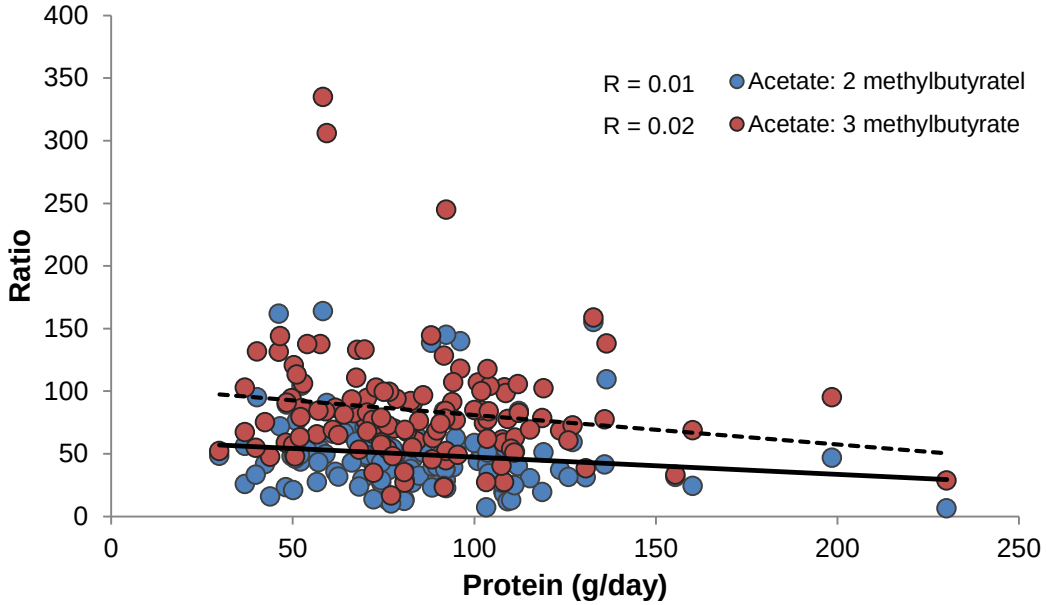
Figure 4.4: Scatter-plot showing correlation of FC-NSP 4 with urinary excretion of acetate (µmol/24 h) of all participants.



R; Spearman rank correlation, FC-NSP 4; fermentable carbohydrate-non starch polysaccharide 4. P-value based on Spearman rank correlation.

The data from all the study participants (n = 122) showed a negative relationship between protein vs. acetate: 2-methylbutyrate, protein vs. acetate: 3-methylbutyrate (Figure 4.5) and protein vs. SCFA: BCFA. On the other hand, an analysis of the data by a multivariate regression model of protein intake and iso-butyrate ($p < 0.001$) and 2-methylbutyrate ($p = 0.003$) as protein urinary excretion biomarkers, with FC-DF and FC-NSP as covariates, showed no significant effect for FC intake on the excretion of biomarkers related to protein intake. However, when the 24 h urinary excretion of acetate was added to the multivariate regression model as a urinary excretion biomarker of FC, there was a significant effect on iso-butyrate ($p = 0.005$) and intake protein disappeared. This effect of the 24 h urinary excretion of acetate (as the predominant SCFA product) might provide evidence that increasing FC intake could decrease protein fermentation products.

Figure 4.5: Scatter-plot showing correlation between protein intake vs. acetate: 3-methylbutrate (dotted line) and acetate: 2-methylbutyrate ratios (solid line).



R; Spearman rank correlation

Table 4.7: Correlation between dietary fibre (AOAC), NSP, FC and urinary biomarkers of colonic fermentation of all the study participants (n = 122).

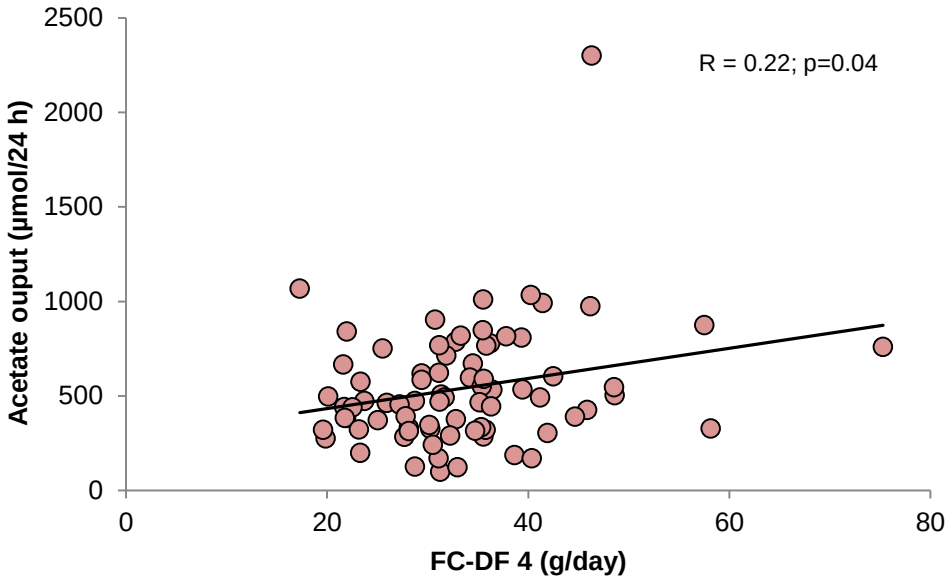
	Correlation*	Acetate	Propionate	Butyrate	SCFA	BCFA	SCFA: BCFA	Acetate:2mB	Acetate:3mB	Iso-butyrate	2mBψ	3mBφ
DF	*R*	0.20	0.04	0.02	0.19	0.07	0.21	0.20	0.13	0.03	0.04	0.15
	p	**0.02**	NS	NS	**0.03**	NS	**0.02**	**0.02**	NS	NS	NS	NS
FC-DF1	*R*	0.15	0.08	0.00	0.15	0.11	0.03	0.06	-0.04	0.09	0.09	0.20
5 % TS & 10 % insoluble DF	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.02**
FC-DF2	*R*	0.22	0.11	0.03	0.21	0.11	0.14	0.15	0.07	0.07	0.08	0.21
5 % TS & 50 % insoluble DF	*P*	**0.01**	NS	NS	**0.01**	NS	NS	NS	NS	NS	NS	**0.02**
FC-DF3	*R*	0.14	0.09	0.01	0.14	0.11	0.01	0.04	-0.04	0.08	0.10	0.19
10 % TS & 10 % insoluble DF	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FC-DF4	*R*	0.20	0.12	0.04	0.20	0.12	0.11	0.11	0.04	0.08	0.10	0.21
10 % TS & 50 % insoluble DF	*p*	**0.02**	NS	NS	**0.02**	NS	NS	NS	NS	NS	NS	NS
NSP	*R*	0.22	0.04	0.02	0.22	0.08	0.22	0.22	0.15	0.04	0.06	0.17
	p	**0.01**	NS	NS	**0.01**	NS	**0.01**	**0.01**	NS	NS	NS	NS
FC-NSP1	*R*	0.19	0.17	-0.03	0.19	0.13	0.05	0.08	0.01	0.15	0.09	0.18
5 % TS &10 % insoluble NSP	*p*	**0.03**	NS	NS	**0.03**	NS	NS	NS	NS	NS	NS	**0.04**
FC-NSP2	*R*	0.22	0.15	0.01	0.22	0.10	0.15	0.16	0.09	0.10	0.08	0.17
5 % TS & 50 % insoluble NSP	*p*	**0.01**	NS	NS	**0.01**	NS	NS	NS	NS	NS	NS	NS
FC-NSP3	*R*	0.16	0.15	0.00	0.16	0.13	0.03	0.06	0.00	0.13	0.10	0.17
10 % TS & 10 % insoluble NSP	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FC-NSP4	*R*	0.18	0.13	0.02	0.18	0.10	0.11	0.12	0.06	0.08	0.08	0.17
10 % TS & 50 % insoluble NSP	*p*	**0.04**	NS	NS	**0.04**	NS	NS	NS	NS	NS	NS	NS
Protein	*R*	0.30	0.31	0.02	0.31	0.47	-0.20	-0.18	-0.13	0.49	0.39	0.48
	p	**0.00**	**0.00**	NS	**0.00**	**0.00**	NS	NS	NS	**0.00**	**0.00**	**0.00**
Alcohol	*R*	0.23	0.04	0.02	0.23	0.09	0.15	0.12	0.14	0.07	0.10	0.11
	p	**0.01**	NS	NS	**0.01**	NS	NS	NS	NS	NS	NS	NS

BCFA, Branched chain fatty acid; SCFA, Short chain fatty acid; ψ2mB, 2-methylbutyrate; φ3mB, 3-methylbutyrate; *R, Spearman correlation coefficients

4.5.4.2 Relationship between DF, FC-DF and urinary biomarkers of colonic fermentation in the valid participants (n =78)

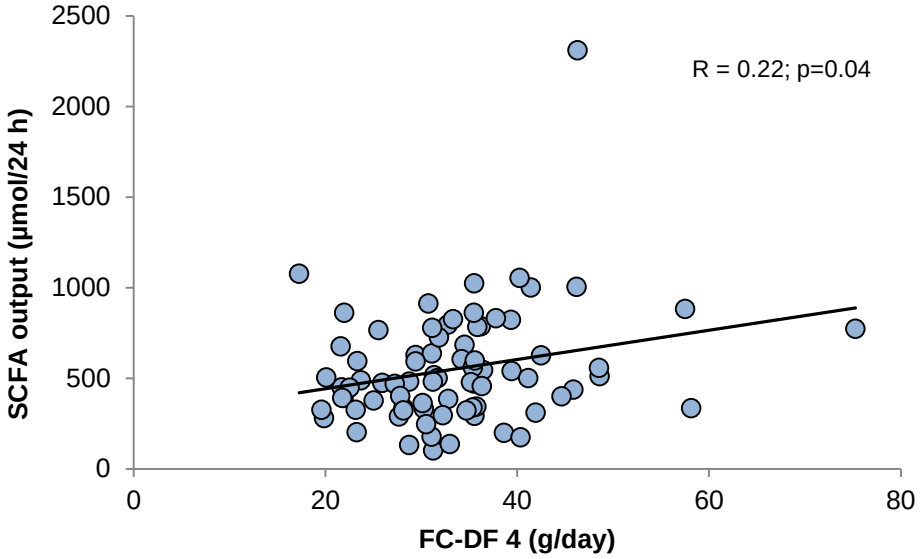
The data of valid participants (n = 78) were analysed to determine the relationships between DF (AOAC 991.43 method), FC-DF, NSP, FC-NSP, protein intake, alcohol intake, and colonic fermentation biomarkers (Table 4.8). Data analysis showed no relationship between DF and the 24 h urinary excretion of colonic fermentation. The only positive relationship that was observed was between DF and urinary excretion of SCFA: BCFA (R = 0.27; p = 0.01). The predicted FC-DF 1 showed a positive relationship with 3-methylbutraye (R = 0.31; p < 0.01). FC-DF 2 and FC-DF 3 showed no relationship with the 24 h urinary excretion of colonic fermentation biomarkers of FC, whereas FC-DF 3 showed a significant positive relationship with 3-methylbutyrate (R = 0.29; p < 0.01). Only FC-DF 4 with the addition of 50 % insoluble fibre and 10 % of TS showed a significant positive relationship with the 24 h urinary excretion of acetate (R = 0.22; p = 0.04; Figure 4.6) and SCFA (R = 0.22; p = 0.04; Figure 4.7); whereas no relationship with other SCFA products was seen.

Figure 4.6: Scatter-plot showing correlation of FC-DF 4 with urinary excretion of acetate (µmol/24 h) of valid participants (n = 78).



R; Spearman rank correlation, FC-DF4; fermentable carbohydrate-dietary fibre 4. P-value based on Spearman rank correlation.

Figure 4.7: Scatter-plot showing correlation of FC-DF 4 with urinary excretion of SCFA (µmol/24 h) of valid participants (n = 78).

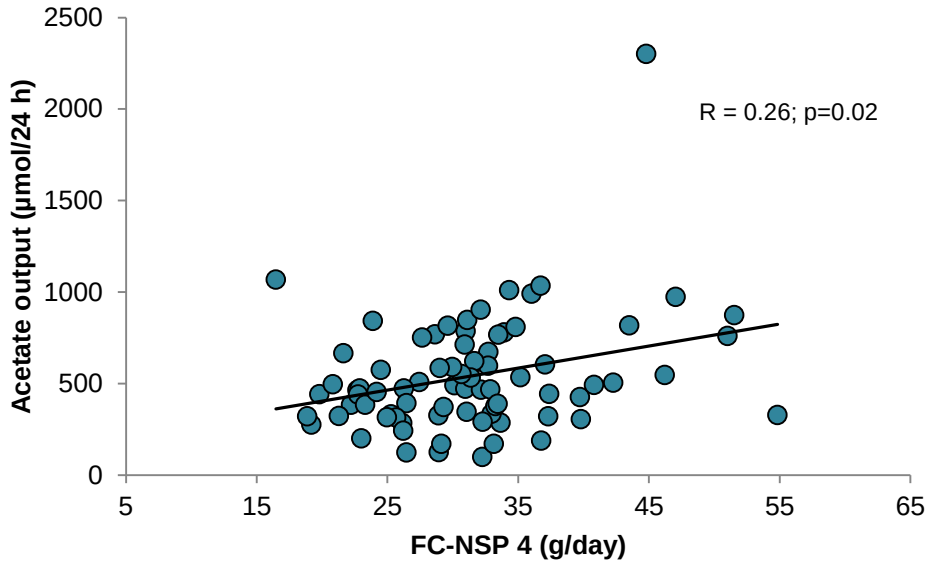


R; Spearman rank correlation, FC-DF4; fermentable carbohydrate-dietary fibre 4. P-value based on Spearman rank correlation.

4.5.4.3 Relationship between NSP and FC-NSP and urinary biomarkers of colonic fermentation in the valid participants (n = 78)

The data of valid participants (n = 78) showed a higher correlation between the predicted FC-NSP and urinary colonic fermentation biomarkers than all participants (n = 122). There was a significant positive relationship between FC-NSP 1 and the 24 h urinary excretion of acetate (R = 0.29; p = 0.01), SCFA (R = 0.29; p = 0.01) and propionate (R = 0.26; p = 0.02). The predicted FC-NSP 2 also showed a positive correlation with 24 h urinary excretion of acetate (R = 0.29; p = 0.01) and SCFA (R = 0.29; p = 0.01). There was also a relationship between FC-NSP 3 and the 24 h urinary FC biomarker of acetate (R = 0.27; p=0.01), SCFA (R = 0.27; p = 0.01) and propionate (R = 0.22; p = 0.05). There was positive relationship between predicted FC-NSP 1 and 2-methylbutrate (R = 0.25; p = 0.03); protein fermentation metabolites and FC-NSP 3 also correlated with 3-methylbutyrate excretion (R = 0.25; p = 0.03). The predicted FC-NSP 4 positively correlated with the 24 h urinary excretion of acetate (R = 0.26; p = 0.02; Figure 4.8) and SCFA (R = 0.26; p = 0.02). There was a significant positive relationship between protein intake and BCFA output in the urine (R = 0.45; p < 0.01). A regression predictive model was developed to assess the association between the predictive FC equations and urinary biomarker, the results showed there was a positive significant relationship between FC-DF 4, FC-NSP 4 and the 24 h urinary excretion of acetate.

Figure 4.8: Scatter-plot showing correlation of FC-NSP 4 with urinary excretion of acetate (µmol/24 h) of valid participants (n = 78).



R; Spearman rank correlation, FC-NSP 4; fermentable carbohydrate of non-starch polysaccharide 4. P-value based on Spearman rank correlation.

The data of valid participants (n = 78) showed an inverse relationship between protein vs. acetate: 2-methylbutyrate, protein vs. acetate: 3-methylbutyrate and protein vs. SCFA: BCFA. On the other hand, an analysis of the data by a multivariate regression model of protein intake and iso-butyrate (p = 0.005) and 2-methylbutyrate (p = 0.02) as protein urinary excretion biomarkers, with FC-DF and FC-NSP as covariates, showed no significant effect of FC intake on excretion of these biomarkers of protein intake. Even when the 24 h urinary excretion of acetate was added to the multivariate regression model as a urinary excretion biomarker of FC, there was still no significant relationship between 2-methylbutyrate (p = 0.085) and 3-methylbutyrate (p = 1.00) and protein intake. This negative relationship might provide evidence that increasing FC intake causes a reduction in the protein fermentation metabolite productions.

Table 4.8: Correlation between Dietary fibre (AOAC), NSP and FC and urinary biomarkers of colonic fermentation of the valid participants (n = 78).

	Correlation*	Acetate	Propionate	Butyrate	SCFA	BCFA	SCFA: BCFA	Acetate:2mB	Acetate:3mB	Iso-butyrate	2mBψ	3mBφ
DF	*R*	0.13	0.00	0.06	0.13	-0.04	0.27	0.21	0.20	-0.10	-0.04	0.06
	p	NS	NS	NS	NS	NS	**0.01**	NS	NS	NS	NS	NS
FC-DF1	*R*	0.20	0.09	-0.03	0.20	0.19	0.00	0.03	-0.08	0.11	0.17	0.31
5 % TS & 10 % insoluble DF	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.00**
FC-DF2	*R*	0.21	0.12	0.06	0.21	0.10	0.17	0.13	0.09	0.01	0.08	0.22
5 % TS & 50 % insoluble DF	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FC-DF3	*R*	0.20	0.10	-0.02	0.20	0.19	0.01	0.03	-0.05	0.09	0.17	0.29
10 % TS & 10 % insoluble DF	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.00**
FC-DF4	*R*	0.22	0.14	0.08	0.22	0.14	0.13	0.09	0.07	0.04	0.13	0.24
10 % TS & 50 % insoluble DF	*p*	**0.04**	NS	NS	**0.04**	NS	NS	NS	NS	NS	NS	0.03
NSP	*R*	0.14	-0.03	0.06	0.14	0.00	0.23	0.18	0.18	-0.08	0.00	0.06
	p	NS	NS	NS	NS	NS	**0.03**	NS	NS	NS	NS	NS
FC-NSP1	*R*	0.29	0.26	-0.08	0.29	0.20	0.05	0.10	0.02	0.20	0.15	0.25
5 % TS & 10 % insoluble NSP	*p*	**0.01**	**0.02**	NS	**0.01**	NS	NS	NS	NS	NS	NS	**0.03**
FC-NSP2	*R*	0.29	0.16	0.03	0.29	0.10	0.22	0.19	0.16	0.04	0.08	0.18
5 % TS & 50 % insoluble NSP	*p*	**0.01**	NS	NS	**0.01**	NS	**0.04**	NS	NS	NS	NS	NS
FC-NSP3	*R*	0.27	0.22	-0.04	0.27	0.20	0.05	0.08	0.03	0.17	0.17	0.25
10 % TS & 10 % insoluble NSP	*p*	**0.01**	**0.05**	NS	**0.01**	NS	NS	NS	NS	NS	NS	**0.03**
FC-NSP4	*R*	0.26	0.16	0.05	0.26	0.12	0.16	0.13	0.12	0.05	0.11	0.19
10 % TS & 50 % insoluble NSP	*p*	**0.02**	NS	NS	**0.02**	NS	NS	NS	NS	NS	NS	NS
Protein	*R*	0.33	0.30	0.08	0.33	0.47	-0.16	-0.12	-0.11	0.50	0.38	0.48
	p	**0.00**	**0.00**	NS	**0.00**	**0.00**	NS	NS	NS	**0.00**	**0.00**	**0.00**
Alcohol	*R*	0.26	0.04	0.08	0.26	0.06	0.22	0.16	0.19	0.03	0.09	0.08
	p	**0.02**	NS	NS	**0.02**	NS	NS	NS	NS	NS	NS	NS

BCFA: Branched chain fatty acid; SCFA Short chain fatty acid; ψ2mB, 2-methylbutyrate; φ3mB, 3-methylbutyrate; *R, Spearman correlation coefficients

4.6 Discussion

Several studies have demonstrated that long term consumption of diets rich in NDCs, such as cereals, fruit and vegetables might be protective against CVD (Estruch et al., 2009), CRC (Bingham et al., 2003), obesity (Bazzano et al., 2005) and diabetes (Lindström et al., 2006). It is proposed that some of the protective effects of NDCs may be attributed to their fermentation to SCFA in the colon by the colonic microbiota (Edwards et al., 1996). However, these studies use traditional dietary analysis methods which depend on old food composition databases, which may not have been updated to include all FC sources and so do not provide accurate data on NDCs intake whether or not it is fermented (Westenbrink et al., 2013b). Therefore, this study describes a comparison between our predictive equations developed to estimate the amount of potential FC from dietary records of secondary analysis study combined with urinary excretion of SCFA.

Our results for both data analyses (n = 122) and (n = 78), showed weak but significant correlation between the 24 h urinary excretion of acetate and estimated FC-DF 4 and FC-NSP 4. In the same manner, the 24 h urinary excretion of SCFA was correlated with estimated FC-DF 4 and FC-NSP 4. It was also observed that FC-DF and FC-NSP had positive correlations with 24h urinary acetate and SCFA compared with DF and NSP alone. Hence, it could be hypothesised that using developed index to estimate FC in the diet form dietary records, might predict SCFA production in the colon *in vivo* in humans. There are a number of potential reasons that might explain the weak correlation between predicted FC intake and 24 h urinary excretion of colonic metabolites.

Firstly, this study examined habitual NDCs intake, which possibly represents the difficulty in assessing FC from dietary intake data, because participants are likely to eat a wide variety of DF, which not all may be readily fermentable, for example wheat bran. On the other hand, the supplementation/trial studies clearly added known and relatively well controlled amounts of fermentable NDCs, for example a randomized crossover block-design study carried out by Muir et al. (2004), fed 12 subjects three diets of wheat bran (WB 12 g/d), WBRS combined diet (WB 12 g/d and RS 22 g/d) or control for three weeks. They found a higher faecal concentration of acetate output (101 %) in the WBRS combined diet compared with WB alone and the control. Ahmed et al. (2000) examined colonic SCFA formation in patients with a

left-sided colectomy. They found a higher SCFA production, and in particular butyrate production after consumption of a specific mixture of high RS diet as compared with a low RS diet. Moreover, this is similar to the fermentation found in rats fed with various single fibre sources, including wheat barn and high amylose cornstarch, or in combinations for 13 days (Henningsson et al., 2002). Henningsson et al. (2002) observed that rats fed with known amount of high amylose cornstarch with wheat barn produced higher faecal excretion of total SCFA (184 ± 19 μmol/d), than rats fed with a single fibre source high amylose cornstarch (77 ± 10 μmol/d) or wheat barn (116 ± 12 μmol/d), which also showed the lowest SCFA producer was wheat bran which is comprised of > 80 % insoluble DF. Additionally, Le Leu et al. (2002) reported that when RS was combined with wheat bran in a rat diet, there was a high production total SCFA was observed. These findings further support that combined known amount of fermentable NDCs showed predictable higher SCFA concentration than habitual diets.

The second reason that might explain the weak correlation between predicted FC intake and 24 h urinary excretion of colonic metabolites, is that approximately 95 % of all SCFA (acetate, propionate and butyrate) components are rapidly absorbed by the colonocytes, while the remaining 5 % are excreted in the faeces. All the metabolites are rapidly absorbed across the colonic epithelium, mediated by the specific cellular transport protein mono-carboxylate transporter isoform 1 (MCT-1) (Miller and Wolin, 1996). However, once they are transported out of the lumen of the colon, each SCFA can have a different fate; acetate and propionate pass through almost entirely into the portal circulation, whereas butyrate is considered as the major fuel driving energy transduction in colonocytes and therefore an unknown quantity reaches the portal circulation (Kimura et al., 2014). Once in the portal circulation, the SCFAs will be transported to the liver where only acetate escapes the first pass of hepatic metabolism to any significant degree; approximately 40 to 60 % of acetate may spill over into the general circulation, whereas only a limited amount of propionate and a very small amount of butyrate appear systemically (Layden et al., 2013). These results suggest that excretion rates are a poor measure of dietary intake.

These results suggest that acetate would to be the ideal systemic marker for NDCs fermentation in the colon, but the fate of hepatic splitting of acetate is complex and not yet completely understood (den Besten et al., 2013). Research has suggested

that approximately 70 % of the acetate production, which is exogenous acetate formed by colonic bacterial fermentation of NDCs and endogenous acetate, rather than formed in the liver during fatty acid oxidation, is taken up by the liver (Tan et al., 2013). The liver uses acetate as an energy source; but also as a substrate for the synthesis of cholesterol and long-chain fatty acids, and as a co-substrate for glutamine and glutamate synthesis; other tissues, including the heart, adipose tissue, kidney and skeletal muscle, metabolize the remainder of the acetate (Bloemen et al., 2009, Layden et al., 2013). It would be predicted that endogenous levels of acetate would scale with the metabolic rate, which would remain fairly constant and independent of NDCs intake, while exogenous acetate would relate to NDCs fermentation, and therefore would be variable depending on NDCs intake. This also might explain why the urinary excretion of SCFA is not well validated to reflect colonic production of SCFA, and the low level of urinary excretion of acetate in the study participants' urine samples.

Thirdly, under-reporting of food intake is one of the main obstacles that inhibit the collection of accurate habitual dietary intake data, which is often used to investigate the relationship between diet and health. Macdiarmid and Blundell (1998) stated that under-reporting ranged between 18 % and 54 % in the large nutritional surveys when considering the whole study group, and it could reach up to 70 % in some subgroups. In particular, women were more likely to under-report their daily dietary intake than men, and under-reporting is also more common among overweight and obese individuals. In our study from all 122 healthy volunteers (n = 122), 36 % under-reported their dietary intake. Of the 36 % under-reporting their dietary intake, 57 % were female, whereas from the data of valid participants (n = 78) 53.8 % were female. This is further supported by a study conducted by Goran and Poehlman (1992), who assessed the relationship between body composition and energy intake in six women and seven men aged 56 - 78 years. They observed that women were underreporting their intake by 32 % and men were underreporting their intake by 13 %. Moreover, Conway et al. (2003) recruited forty nine normal weight, overweight and obese women aged 21 - 65 years old to assess validity of the US Department of Agriculture (USDA) 5-step multiple-pass method for 24 h dietary recall. They observed that normal weight and overweight women significantly ($p < 0.01$) overestimated their energy, protein and carbohydrate intake.

The analysis of the 24 h urinary excretion outputs of SCFA products of all healthy volunteers (n = 122) and those valid participants only (n = 78) were higher in males compared to females, this can partly by explained by the frequent daily bowel movements in the females compared to the males, as this may account for the lower urinary SCFA product excretion in females. As the majority of NDCs are fermented in the colon, transit time can influence changes in the microbiota and SCFA absorption, which may impact SCFA availability and metabolism (Lampe et al., 1993b). There are several studies that have suggested a longer transit time in women compared to men (Lampe et al., 1993a, Fisher and Yousef, 1973, Gear et al., 1981, Degen and Phillips, 1996). Lampe et al. (1993) stated that men displayed faster transit time ($p = 0.02$) and greater stool bulk ($p = 0.005$) than women when they consumed identical dietary fibre, and men excreted more bile acid in the faeces than women ($p < 0.05$) (Lampe et al., 1993a). Other studies have reported similar results even when men and women consumed their habitual diet (Metcalf et al., 1987, Davies et al., 1986, Kelsay and Clark, 1984, Rao et al., 1987). Faecal output was reported to be positively correlated, and transit time negatively correlated, with energy intake and food consumption (Eastwood et al., 1984, Kelsay and Clark, 1984).

The data from all study participants (n = 122) and valid participants (n = 78) showed that formation of SCFA from high FC intake might decrease production of protein fermentation products. It is believed that high FC intake might reduce the production of toxic metabolites in the large intestinal derived from the fermentation of fermentable proteins in humans and animals (Ross et al., 2013, Pieper et al., 2012). This has been shown in a study of seventeen healthy adults who participated in randomized crossover trial and received either wheat grain or refined grain for two weeks. The results showed that wheat grain increased acetate and butyrate production and decreased urinary excretion of protein fermentation products (Ross et al., 2013). Moreover, Pieper et al. (2012) showed that feeding thirty two weaned piglets a high FC diet increased the acetate to propionate ratio toward acetate ($p < 0.05$), and reduced ammonia ($p < 0.05$) production. Overall these studies show that formation of SCFA derived from fermentable NDCs play a role in reducing the production of toxic metabolites in the large intestinal derived from the fermentation of fermentable proteins.

In conclusion, the data of this study showed a weakly significant correlation between the 24 h urinary excretion of acetate and SCFA, and estimated FC-DF and FC-NSP. FC-DF and FC-NSP also had positive correlations with 24 h urinary acetate and SCFA compared to DF and NSP alone. The weak correlation between the 24 h urinary acetate, SCFA and FC estimated intake suggested that using urinary output may not be an appropriate index to reflect colonic production of SCFA. This might be explained by the high uptake of these substrates; approximately 70 % of the exogenous acetate formed by colonic bacterial fermentation of NDCs and endogenous acetate formed in the liver during fatty acid oxidation is taken up by the liver (Layden et al., 2013). Urinary excretion of SCFA was not validated well as it didn't reflect the colonic production of SCFA as hoped. Therefore, an *in vitro* enzymatic digestion and fermentation study was conducted to investigate the relationship between NDCs and SCFA products (Chapter 5).

5 Chapter Five

Prediction of SCFA production from a single fibre and mixtures of fermentable carbohydrates using *in vitro* small intestinal digestion and human colon fermentation models and the equations for fermentable carbohydrate

5.1 Introduction

This chapter will examine the validity of the FC equations developed in chapter 3 and tested on *in vivo* data in chapter 4, using *in vitro* models of small intestinal digestion and human colon fermentation. These equations were based on the content of dietary fibre in foods by using both the AOAC method and the NSP, with 50 % of insoluble and 100 % of soluble fibre. Ten percent of the total starch was chosen to represent the amount of RS, and all NDOs were assumed to enter the colon for fermentation in the experiments. In chapter 4, these equations were applied to three day weighed dietary records of 122 healthy subjects on their habitual diet, and correlated with urinary SCFA production. It was observed weak but significant correlations between FC-DF 4, FC-NSP 4 with urinary acetate and SCFA. However, it was clear that urinary SCFA may not reflect the colonic production of SCFA as efficiently as required. The results of chapter 4 were not sufficient to test the robustness of our proposed equations to predict FC; therefore, this study was designed to use *in vitr*o digestion models for mimicking the physiological actions of the upper intestine and the bacterial fermentation of NDCs in the human colon.

Human colonic bacteria exhibit a range of fermentation activities, as they act on a wide variety of diet based fermentable carbohydrate or protein substrates (Tan et al., 2013). It has been estimated that there are more than 1000 - 1150 different species of bacteria in the colon, which may belong to more than 70 genera (Sekirov et al., 2010). The numerically predominant bacteria are anaerobes, including the species *bacteroides, bifidobacteria, eubacteria, streptococci and lactobacilli*. Studies have shown that *bacteroides* can constitute 30 % of the total bacteria in the gut (den Besten et al., 2013).

Colonic anaerobic bacterial degradation of FC produces energy for bacterial growth, and SCFAs (acetate, propionate and butyrate) as the major end products of NDCs fermentation, with mean ratio of 60:20:20 acetate: propionate: butyrate (Tan et al., 2013). The proportion of SCFAs may differ between individuals, due to their type of diet, gut microflora, gut transit time, and the presence of any gastrointestinal diseases, such as inflammatory bowel disease. The amount and proportion of these SCFAs may also differ with the type of NDCs available for fermentation (den Besten et al., 2013, Flint et al., 2012), as the main components of NDCs that pass the small

intestine are NSPs, RS, and NDOs (Roy et al., 2006). In Western societies, the mean NDCs intake is approximately 20 - 25 g/d (Bingham et al., 2003), which leads to NDCs fermentation in colon to produce 400 - 600 mmol of SCFAs/d, which may provide approximately 10 % of the human energy needs (Musso et al., 2011). SCFA may play a significant role in many metabolic diseases such as obesity, metabolic syndrome, and diabetes, as well as gastrointestinal diseases like CRC and inflammatory bowel disease (Sekirov et al., 2010). An increase in SCFA concentration can also have beneficial properties, for example propionate has potential health promoting properties that include anti-lipogenic, cholesterol lowering, anti-inflammatory and anti-carcinogenic action (Hosseini et al., 2011). Additionally, propionate has a role in improving satiety through up regulation of the gut hormone PYY, and appetite suppression through increased leptin levels (Arora et al., 2011). Of the SCFAs produced butyrate has been investigated the most due to its potential anti-tumorigenic properties, whereas acetate is the most abundant colonic SCFA; butyrate is considered to be the main energy source for large human intestine epithelial cells. Studies have shown it inhibits proliferation, induces differentiation and apoptosis of CRC cells *in vitro* at concentrations similar to those found in the large bowel *in vivo* (Hamer et al., 2008).

Despite the physiological importance of the colonic anaerobic bacteria and their degradation of fermentable carbohydrate (chapter 1), the main site of FC is the proximal colon, as it has the greatest substrate availability and a higher liquid content the other parts of the colon, which means most of the SCFA are produced and absorbed here (den Besten et al., 2013). Proximal colonic SCFA cannot be measured easily in humans because it is very difficult to take samples from proximal colon in healthy subjects (Edwards et al., 1990). The limitations of using faecal SCFA to estimate fermentation have been discussed (chapter 4), but there is no validated method to measure the extent of colonic fermentation *in vivo*, especially as 95 % of the SCFA produced are rapidly absorbed by the colonocytes, while only 5 % is excreted in the faeces making faecal SCFA a poor index of colonic production of SCFA (Topping and Clifton, 2001).

Therefore, it may be more useful to use an *in vitro* fermentation model using a human faecal sample as its inoculum to determine SCFA levels. These models are cheap and easy to use for measuring the level of NDCs fermentability and SCFA profiles produced (Edwards et al., 1996). Faecal sample collection is easy but

unpleasant (Edwards and Rowland, 1992). In terms of mimicking human colonic conditions, the sample of faecal bacteria will provide most of the bacteria present in the colon and with a medium mimicking the large intestine (buffer, macronutrients and micronutrients) however, there are several potential differences with the *in vivo* situation, most importantly the lack of absorption of SCFA and other products (Cummings, 1997). For the purposes of this thesis, it was important to determine the fermentation capacity of the different carbohydrate sources and their digestibility before fermentation. Therefore, it was important to remove any carbohydrates and other macronutrients which would normally be digested and absorbed in the small intestine. It is important to perform an *in vitro* model enzymatic digestion to hydrolyse starch and protein before *in vitro* fermentation, because a high amount of normally digestible starch may mask the *in vitro* fermentation of the dietary fibre (McBurney et al., 1988).

Overall this suggests an *in vitro* fermentation model using human faeces as inoculum is suitable and easy method to measure NDCs fermentability and SCFAs product (Edwards et al., 1996). This model would allow testing of the equations to predict FC in the diet by allowing direct comparison of SCFA from the carbohydrates that escape simulated small intestinal digestion. Therefore, the aim of this study was to assess the digestibility of different dietary carbohydrates, to see if the different FC produced predictable amounts of SCFA using an *in vitro* human colon fermentation model.

5.2 Objectives

The objectives of the present study were:

1. To use an *in vitro* enzymatic digestion model for the removal of starch and other digestible macronutrients prior to the *in vitro* fermentation of FC samples
2. To use an *in vitro* human colon fermentation model, to study the metabolic capacity of the colonic bacteria when single fibre, mixtures of isolated carbohydrates and food mixtures based on the habitual diets of subjects were fermented, and to test our equations against the SCFA amounts produced from each carbohydrate.
3. To compare the SCFA concentration produced via *in vitro* fermentation of FC against the predictable amounts of SCFA using our prediction equations.

5.3 Methods

5.3.1 Substrates used for *in vitro* upper gastrointestinal digestion

These substrates were subjected to *in vitro* upper gastrointestinal digestion (section 2.3.1).

5.3.1.1 Single dietary fibre sources (raw potato starch, raftiline, wheat bran, psyllium and a mixture of all four)

Raw potato starch was obtained from Avebe (Foxhol, The Netherlands; samples I/P), un-treated non-pre-digested wheat bran was obtained from Infinity foods co. (UK). Raftiline Orafti® HP, a long chain inulin fructo-oligosaccharide with a degree of polymerization (DP) > 23 which was extracted from chicory root (*cichorium intybus*), which was obtained from Beneo Orafti (Tienen Belgium), and psyllium husk seed was obtained from Sat-Isabgol factory (India). The four fibre mixture was 250 mg of each fibre.

5.3.1.2 Three different NDCs mixtures (low, medium and high)

These NDCs mixture were obtained from habitual dietary records in the previous study, which was likely to contain varied amounts of DF, RS and NDOs. Three different NDCs mixture were selected as low, medium and high NDCs. The fibre content in mixtures was reassessed using similar products in a supermarket. For each NDCs mixture, all food items in each mixture were weighed on a digital kitchen scales (Brabanita, UK) onto a plate and mixed in a blender (Braun™). Some food items in each mixture were cooked based on the most commonly eaten form, before they were mixed in the blender. After that, each NDCs mixture were freeze-dried and milled before use in the *in vitro* digestion study (Table 5.1).

Table 5.1: Dietary composition of the three different mixtures of NDCs from habitual dietary records in the previous study used for *in vitro* digestion (g/d).

Low FC					Medium FC					High FC				
Food item	**g/d**	**Starch**	**NDO**	**DF**	**Food item**	**g/d**	**Starch**	**NDO**	**DF**	**Food item**	**g/d**	**Starch**	**NDO**	**DF**
Strawberries, raw	66.0	-		1.3	Fresh orange juice	178.0	-		0.2	Bran Flakes	96.0	46.5	2.32	14.4
Orange juice, freshly squeezed	125.0	-		0.1	Wholemeal bread	181.0	77.3	4.86	13.8	Wholemeal bread	256	100.1	13.79	16.2
Porridge made with whole milk	130.0	10.1		1.0	Lettuce, raw	9.0	-	0.19	0.1	Fruit jam	20.0	-		0.2
Satsumas	89.0	-		1.5	Olives	25.0	0.0		1.0	Yeast extract	10.0	0.2		-
Kiwi fruit	67.0	0.2		-	Roasted cashew nuts	46.0	6.1		-	Apples, raw	432.0	-	0.03	8.6
Lettuce, raw	382.0	-	0.13	4.9	Anchovy sauce	156.0	0.2		-	Digestive biscuits, chocolate	45.0	17.1		1.4
Chocolate cake	53.0	11.5		2.0	Plain pasta cooked	179.0	55.0		-	White rice, boiled	500.0	148.0		4.0
Breadcrumbs, homemade	6.0	4.5		0.3						New potatoes, boiled	500.0	83.5		6.0
New potatoes, boiled	93.0	15.5		1.1						Orange juice	600.0	-		0.6
Lettuce, raw	257.0	-	0.06	3.3										
Sweet pickle	69.0	1.4		1.0										
Low fat yogurt	175.0	0.5		-										
Oatcakes	25.0	15.0		0.9										
Total	**1537**	**58.7**	**0.19**	**17.4**		**774**	**138.6**	**5.05**	**15.1**		**2459**	**395.4**	**16.14**	**51.4**

NDCs, non-digestible carbohydrates; DF, dietary fibre; NDO, non-digestible oligosaccharide

5.3.1.3 Mixtures of different isolated NDCs (RS, Soluble DF, Insoluble DF and NDOs)

Sample foods were digested *in vitro* either raw or cooked, based on the most commonly form eaten (Table 5.2). For example, apple was *in vitro* digested raw, while red kidney beans were cooked. The food samples were purchased from Tesco supermarket. Food samples were categorized into four groups:

1. **RS mixture**
   a) 400 mg of banana
   b) 200 mg of red kidney beans
   c) 200 mg of rye bread
   d) 200 mg of apple
2. **Soluble DF mixture**
   a) 400 mg of carrot, raw fresh
   b) 200 mg of red kidney beans
   c) 200 mg of new potatoes, boiled
   d) 200 mg of apple
3. **Insoluble DF mixture**
   a) 400 mg of all bran
   b) 200 mg of red kidney beans
   c) 200 mg of new potatoes, boiled
   d) 200 mg of apple
4. **NDOs mixture**
   a) 400 mg of wheat bread
   b) 200 mg of red kidney beans
   c) 200 mg of new potatoes, boiled
   d) 200 mg of Ryvita, high fibre

Table 5.2: Dietary composition of isolated NDCs mixtures used for *in vitro* digestion

Items g/100g	Red Kidney Beans	All bran	Ryvita	Carrot	Wheat bread	Apple	Banana	New potatoes, boiled	Rye bread
Starch	12.8	28.6	58.0	0.3	39.3	trace	54.2	16.7	44
DF*	19.1	25.3	18.9	19.5	9.1	9.9	3.2	5.8	13.3
Soluble DF	9.1	4.2	14.2	11.4	6.0	3.6	2.2	3	6.6
Insoluble DF	9.9	21.1	4.7	8.1	3.1	6.3	1.0	5.8	6.7
NDOs	0.01	trace	trace	0.02	2.8	0.01	1.0	0.5	1.4

*DF, dietary fibre; NDOs, non-digestible oligosaccharides

5.3.2 *In vitro* upper gastrointestinal digestion model

Three *in vitro* digestion and fermentation studies are included in this chapter (section 2.3.1). The *in vitro* upper gastrointestinal digestion was based on a model previously described by Aura et al. (1999).

Briefly, all NDC samples were freeze-dried and milled before *in vitro* digestion. All incubation steps were performed at 37 °C, with stirring and shaking in a water-bath. 1 g of each NDC was weighed into a 100 ml beaker containing mixture of 10 ml distilled water and 7 ml of a 0.85 wt % sodium chloride solution. Salivary α-amylase (50 U/50 µl) was dissolved with sodium phosphate buffer (0.02 M, pH 6.9) and added to the mixture. The mixture was then incubated for 5 minutes to mimic the digestive actions in the mouth. To mimic events in the stomach hydrochloric acid (0.15 M, 4 ml) was added to adjust the pH to 2.5. 2 mg of pepsin was dissolved in 1 ml of 0.02 M of HCL acid/ sample, and added to the sample, which was then incubated for 2 h. To mimic events in the small intestine porcine extract bile acid (400-600 mg/sample) was dissolved in 4 ml of 0.15M sodium bicarbonate and added to the mixture. 75 mg pancreatin (which contains amylase, proteases and lipase) was dissolved in 4 ml of 0.15 M of sodium bicarbonate solution and added to the mixture. The pH was adjusted to 6.5 using 6 M NaOH. Each digestion mixture at this stage was placed into a dialysis tube (Biotech Cellulose Ester Membrane; 35 cm long with flat width 31 mm, Dia 20 mm; cut-off: 500-1000 MWCO), which was placed in a 2 L beaker and filled with distilled water and incubated for 6 h at 37 °C with stirring. After dialysis, the retentates were freeze-dried and used as substrates for *in vitro* fermentation.

5.3.3 Subjects used as donors of faecal inoculum

Fresh faeces were obtained from five healthy adults (males aged 25-45 years), the same subjects were donors of fresh faecal samples for all three *in vitro* fermentation experiments (section 2.4.1).

5.3.4 *In vitro* fermentation model

After *in vitro* upper gastrointestinal digestion, the freeze-dried remnants of all four different isolated NDCs mixtures (RS, Soluble NSP, Insoluble NSP and NDOs), single dietary fibre (raw potato starch, raftiline, wheat bran, psyllium and mixture)

and different NDCs mixtures (low FC, medium FC and high FC), were used as a source of carbohydrate in these experiments. One control culture without any added substrate was used with each set of incubations to calculate the fermentation of the indigestible substrates in the original inoculum. Samples were taken from each incubation at 0, 4, and 24 hours. A 3 ml sample was transferred by syringe into bijoux tubes at 0 h to measure pH and then was frozen at -20°C for later SCFA analysis. Then at 4 h and 24 h, a further 3 ml of each fermented sample was removed and analysed for pH and fermentation products.

5.3.5 Non-digestible fraction measurement

After *in vitro* upper gastrointestinal digestion, the freeze-dried substrates of all four different isolated NDCs mixtures (RS, Soluble NSP, Insoluble NSP and NDOs), single dietary fibres (raw potato starch, raftiline, wheat bran, psyllium and mixture of these) and different NDCs mixtures (low FC, medium FC and high FC), were subject to content analysis. The RS content was analysed using the Megazyme RS assay kit (AOAC Method 2002.02) (McCleary et al., 2002), the fructan content using the Megazyme fructans assay kit (AOAC Method 999.03). The non-digestible fractions analyses were performed in duplicate. Soluble DF and insoluble DF fractions present in NDCs were estimated approximately from the GLOBALRPH website dietary fibre calculator, and the studies by Windiets 2005, Lee et al. (1992), and Anderson and Bridges (1988) (section 2.6.1).

5.3.6 SCFA Analysis

SCFAs were analysed by gas chromatography (GC) according to Laurentin and Edwards (section 2.5).

5.4 Statistical analysis

Statistical analysis was performed for all data using Minitab Version 16 (Minitab® V. 16 Inc. USA). The Anderson-Darnley test of normality was applied to evaluate the normal distribution of continuous variables, and all data were expressed as standard deviation (SD). Paired test and two sample t-tests for the normally distributed data were used to explore the differences between more than two independent variables. Pearson correlation coefficients was used to find correlations between different continuous variables, NDCs, and predicted FC with SCFA concentration. Linear

regression analysis was used to estimate predicted SCFA concentration of FC by the respective SCFA concentration produced via *in vitro* fermentation of FC. The agreement between the two methods, was evaluated with Bland-Altman plots using MedCalc Version 15.8 (MedCalc® V. 15.8 Belgium), by plotting the individual differences of a measure, between the two methods, against their mean. The bias of the method is equal to the mean difference of the methods. Limits of agreement between predicted SCFA concentration of FC and SCFA concentration produced via *in vitro* fermentation were calculated as the mean difference of the two methods ± 1.96 times the SD of the mean difference (van Stralen et al., 2008). Any p-values of 0.05 or below were considered significant.

5.5 Results

5.5.1 Residue dry weight of single dietary fibre sources (raw potato starch, raftiline, wheat bran, psyllium and a mixture of all four) after *in vitro* digestion vs. predicted fibre

The amount of the non-digestible fractions of raw potato starch, raftiline, wheat bran, psyllium and the four fibre mixture, which remained after removing the digestible starch and other macronutrients from the samples using an extensive *in vitro* enzymatic digestion model, vs. the predicted amount of the same fibre measured using the developed FC equations was analysed (Figure 5.3). Our model equation for predicting FC was: soluble DF was 100 % fermented and insoluble DF was 50 % fermented, and NDOs were 100 % fermented. Finally, the amount of RS was estimated as 10 % of total starch, which is believed to be totally fermented. Where a mostly pure source of RS was used this was considered to be 100 % fermented.

Table 5.3: FC fractions of raw potato starch, raftiline, wheat bran, psyllium and 4 fibre mixtures vs. predicted (mg/g)

NDCs	Raw potato starch		Raftiline		Wheat bran		Psyllium		Mixture	
	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted**
RS	482.17 ± 19.8*	642.95 ± 58.1	0.0 ± 0.0	0.00 ± 0.0	3.83 ± 0.1	4.71 ± 0.2	0.41 ± 0.02	0.32 ± 0.0	13.97 ± 0.8	15.62 ± 0.9
NDOs	0.00 ± 0.0	0.00 ± 0.0	872 ± 29.9	901 ± 30.9	39.68 ± 1.3	31.61 ± 1.0	0.39 ± 0.02	0.34 ± 0.0	235.03 ± 14.4	239.33 ± 14.6
Insoluble DF	28.82 ± 1.2	28.54 ±1.2	0.0 ± 0.0	0.0 ± 0.0	142.57 ± 4.6	142.57 ± 4.7	34.48 ± 1.6	34.48 ± 1.6	64.89 ± 3.9	65.9 ± 4.0
Soluble DF	14.27 ± 0.6	14.41 ±0.6	0.0 ± 0.0	0.0 ± 0.0	24.21 ± 0.8	24.21 ± 0.8	699.35 ± 32.0	699.35 ± 32.2	182.16 ± 11.8	192 ± 11.7

* The values are reported as mean (standard deviation) for actual (n = 5) vs. predicted (n = 5); ** Predicted FC-DF 4 equation: 100 % Soluble DF + 50 % insoluble DF + 100 % NDOs + 10 % TS

5.5.2 Differences in the production of total and major SCFA after 24 h of *in vitro* fermentation of single dietary fibre sources (raw potato starch, raftiline, wheat bran, psyllium and a mixture of all four).

Total SCFA produced after 24 h *in vitro* fermentation were used to characterise the fermentation pattern of single dietary fibre sources (raw potato starch, raftiline, wheat bran, psyllium and a mixture of all four) (Table 5.4). SCFAs production was also used to compare the predictive capacity of the equations for FC against the individual NDC dietary models in this experiment.

The results (Table 5.4) show there was no significant difference in the total SCFA concentration in these single fibres, except for in the 4 fibre mixtures culture (4.05 ± 0.6 µmol/ml) at 0 h of incubation was higher than raw potato starch, raftiline and wheat bran. It was observed that the rapidly fermented raftiline had highest total SCFA concentration (57.82 ± 6.2 µmol/ml) followed by the 4 fibre mixture fibre (53.19 ± 3.7 µmol/ml) at 4 h, whereas the fermentation of wheat bran had lowest total SCFA concentration followed by psyllium at this time (Table 5.4). The total SCFA concentration in cultures containing raw potato starch, raftiline and psyllium were not significantly different from each other at 24 hours. The mixture of dietary fibre was ranked as having the highest total SCFA concentration (89.07 ± 2.1 µmol/ml; Figure 5.1) followed by raw potato starch (84.75 ± 4.9 µmol/ml), and raftiline (82.18 ± 1.4 µmol/ml), which was significantly higher when compared with that in the cultures of wheat bran and psyllium at 24 hours. At this period of incubation, fermentation of wheat bran continued to produce a lower total SCFA concentration (49.29 ± 3.2 µmol/ml).

Figure 5.1: Mean (±SEM) concentrations of total SCFA (µmol/ml) after 24 h incubation of single dietary fibre sources (raw potato starch (RPS), raftiline (RF), wheat bran (W.Bran) and psyllium (PSY)) and mixture of all four (MIX) with the faecal inoculum (n= 5).

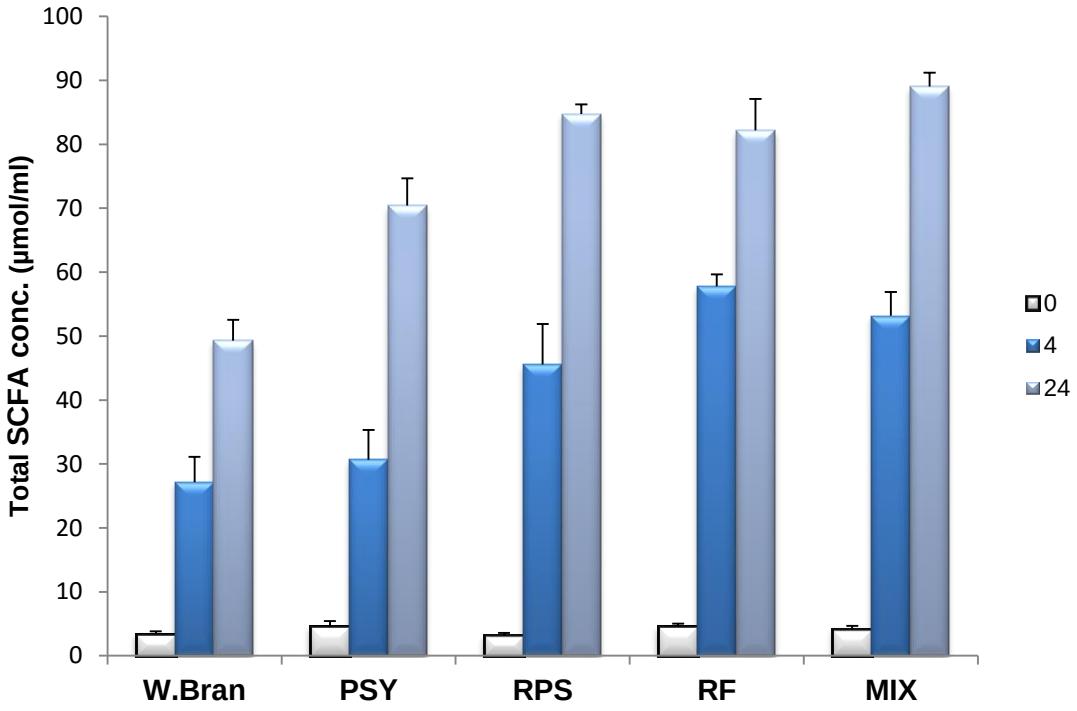


Table 5.4: Concentrations of total and major individual SCFA (µmol/ml) between 0 and 24 h of *in vitro* fermentation using human faecal samples (n = 5) with single dietary fibre substrates.

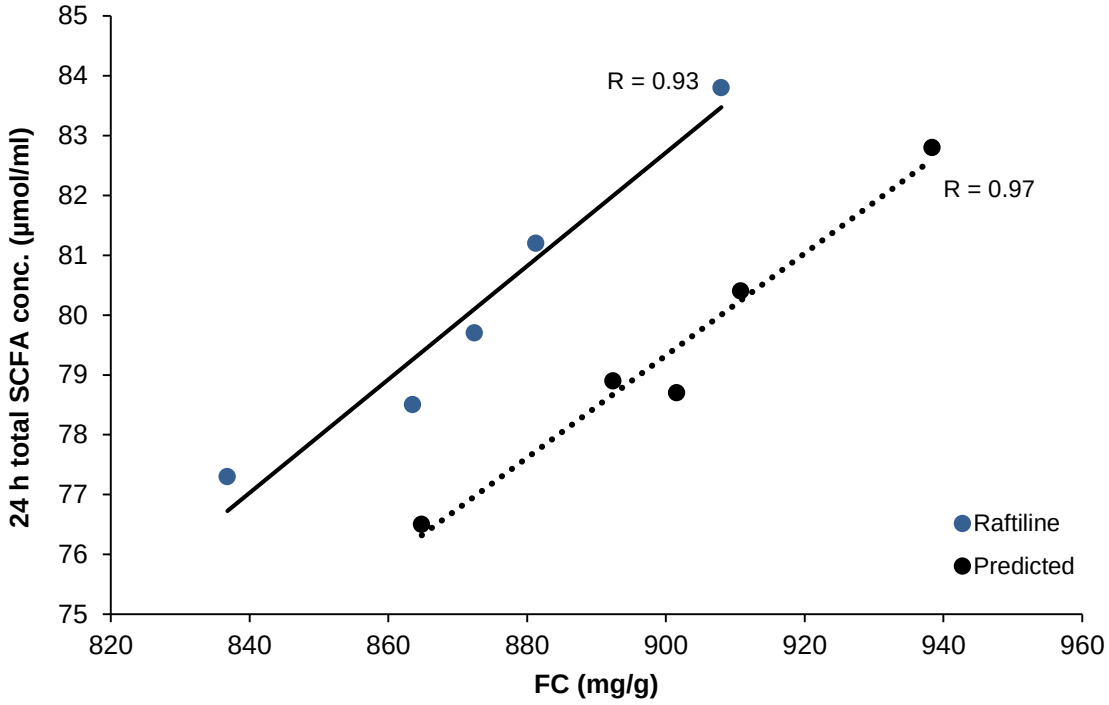
SCFA	NDC				
	Raw potato starch Mean ± SEM	**Raftiline Mean ± SEM**	**Wheat bran Mean ± SEM**	**Psyllium Mean ± SEM**	**4 fibre Mixture Mean ± SEM**
Acetate					
0 h	2.36 ± 0.4 [a]	2.29 ± 0.4 [a]	2.66 ± 0.6 [a]	3.95 ± 0.8 [b]	3.27 ± 0.6 [b]
4 h	28.16 ± 2.3 [c]	36.52 ± 5.8 [c]	15.23 ± 1.7 [d]	18.06 ± 2.7 [d]	31.67 ± 3.9 [c]
24 h	51.08 ± 6.8 [a]	49.81 ± 7.0 [a]	25.44 ± 1.0 [b]	38.81 ± 3.0 [a]	45.82 ± 5.7 [a]
Propionate					
0 h	0.66 ± 0.1 [a]	0.61 ± 0.1 [a]	0.57 ± 0.1 [a]	0.50 ± 0.2 [a]	0.66 ± 0.1 [a]
4 h	9.21 ± 1.6 [a]	15.60 ± 1.1 [b]	6.16 ± 0.5 [a]	7.53 ± 1.1 [a]	14.62 ± 1.4 [b]
24 h	11.97 ± 1.4 [a]	20.70 ± 5.3 [a, b]	11.00 ± 0.8 [a]	23.46 ± 0.5 [b]	21.39 ± 1.7 [b]
Butyrate					
0 h	0.32 ± 0.09 [a]	0.28 ± 0.1 [a]	0.32 ± 0.09 [a]	0.27 ± 0.1 [a]	0.31 ± 0.1 [a]
4 h	5.20 ± 0.8 [a]	3.25 ± 0.2 [b]	2.44 ± 0.5 [b]	2.11 ± 0.2 [b]	4.79 ± 0.5 [a]
24 h	16.18 ± 2.3 [a]	4.96 ± 0.3 [b]	6.32 ± 0.62 [b]	3.55 ± 0.3 [b]	13.03 ± 0.6 [a]
Total SCFA					
0 h	3.15 ± 0.4 [a]	3.00 ± 0.0 [a]	3.36 ± 0.4 [a]	4.5 ± 0.8 [a, b]	4.05 ± 0.6 [b]
4 h	45.60 ± 1.8 [a]	57.82 ± 6.2 [a, d]	27.15 ± 3.9 [b]	30.67 ± 4.6 [c, b]	53.19 ± 3.7 [d]
24 h	84.75 ± 4.9 [a, d]	82.18 ± 1.4 [a, b]	49.29 ± 3.2 [c]	70.39 ± 4.2 [a, b]	89.07 ± 2.1[d]

The values with different letters are significantly different within a row. SCFA was compared with paired t-test between 0, 4 and 24 hours for the same type of culture ($P < 0.05$).

5.5.2.1 Correlation of *in vitro* total SCFA concentration (µmol/ml) of single fibre sources (n = 5; raw potato starch, raftiline, wheat bran, psyllium and mixture of all four) after 24 h of incubation vs. predicted FC (n = 5) using the predictive equation

The Pearson correlation coefficient was used to determine the relationship between *in vitro* total SCFA concentration of single dietary fibre (raw potato starch, raftiline, wheat bran, psyllium) and mixture of all four fibres with the predicted FC (Table 5.5). The *in vitro* fermentation total SCFA concentration of raw potato starch and raftiline were compared against the predicted FC, and the result (Figure 5.2) of raftiline showed an excellent relationship with predicted FC of total SCFA concentration. The bland Altman plot showed that the bias between raftiline SCFA concentration and predicted SCFA concentration was small (0.04 µmol/ml; Figure 5.3), with relatively wide limits of agreement were 1.80 and -1.72 µmol/ml. Wheat bran also showed a strong correlation with total SCFA concentration (R = 0.96; p = 0.009) when compared with the predicted FC (R = 0.93; p = 0.02). Similarly, mixture of all 4 fibres showed an excellent correlation with total SCFA concentration (R = 0.98; p = 0.002; Figure 5.4) compared with predicted FC (R = 0.95; p = 0.01). The bland Altman plot showed that the bias between mixture of all four fibres SCFA concentration and predicted SCFA concentration of all four fibres was small (0.2 µmol/ml; Figure 5.5), although with wide limits of agreement were 1.66 and -1.26 µmol/ml. Moreover, the predicted FC had a better correlation with total SCFA concentration (R = 0.94; p = 0.01) when compared with the psyllium fibre (R = 0.89; p = 0.04). These results show a good agreement correlation between single DF and mixture of all 4 fibres with total SCFA concentration and with those predicted FC.

Figure 5.2: Scatter-plot showing correlation of 24 h total SCFA concentration (µmol/ml) of raftiline fermentation (n = 5) vs. predicted raftiline (n = 5). Dotted line indicates predicted raftiline; solid line indicates raftiline.



Black circle dot indicates predicted SCFA of raftiline (µmol/ml). Blue circle dot indicates SCFA produced from *in vitro* fermentation of raftiline (µmol/ml).

Figure 5.3: Bland Altman plot of 24 h total SCFA concentration (µmol/ml) of raftiline fermentation (n = 5) vs. predicted raftiline.

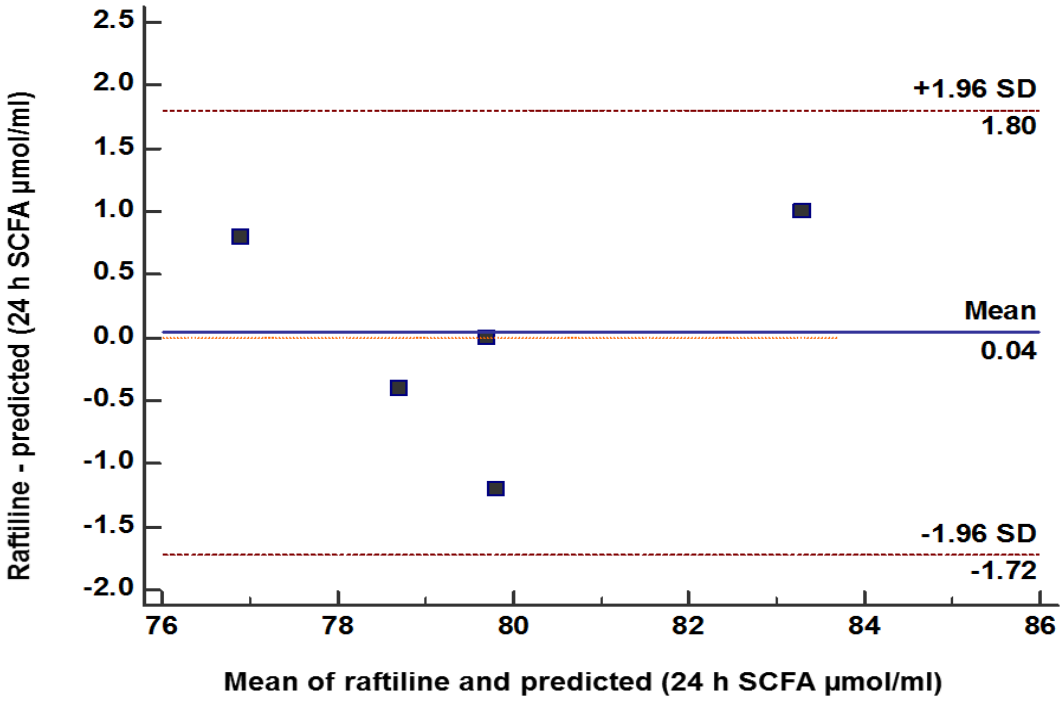
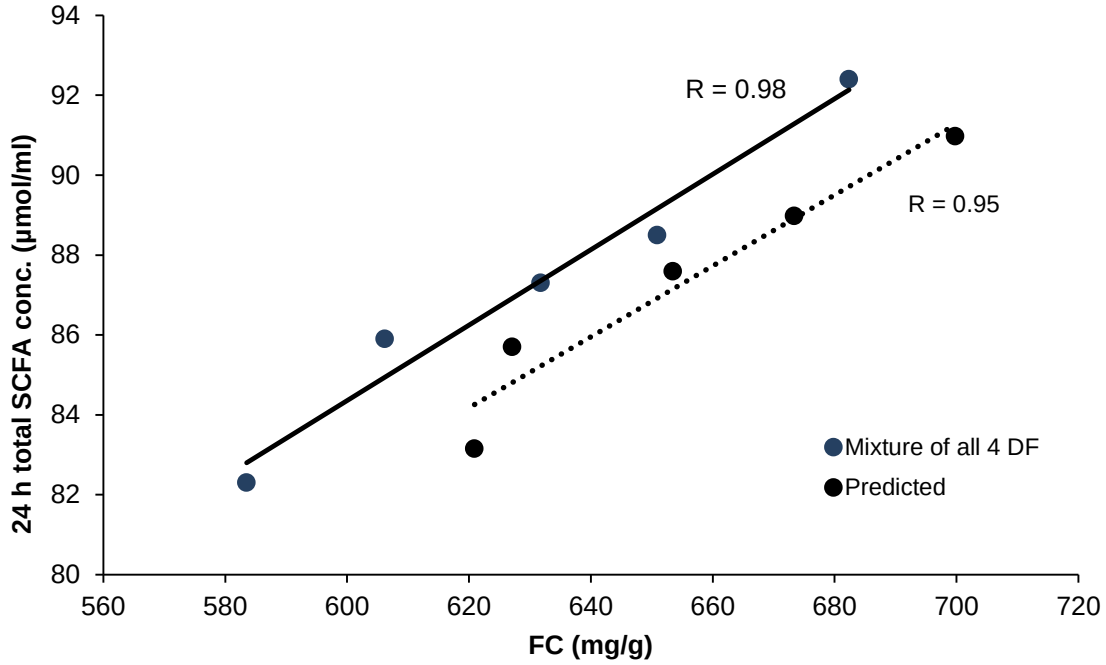


Figure 5.4: Scatter-plot showing correlation of 24 h total SCFA concentration (µmol/ml) of mixture of all 4 fibres fermentation (n = 5) vs. predicted mixture of all 4 fibres (n = 5). Dotted line indicates predicted mixture of all 4 fibres; solid line indicates mixture of all 4 fibres.



Black circle dot indicates predicted SCFA of mixture of all 4 fibres (µmol/ml). Blue circle dot indicates SCFA produced from *in vitro* fermentation of mixture of all 4 fibres (µmol/ml).

Figure 5.5: Bland Altman plot of 24 h total SCFA concentration (µmol/ml) of mixture of all 4 fibres fermentation (n = 5) vs. predicted mixture of all 4 fibres (n = 5).

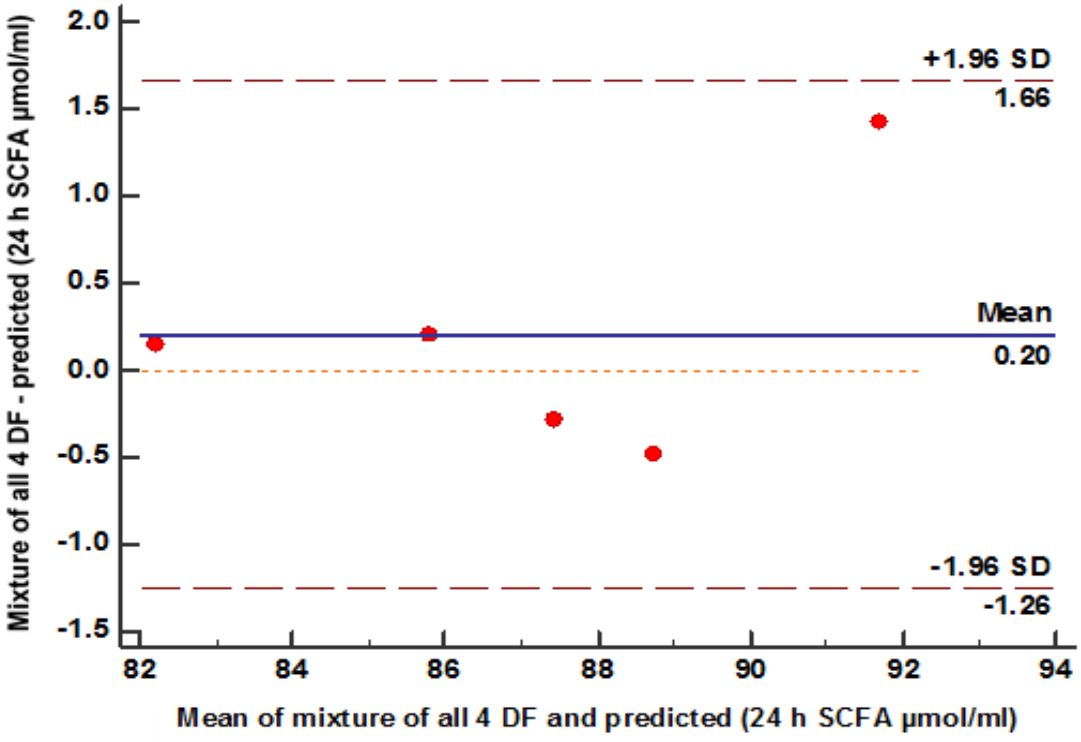


Table 5.5: Correlation between actual single fibre and mixture fermentation (n = 5) with total and major individual SCFA concentration (µmol/ml) after 24 h of *in vitro* fermentation using human faecal microbiota vs. predicted FC (n = 5).

SCFA	Raw potato starch				Raftiline				Wheat bran				Psyllium				Mixture			
	Actual		Estimated		Actual		Estimated		Actual		Estimated		Actual		Estimated		Actual		Estimated	
	R *	p	R	p**	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p
SCFA	0.96	0.008	0.96	0.008	0.93	0.02	0.97	0.004	0.96	0.009	0.93	0.02	0.89	0.04	0.94	0.01	0.98	0.002	0.95	0.01
Acetate	0.97	0.004	0.97	0.004	0.87	0.06	0.89	0.04	0.90	0.03	0.95	0.01	0.95	0.01	0.96	0.01	0.88	0.06	0.84	0.07
Propionate	0.99	0.001	0.99	0.001	0.93	0.02	0.93	0.01	0.86	0.06	0.93	0.02	0.90	0.03	0.96	0.01	0.98	0.002	0.96	0.007
Butyrate	0.99	0.001	0.99	0.001	0.98	0.002	0.98	0.002	0.94	0.01	0.97	0.005	0.87	0.057	0.86	0.06	0.96	0.009	0.98	0.003

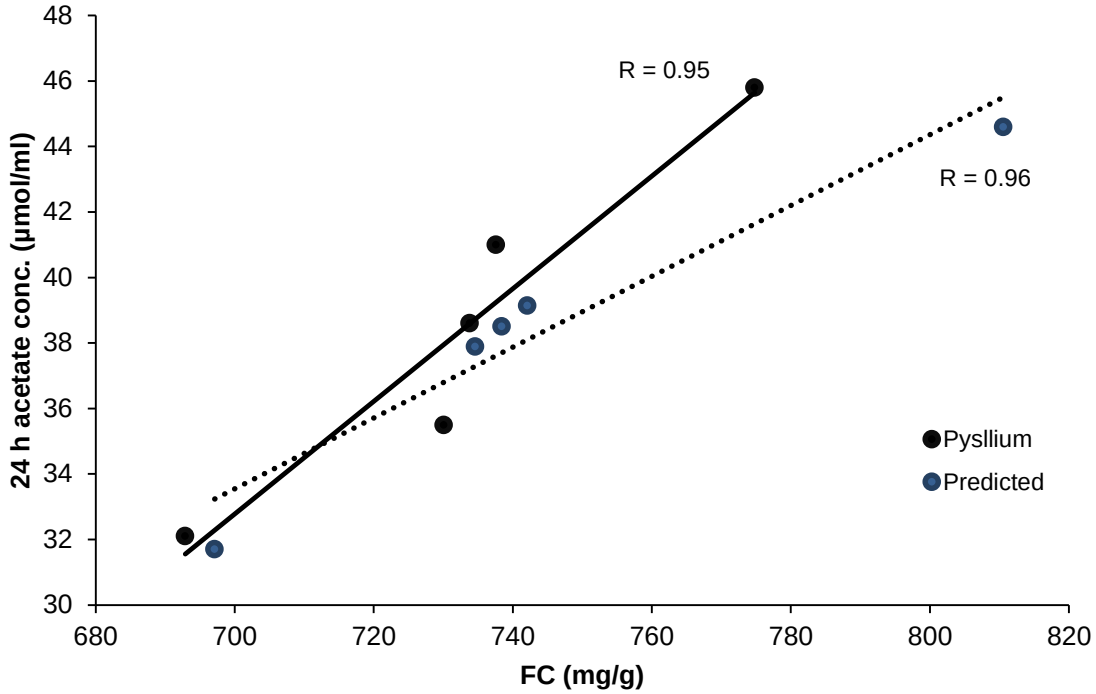
* R; Pearson correlation coefficient, ** p; value significant ($p < 0.05$)

5.5.2.2 Correlation of acetate concentration (µmol/ml) of single fibre sources (n = 5; raw potato starch, raftiline, wheat bran, psyllium and mixture of all four) after 24 h of incubation vs. predicted FC (n = 5) using the predictive equation.

At 24 hours, fermentation of wheat bran continued to produce the lowest acetate concentration (25.44 ± 1.0 µmol/ml), followed by psyllium (38.81 ± 3.0 µmol/ml) compared with the other fibres (Table 5.4). Also at this time point it was observed that raw potato starch had the highest acetate concentration (51.08 ± 6.8 µmol/ml) followed by raftiline (49.81 ± 7.0 µmol/ml). Wheat bran was significantly lower in acetate concentration than other fibres ($p < 0.05$).

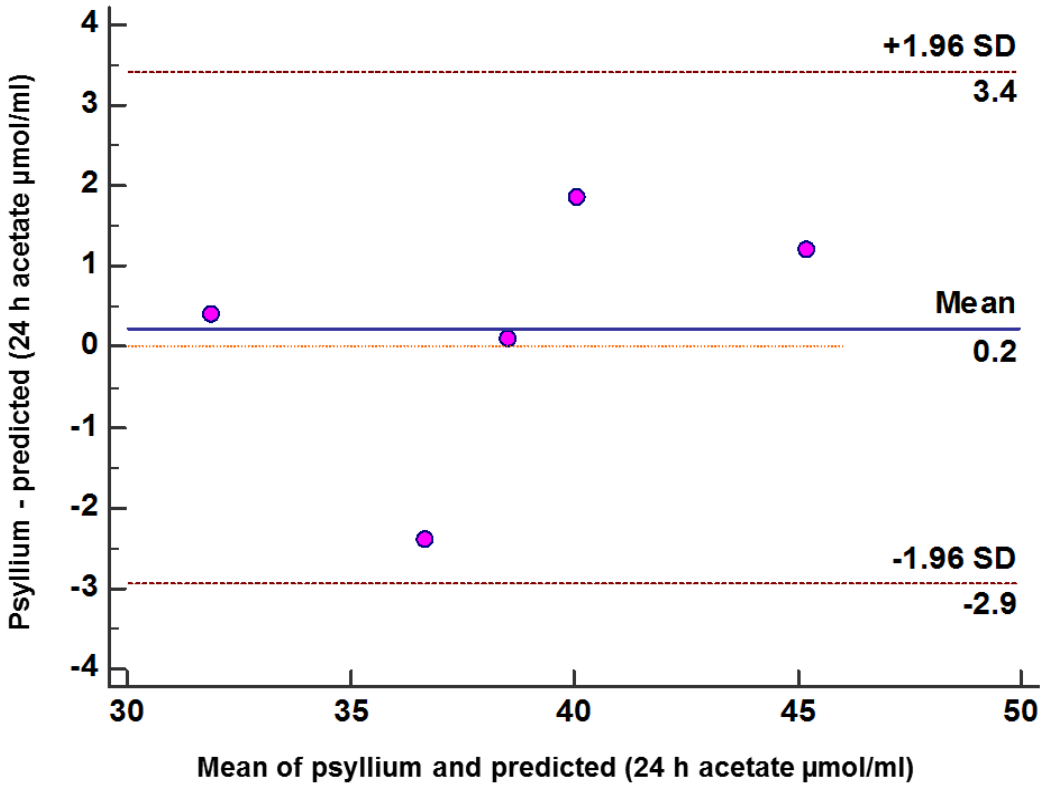
In the comparison of the acetate concentration of raw potato starch and predicted FC (Table 5.5), the results showed an excellent equal relationship in acetate concentration with predicted FC. The predicted FC showed better correlation with acetate concentration ($R = 0.95$; $p = 0.01$) compared with wheat bran fermentation ($R = 0.90$; $p = 0.03$). On the other hand, the mixture of all four ($R = 0.88$; $p = 0.06$) and predicted FC ($R = 0.84$; $p = 0.07$) were unable to show any correlation with acetate concentration. The predicted FC ($R = 0.96$; $p = 0.01$) and psyllium ($R = 0.95$; $p = 0.01$; Figure 5.6) showed a stronger correlation with acetate concentration. Additionally, the bias between psyllium acetate concentration and predicted acetate concentration of psyllium was fairly small (0.2 µmol/ml; Figure 5.7), but with wide limits of agreement (3.4 and -2.9 µmol/ml), which suggest the comparison is not acceptable.

Figure 5.6: Scatter-plot showing correlation of acetate concentration (µmol/ml) of psyllium (n = 5) after 24 h incubation vs. predicted psyllium (n = 5). Dotted line indicates predicted psyllium; solid line indicates psyllium.



Blue circle dot indicates predicted SCFA of psyllium (µmol/ml). Black circle dot indicates SCFA produced from *in vitro* fermentation of psyllium (µmol/ml).

Figure 5.7: Bland Altman plot of acetate concentration (µmol/ml) of psyllium fibre after 24 h incubation vs. predicted psyllium.



5.5.2.3 Correlation of propionate concentration (µmol/ml) of single fibre sources (raw potato starch, raftiline, wheat bran, psyllium and mixture of all four) after 24 h incubation vs. predicted FC using the predictive equation

There was no significant difference in the propionate concentration in raw potato starch, wheat bran and psyllium at 4 hours. However, psyllium had the highest propionate concentration (23.46 ± 0.5 µmol/ml), followed by the mixture of fibres (21.39 ± 1.7 µmol/ml), and raftiline (20.70 ± 5.3 µmol/ml), which had a significantly higher concentration compared with that in the cultures of wheat barn and raw potato starch at 24 hours.

Similar to the SCFA and acetate concentrations, raw potato starch and raftiline have an excellent relationship with *in vitro* propionate concentration (Table 5.5) and that of predicted FC. No correlation was observed between wheat bran fermentation and propionate concentration ($R = 0.86$; $p = 0.06$), while predicted FC had a good relationship with propionate concentration ($R = 0.93$; $p = 0.02$). The mixture of all 4 fibres fermentation showed an excellent correlation with propionate concentration ($R = 0.98$; $p = 0.002$) compared with the predicted FC ($R = 0.96$; $p = 0.007$), and the predicted FC ($R = 0.96$; $p = 0.01$) also had a good relationship with propionate concentration when compared with the psyllium fermentation ($R = 0.90$; $p = 0.03$). These results show a good agreement correlation between psyllium with propionate concentration and with those predicted FC.

5.5.2.4 Correlation of butyrate concentration (µmol/ml) of single fibre sources (raw potato starch, raftiline, wheat bran, psyllium and mixture of all four) after 24 h incubation vs. estimated FC using the predictive equation

At 24 h, fermentation of raw potato starch generated the highest concentrations of butyrate (16.18 ± 2.3 µmol/ml), followed by the mixture of fibres (13.03 ± 0.6 µmol/ml) and was significantly higher than raftiline, wheat bran and psyllium.

The analysis of the correlation of the single fibres and predicted results (Table 5.5) showed that the raw potato starch and raftiline fermentation have an excellent relationship with butyrate concentration, which is similar to that of predicted FC. The predicted FC showed a strong relationship with butyrate concentration ($R = 0.96$; $p = 0.007$) compared with wheat barn fermentation ($R = 0.94$; $p = 0.01$). The psyllium fermentation and predicted FC were showed no correlation with butyrate

concentration, whereas the mixture of all 4 fibres fermentation (R = 0.96; p = 0.009) and predicted FC (R = 0.98; p = 0.003) showed an excellent correlation with butyrate concentration.

5.5.2.5 *In vitro* fermentation of single fibres and 4 mixture DF pH changes

At 0 h, as expected no pH differences were observed between all cultures (Table 5.6). The pH then dropped rapidly during the fermentation of raw potato starch, raftiline, wheat bran, psyllium and 4 fibre mixture up to 4 hours, but then did not change appreciably for the cultures of wheat bran during the prolonged incubation. A progressive fall in pH from 0 hour to 24 hours of fermentation was seen in cultures containing raw potato starch, raftiline, psyllium and mixture, and the raw potato starch and raftiline consistently produced lower pH valued compared to wheat bran, psyllium and mixture fibres cultures (Table 5.6).

Table 5.6: Changes in pH (mean ± SEM) of human faecal cultures containing raw potatoes starch, raftiline, wheat bran, psyllium and 4 mixture DF over 24 h (n = 5) after *in vitro* anaerobic incubation.

	pH at 0 h	pH at 4 h	pH at 24 h
Raw potato starch	7.00 ± 0.08 [a]	5.8 ± 0.02 [a]	4.60 ± 0.01 [a]
Raftiline	6.98 ± 0.07 [a]	4.80 ± 0.08 [a]	4.27 ± 0.18 [a]
Wheat bran	7.01 ± 0.07 [a]	6.66 ± 0.10 [b]	6.40 ± 0.07 [c]
Psyllium	6.98 ± 0.07 [a]	6.51 ± 0.09 [b, 2]	5.40 ± 0.12 [b]
MIX	7.03 ± 0.08 [a]	5.54 ± 0.15 [a]	4.9 ± 0.10 [b]

Values with different letters within a column are significantly (P < 0.05) different. There are significant (P < 0.05) differences between time points within a row except Raftiline (4 h vs 24 h).

5.5.3 Residue dry weight of different mixtures (low, medium and high FC) NDCs after *in vitro* digestion vs. predicted fibre

The amount of the different mixtures (low FC, medium FC and high FC) NDCs after removing the digestible starch from the samples using an extensive *in vitro* enzymatic digestion model, was compared to the predicted amount of the same fibre measured using developed FC equations (Table 5.7).

Table 5.7: Non-digestible fractions of different mixture NDCs (low, medium and high FC) vs. predicted (mg/g)

NDC	Low-FC		Medium-FC		High-FC	
	Actual	**Predicted****	**Actual**	**Predicted**	**Actual**	**Predicted**
Resistant starch	12.66 ± 0.5*	11.99 ± 0.5	14.80 ± 0.5	16.94 ± 0.6	27.58 ± 1.3	30.05 ± 1.4
NDOs	3.74 ± 0.2	3.52 ± 0.1	5.86 ± 0.2	6.13 ± 0.2	9.65 ± 0.4	9.43 ± 0.4
Insoluble DF	8.79 ± 0.4	8.81 ± 0.4	11.85 ± 0.4	10.85 ± 0.4	12.86 ± 0.6	12.86 ± 0.6
Soluble DF	5.83 ± 0.2	5.83 ± 0.2	10.82 ± 0.3	11.22 ± 0.4	12.87 ± 0.6	12.87 ± 0.6

* The values are reported as mean (standard deviation) for actual (n = 5) vs. predicted (n = 5); ** Predicted FC-DF 4 equation: 100 % Soluble DF + 50 % insoluble DF + 100 % NDOs + 10 % TS

5.5.4 *In vitro* fermentation of different NDCs (low, medium and high FC) mixtures

Total SCFA concentrations during *in vitro* fermentation were used to characterise the individual different mixed fermentable diets in this experiment. It was also used to compare the equation for FC against the individual NDC dietary models in this experiment (Table 5.8). At 4 h of fermentation, it was observed that the high FC samples had the highest total SCFA concentration (63.48 ± 2.1 µmol/ml), compared to the low and medium FC mixture of NDCs (Table 5.8). At 24 hours, fermentation of high FC mixture of NDCs continued to generate a higher total SCFA concentration (142.53 ± 5.7 µmol/ml), followed by the medium (107.50 ± 4.5 µmol/ml) and low FC mixtures (85.81 ± 5.0 µmol/ml) (Figure 5.8). The total SCFA concentration in the low FC mixture of NDCs culture at 24 h was lower than other NDCs mixture cultures.

Figure 5.8: Mean (±SEM) concentrations of total SCFA (µmol/ml) produced after 24 h incubation of different mixed (low, medium and high FC) NDCs with the faecal inoculum (n= 5).

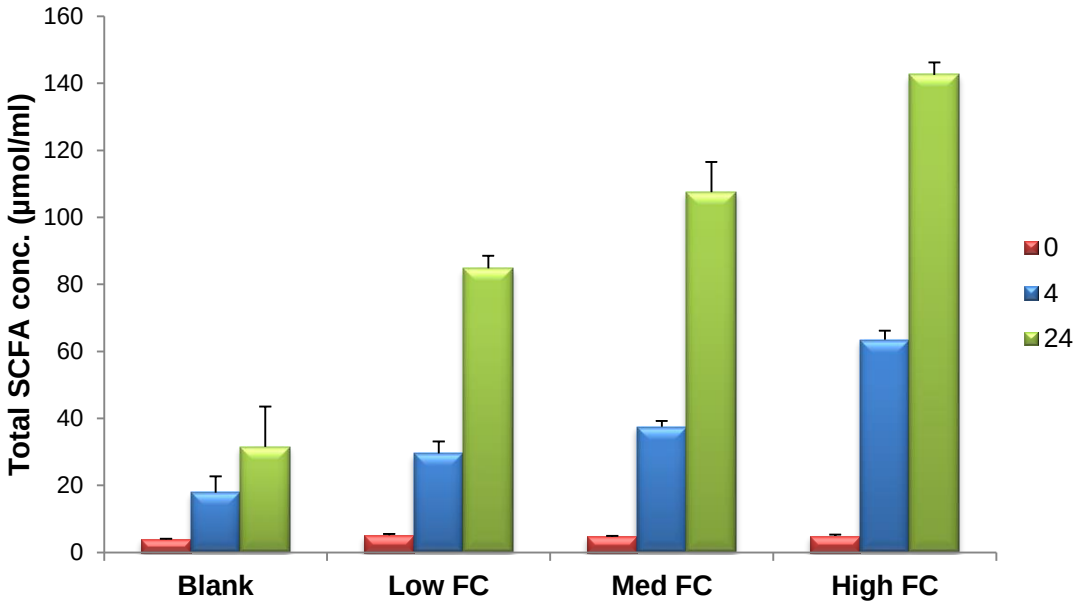


Table 5.8: Concentration of total and major individual SCFA (µmol/ml) between 0 and 24 h of *in vitro* fermentation using human faecal samples (n = 5) with NDC substrates.

SCFA	NDC			
	Low-FC	**Medium-FC**	**High-FC**	**Blank**
Acetate				
0 h	3.78 ± 0.2 [a]	3.41 ± 0.15 [a]	3.49 ± 0.2 [b]	2.67 ± 0.2 [c]
4 h	19.96 ± 0.8 [a]	28.96 ± 2.6 [b]	51.92 ± 2.3 [c]	11.36 ± 1.1 [d]
24 h	50.89 ± 2.2 [a]	67.05 ± 2.4 [b]	97.13 ± 2.0 [c]	15.73 ± 1.5 [d]
Propionate				
0 h	0.87 ± 0.1 [a]	0.95 ± 0.1 [a, b]	0.91 ± 0.1 [b]	0.86 ± 0.1 [a, c]
4 h	8.75 ± 2.2 [a]	7.84 ± 2.4 [a, b]	10.13 ± 2.7 [a, b]	3.26 ± 0.6 [b]
24 h	26.00 ± 3.3 [a]	31.37 ± 4.0 [a]	36.61 ± 5.1 [a]	5.98 ± 1.4 [b]
Butyrate				
0 h	0.48 ± 0.05 [a]	0.47 ± 0.09 [a]	0.50 ± 0.06 [a]	0.22 ± 0.01 [b]
4 h	0.83 ± 0.3 [a]	1.41 ± 0.2 [a]	2.50 ± 0.3 [b]	0.63 ± 0.1 [a]
24 h	5.38 ± 1.5 [a]	6.13 ± 1.2 [a]	7.07 ± 0.9 [a]	4.05 ± 0.6 [a]
Total SCFA				
0 h	4.93 ± 0.2 [b]	4.84 ± 0.3 [a, b]	5.17 ± 0.2 [a]	3.97 ± 0.3 [c]
4 h	29.58 ± 2.4 [a]	37.49 ± 1.8 [b]	63.48 ± 2.1 [c]	17.86 ± 2.3 [d]
24 h	85.81 ± 5.0 [a]	107.50 ± 4.5 [b]	142.53 ± 5.7 [c]	31.47 ± 3.5 [d]

The values are reported as mean (standard error) of two duplicate. Values with different letters are significantly different within a row. SCFA was compared with paired t-test between 0, 4 and 24 hours for the same type of culture ($P < 0.05$).

Table 5.9: Correlation of different mixtures NDC (n = 5) fermentation of total and major individual SCFA concentration (µmol/ml) after 24 h of *in vitro* fermentation using human faecal microbiota vs. predicted FC (n = 5).

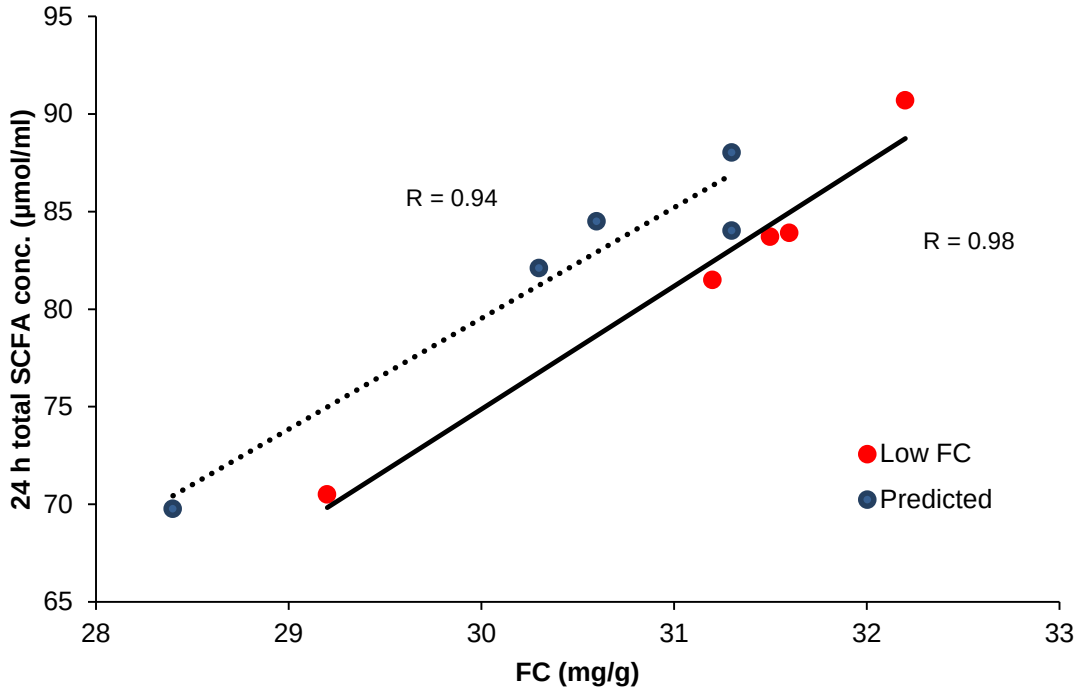
SCFA	Low-FC				Medium-FC				High-FC			
	Actual		Estimated		Actual		Estimated		Actual		Estimated	
	R *	p**	R	p	R	p	R	p	R	p	R	p
SCFA	0.98	0.003	0.94	0.01	0.90	0.03	0.94	0.01	0.94	0.01	0.90	0.03
Acetate	0.87	0.07	0.81	0.09	0.98	0.004	0.90	0.03	0.94	0.008	0.96	0.009
Propionate	0.83	0.08	0.85	0.06	0.90	0.03	0.94	0.01	0.93	0.02	0.87	0.06
Butyrate	0.65	0.23	0.62	0.25	0.97	0.004	0.90	0.03	0.92	0.02	0.86	0.06

* R; Pearson correlation coefficient, ** p value significant (P < 0.05)

5.5.4.1 Correlation of total SCFA concentration (µmol/ml) of different mixture NDCs (low, medium and high FC) after 24 h incubation vs. predicted FC using the predictive equation

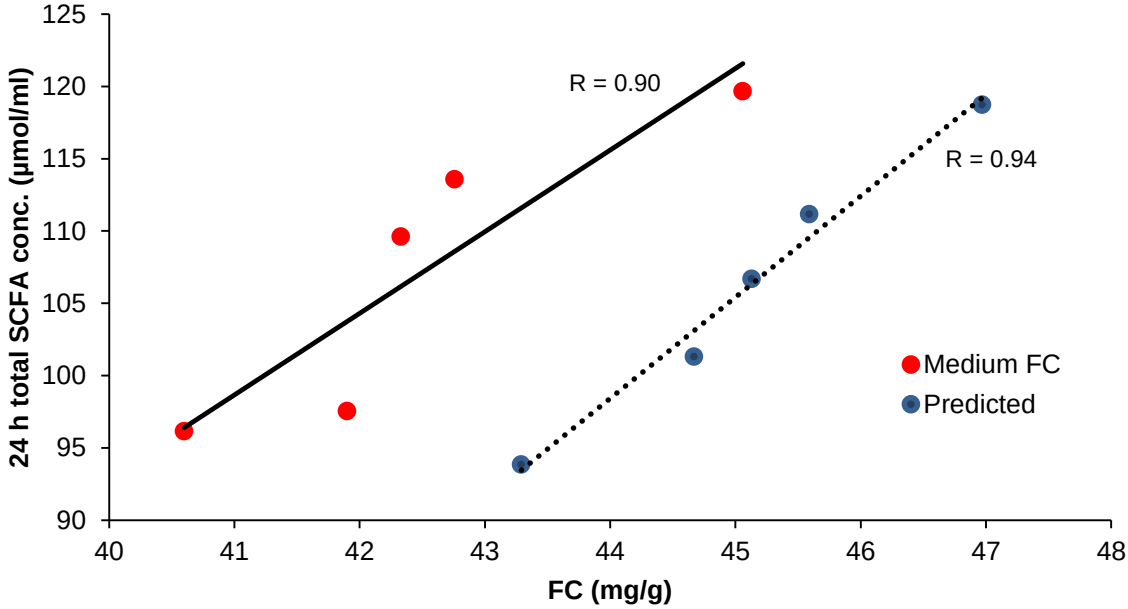
The results (Table 5.9) showed that total SCFA concentration was strongly correlated with low-FC mixture NDCs fermentation (R = 0.98; p = 0.003) when compared with the predicted FC (R = 0.94; p = 0.01; Figure 5.9). Whereas, predicted FC had a better correlation with total SCFA concentration (R = 0.94; p = 0.01) when compared with medium-FC mixture NDCs (R = 0.90; p = 0.03; Figure 5.10). The bland Altman plot showed that the bias between medium mixture NDCs total SCFA concentration and predicted total SCFA concentration was fairly small (1.0 µmol/ml; Figure 5.11), with wide limits of agreement (6.3 and -4.4 µmol/ml). Moreover, high-FC mixture NDCs fermentation (R = 0.94; p = 0.01) and predicted FC (R = 0.90; p = 0.03) showed a good relationship with total SCFA concentration. Overall these results show a good correlation between different mixture of NDCs (low, medium and high FC) with total SCFA concentration and with those predicted FC. Therefore, we can conclude that the newly developed predicted equations deemed a valid and practical tool to assess SCFA production in in vitro fermentation.

Figure 5.9: Scatter-plot showing correlation of total SCFA concentration (µmol/ml) of mixture low-FC (n = 5) after 24 h incubation vs. predicted mixture low-FC (n = 5). Dotted line indicates mixture low-FC; solid line indicates predicted mixture low-FC.



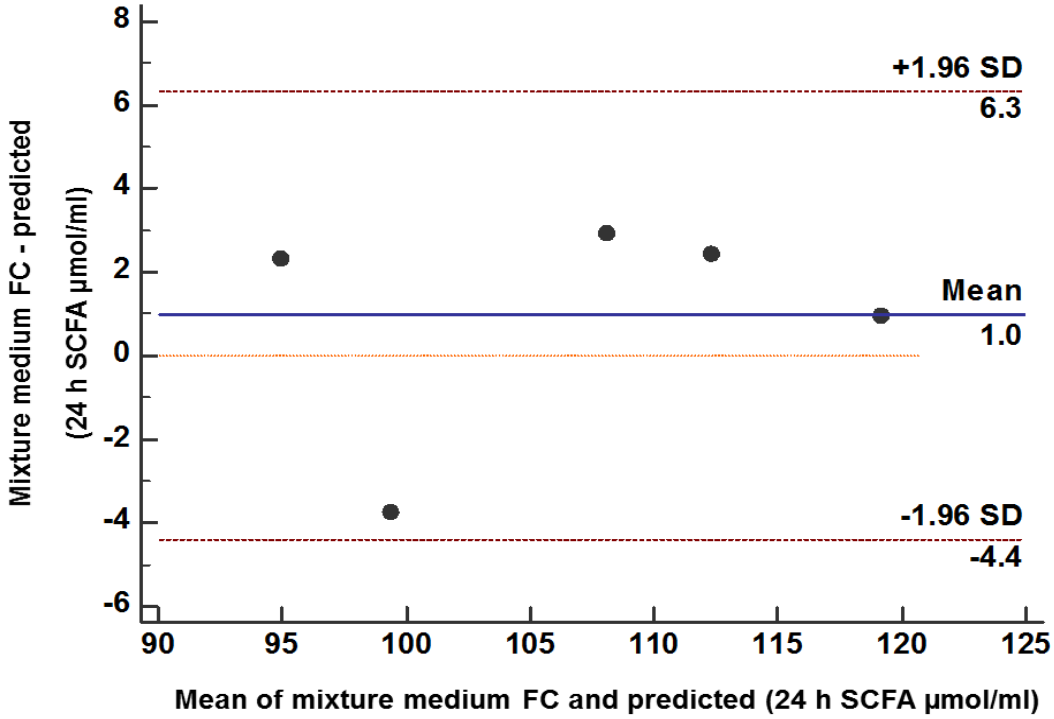
Blue circle dot indicates predicted SCFA of mixture low-FC (µmol/ml). Red circle dot indicates SCFA produced from *in vitro* fermentation of mixture low-FC (µmol/ml).

Figure 5.10: Scatter-plot showing correlation of total SCFA concentration (µmol/ml) of mixture Medium-FC (n = 5) after 24 h incubation vs. predicted mixture Medium-FC (n = 5). Solid line indicates medium mixture of FC; Dotted line indicates predicted mixture Medium-FC.



Blue circle dot indicates predicted SCFA of mixture Medium-FC (µmol/ml). Red circle dot indicates SCFA produced from *in vitro* fermentation of mixture Medium-FC (µmol/ml).

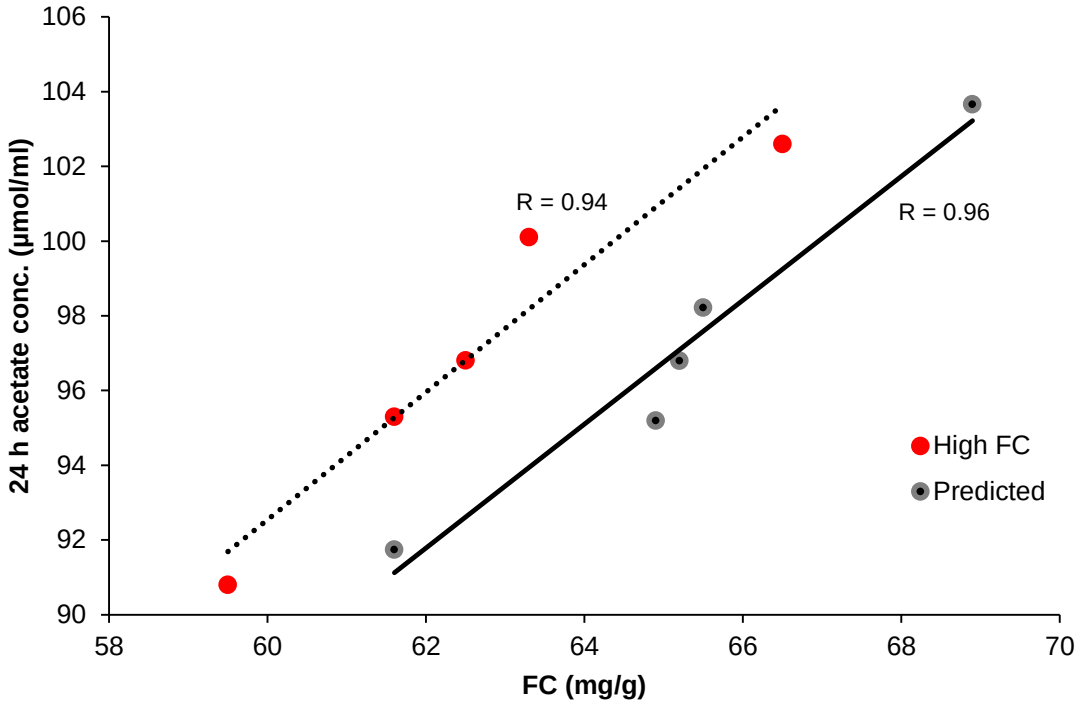
Figure 5.11: Bland Altman plot of total SCFA concentration (µmol/ml) of mixture Medium-FC (n = 5) after 24 h incubation vs. predicted mixture Medium-FC (n = 5).



5.5.4.2 Correlation of acetate concentration (µmol/ml) of different NDCs mixture (low, medium and high FC) after 24 h incubation vs. predicted FC using the predictive equation

At 24 hours, fermentation of the high FC mixture induced the highest acetate concentration (97.13 ± 2.0 µmol/ml), followed by the medium mixture (67.05 ± 2.4 µmol/ml). At 4 and 24 hours, there was significant difference in the acetate concentration between high, medium and low FC mixture of NDCs respectively ($p<0.05$). The results (Table 5.9) show there was no relationship observed between the acetate concentration and low-FC mixture of NDCs fermentation, and the predicted FC, whereas the medium-FC mixture of NDCs had an excellent correlation with the acetate concentration ($R = 0.98$; $p = 0.004$) compared with predicted FC ($R = 0.93$; $p = 0.02$). The high-FC mixture of NDCs fermentation and predicted FC, showed significant relationship with acetate concentration (Figure 5.12).

Figure 5.12: Scatter-plot showing correlation of acetate concentration (µmol/ml) of High-FC mixture (n = 5) after 24 h incubation vs. predicted High-FC mixture (n = 5). Dotted line indicates High-FC mixture; solid line indicates predicted High-FC mixture.



Black circle dot indicates predicted SCFA of High-FC mixture (µmol/ml). Red circle dot indicates SCFA produced from *in vitro* fermentation of High-FC mixture (µmol/ml).

5.5.4.3 Correlation of propionate concentration (µmol/ml) of different NDCs (low, medium and high FC) mixtures after 24 h incubation vs. predicted FC using the predictive equation

The results showed there was no significant difference in the propionate concentration between any of the NDCs mixtures after 4 h of incubation. After 24 hours of fermentation, all the NDCs fermentable mixtures had a higher propionate concentration than at 4 hours, but there were still no significant differences observed between the different fermentable NDCs mixtures.

By comparing the different FC mixtures and predicted values, the results (Table 5.9) show only the medium-FC mixture of NDCs (R = 0.90; p = 0.03) and the predicted FC (R = 0.94; p = 0.01) had significant relationship with the propionate concentration. The high FC mixtures of NDCs (R = 0.93; p = 0.02) showed a good correlation with propionate concentration, but the predicted FC did not have a good relationship with the propionate concentration. Overall these results show a good agreement correlation between different mixture of NDCs (low, medium and high FC) with propionate concentration and with those predicted FC.

5.5.4.4 Correlation of butyrate concentration (µmol/ml) of different NDCs (low, medium and high FC) mixtures after 24 h incubation vs. predicted FC using the predictive equation

At 4 h, the high-FC mixture of NDCs showed a significantly higher butyrate concentration (2.50 ± 0.3 µmol/ml) than low and medium FC mixtures. At 24 h of fermentation, there was also no significant differences observed in butyrate concentration between all the FC mixtures of NDCs.

In term of the relationship between the FC mixtures of NDCs and the predicted FC with butyrate concentration, the butyrate concentration was strongly correlated with the medium FC mixture NDCs fermentation ($R = 0.97$; $p = 0.004$) compared with the predicted FC ($R = 0.90$; $p = 0.03$; Table 5.9).

5.5.4.5 *In vitro* fermentation of different NDCs (low, medium and high FC) mixtures pH changes

The analysis of the pH of the cultures (Table 5.10) showed that the pH dropped rapidly during the fermentation of low, medium and high FC mixtures of NDCs up to 4 hours. The pH in culture of medium FC then only showed relatively small decreases in pH during prolonged incubation, whereas there was a progressive fall in pH from 0 hour to 24 hours of fermentation for the cultures containing high FC. Overall, the high FC consistently produced a lower pH compared to the low and medium FC cultures.

Table 5.10: Changes in pH (mean ± SEM) of human faecal cultures containing low, medium and high FC mixtures of NDCs over 24 h (n=5) after *in vitro* anaerobic incubation with human faeces.

	pH at 0 h	pH at 4 h	pH at 24 h
Low-FC	7.22 ± 0.02 [a]	6.43 ± 0.11 [a]	5.93 ± 0.12 [a]
Medium-FC	7.20 ± 0.05 [a, c]	6.45 ± 0.08 [a, e]	6.13 ± 0.09 [a, d]
High-FC	7.18 ± 0.03 [c]	6.42 ± 0.11 [a, d]	5.41 ± 0.14 [b, e]
Blank	7.22 ± 0.07 [a, c]	7.32 ± 0.05 [b]	7.33 ± 0.04 [c]

Values with different letters within a column are significantly ($P < 0.05$) different. There are significant ($P < 0.05$) differences between time points within a row except Blank (0h vs. 4h and 4h vs 24h).

5.5.5 Residue dry weight of isolated FC mixtures of (RS, soluble DF, insoluble DF and oligosaccharide) after in vitro digestion vs. predicted fibre

The results (Table 5.11) for the amount of isolated RS, soluble DF, insoluble DF and NDOs after removing the digestible starch from the samples using an extensive *in vitro* enzymatic digestion model, vs. the predicted amount of the same fibre measured using developed FC equations showed the differences between the isolated mixtures and predicted values produced.

Table 5.11: Non-digestible fractions of isolated FC mixtures of (RS, soluble DF, insoluble DF and oligosaccharide) vs. predicted (mg/g).

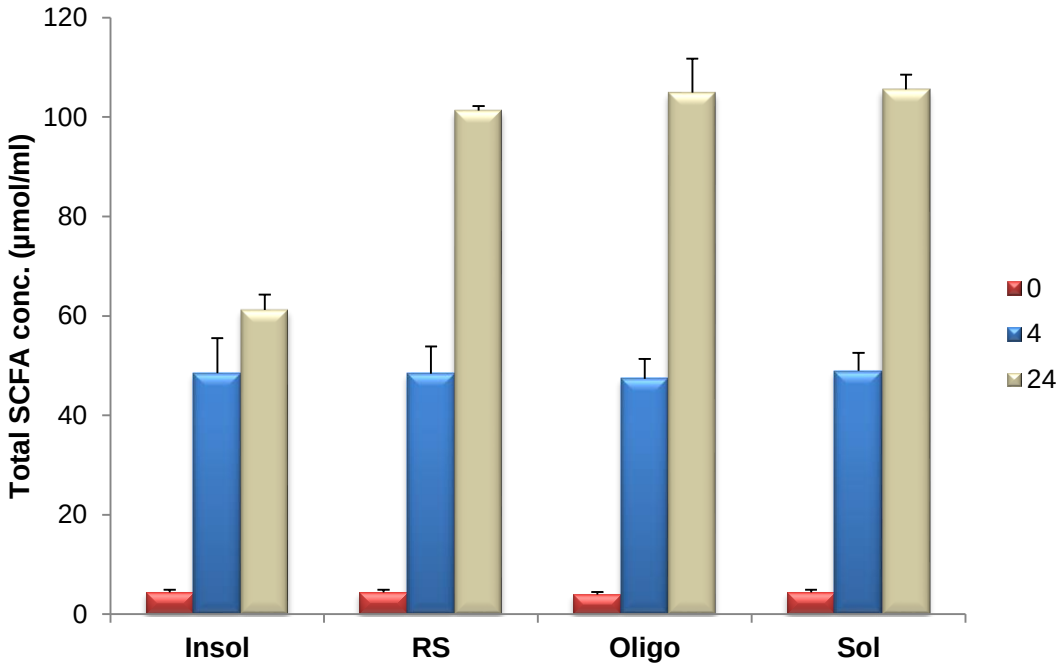
NDC	Isolated mixture of RS		Isolated mixture of soluble DF		Isolated mixture of insoluble DF		Isolated mixture of oligosaccharide	
	Actual	Predicted**	Actual	Predicted	Actual	Predicted	Actual	Predicted
Resistant Starch	336.47 ± 16.9	378.56 ± 19.0	14.18 ± 0.7	5.02 ± 0.2	10.53 ± 0.5	27.95 ± 1.4	44.72 ± 2.0	46.01 ± 2.3
Fructan	5.27 ± 0.2*	5.95 ± 0.3	2.43 ± 0.1	5.95 ± 0.3	2.1 ± 0.1	0.84 ± 0.4	9.36 ± 0.4	21.24 ± 1.0
Soluble DF	12.37 ± 0.6	12.37 ± 0.6	108.54 ± 5.5	108.54 ± 5.5	56.05 ± 2.8	56.05 ± 2.8	86.35 ± 4.3	86.35 ± 4.3
Insoluble DF	25.84 ± 1.3	25.84 ± 1.3	23.67 ± 1.1	23.67 ± 1.2	47.36 ± 2.4	47.36 ± 2.39	13.91 ± 0.7	13.91 ± 0.7

* The values are reported as mean (standard deviation) for actual (n = 5) vs. predicted (n = 5); ** Predicted FC-DF 4 equation: 100 % Soluble DF + 50 % insoluble DF + 100 % NDOs + 10 % TS.

5.5.6 *In vitro* fermentation of mixtures of different isolated FC (RS, Soluble DF, Insoluble DF and NDOs)

Total SCFA concentrations during in vitro fermentation were used to compare the equations for FC against the individual FC dietary models in this experiment (Table 5.12). The results show there was no significant difference in the total SCFA concentration in these models at 4 h (Figure 5.13), whereas at 24 h, it was observed that insoluble DF isolated mixture had lower SCFA concentration (61.19 ± 3.0 µmol/ml) compared with RS, soluble and oligosaccharide isolated mixtures (Table 5.12).

Figure 5.13: Mean (±SEM) concentrations of total SCFA (µmol/ml) produced after 24 h incubation of mixtures of different isolated FC (RS, Soluble DF (Sol), Insoluble DF (Insol) and NDOs (Oligo)) with the faecal inoculum (n= 5).



5.5.6.1 Correlation of total SCFA concentration (µmol/ml) of mixtures of different isolated FC (n = 5; RS, Soluble DF, Insoluble DF and NDOs) after 24 h incubation vs. predicted FC (n = 5) using the predictive equation

The results (Table 5.13) show the isolated RS mixture fermentation was strongly correlated with total SCFA concentration (R = 0.99; p = 0.001), when compared with predicted FC (R = 0.97; p = 0.005). No correlation was observed for the isolated mixture of soluble DF and predicted FC with total SCFA concentration. The isolated mixture of insoluble DF showed a higher correlation with total SCFA concentration (R = 0.96; p = 0.007) than the predicted FC (R = 0.93; p = 0.01), whereas the isolated

NDOs mixture showed a strong correlation with total SCFA concentration (R = 0.96; p = 0.007), when compared with predicted FC (R = 0.94; p = 0.01) (Table 5.13).

Table 5.12: Concentration of total and major individual SCFA (µmol/ml) between 0 and 24 h of *in vitro* fermentation using human faecal samples (n = 5) with NDC substrates.

SCFA	NDC			
	Isolated mixture of RS	**Isolated mixture of soluble DF**	**Isolated mixture of insoluble DF**	**Isolated mixture of NDOs**
Acetate				
0 h	3.49 ± 0.3 [a]	3.64 ± 0.2 [a]	3.40 ± 0.3 [a]	3.00 ± 0.2 [b**]
4 h	30.48 ± 2.2 [a]	32.91 ± 3.0 [a]	31.73 ± 2.6 [a]	29.48 ± 3.2 [a]
24 h	50.70 ± 4.6 [a]	61.77 ± 3.1 [b]	42.77 ± 1.4 [c]	56.04 ± 3.9 [c, d]
Propionate				
0 h	0.53 ± 0.2 [a*]	0.48 ± 0.2 [a]	0.56 ± 0.2 [a]	0.30 ± 0.1 [a]
4 h	16.02 ± 5.6 [a, b]	17.88 ± 3.7 [a, b]	9.00 ± 3.4 [a]	25.38 ± 2.8 [b]
24 h	36.59 ± 4.3 [a]	36.45 ± 2.0 [a]	37.52 ± 3.8 [a, b]	48.46 ± 4.7 [b]
Butyrate				
0 h	0.36 ± 0.1 [a]	0.41 ± 0.1 [a]	0.41 ± 0.1 [a]	0.22 ± 0.1 [a]
4 h	1.77 ± 0.6 [a]	1.36 ± 0.4 [a]	1.1 ± 0.1 [a]	0.88 ± 0.2 [a]
24 h	7.96 ± 0.9 [a]	5.55 ± 1.0 [a]	3.04 ± 0.4 [b]	5.25 ± 1.0 [a]
Total SCFA				
0 h	4.42 ± 0.5 [a]	12.96 ± 1.5 [b]	4.40 ± 0.5 [a]	3.94 ± 0.6 [c]
4 h	48.40 ± 5.4 [a]	48.94 ± 3.6 [a]	48.52 ± 7.0 [a]	47.38 ± 3.9 [a]
24 h	101.30 ± 0.9 [a]	105.58 ± 2.9 [a]	61.19 ± 3.0 [b]	104.91 ± 6.8 [a]

*Values with different letters are significantly different within a row. **SCFA was compared with paired t-test between 0, 4 and 24 hours for the same type of culture ($P < 0.05$).

Table 5.13 Correlation of actual NDC fermentation of total and major individual SCFA concentrations (µmol/ml) after 24 h of incubation using human faecal microbiota vs. predicted FC (n = 5).

SCFA	Isolated mixture of RS				Isolated mixture of soluble DF				Isolated mixture of insoluble DF				Isolated mixture of oligosaccharide			
	Actual		Predicted		Actual		Predicted		Actual		Predicted		Actual		Predicted	
	R	p	R	p	R	P	R	p	R	p	R	p	R	p	R	p
SCFA	0.99	0.001	0.97	0.005	0.78	0.13	0.77	0.12	0.96	0.007	0.93	0.01	0.96	0.007	0.94	0.01
Acetate	0.78	0.11	0.75	0.14	0.82	0.09	0.82	0.09	0.94	0.01	0.96	0.009	0.94	0.01	0.94	0.01
Propionate	0.81	0.09	0.80	0.10	0.72	0.16	0.74	0.15	0.99	0.00	0.99	0.00	0.93	0.02	0.92	0.02
Butyrate	0.97	0.006	0.94	0.01	0.77	0.12	0.78	0.11	0.95	0.01	0.92	0.02	0.97	0.004	0.93	0.02

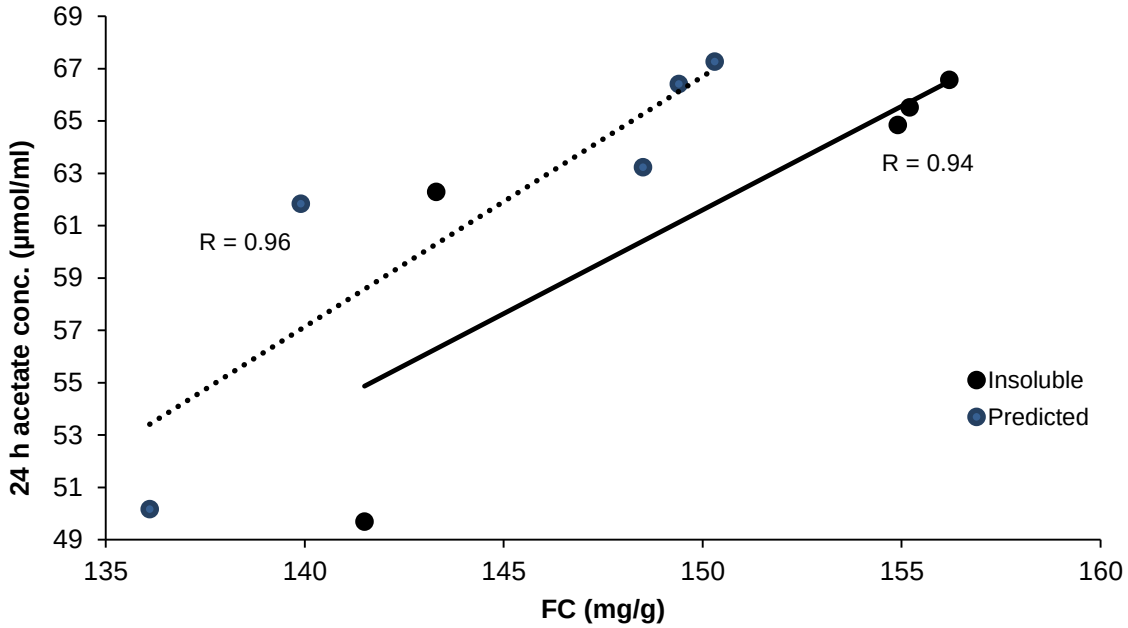
* R; Pearson correlation coefficient, ** p value significant (P < 0.05)

5.5.6.2 Correlation of acetate concentration (µmol/ml) of mixtures of different isolated FC (RS, Soluble DF, Insoluble DF and NDOs) after 24 h incubation vs. predicted FC using the predictive equation

The results (Table 5.12) show there was no significant difference in the acetate concentration at 4 h of fermentation in all these models. After 24 h there was a consistent but small increase in acetate in the isolated mixture containing RS (50.70 ± 4.6 µmol/ml), soluble DF (61.77 ± 3.1 µmol/ml) and NDOs (56.04 ± 3.9 µmol/ml), with a significantly higher acetate concentration compared with insoluble DF (Table 5.12).

The isolated RS (Table 5.13), soluble DF mixtures and predicted FC showed no relationship with the acetate concentration, whereas the predicted FC showed an excellent correlation with acetate concentration (R = 0.96; p = 0.009), which was better than the isolated mixture of insoluble DF (R = 0.94; p = 0.01; Figure 5.14). The isolated NDOs mixture and predicted FC showed a similar correlation with the acetate concentration (R = 0.94; p = 0.01) (Table 5.13).

Figure 5.14: Scatter-plot showing correlation of acetate concentration (µmol/ml) of isolated FC mixture of insoluble DF (n = 5) after 24 h incubation vs. predicted isolated FC mixture of insoluble DF (n = 5). Solid line indicates isolated FC mixture of insoluble DF; Dotted line indicates predicted isolated FC mixture of insoluble DF.



Blue circle dot indicates predicted SCFA of isolated FC mixture of insoluble DF (µmol/ml). Black circle dot indicates SCFA produced from *in vitro* fermentation of isolated FC mixture of insoluble DF (µmol/ml).

5.5.6.3 Correlation of propionate concentration (µmol/ml) of mixtures of different isolated FC (n = 5; RS, Soluble DF, Insoluble DF and NDOs) after 24 h incubation vs. predicted FC (n = 5) using the predictive equation

The results (Table 5.13) show there was a significant difference in the propionate concentration between the isolated FC mixture of insoluble DF and RS, soluble DF and NDOs FC mixtures at 4 hours. At 24 h, the isolated FC mixture of NDOs continued to have a higher propionate concentration (48.46 ± 4.7 µmol/ml) than the other mixtures. RS and soluble DF isolated mixtures had almost similar propionate concentrations at 24 hours of fermentation.

The isolated RS (Table 5.13), soluble DF mixtures and predicted FC showed no relationship with the propionate concentration, whereas the predicted FC showed an excellent correlation with the propionate concentration ($R = 0.99$; $p = 0.0001$), and the isolated mixture of insoluble DF ($R = 0.99$; $p = 0.0001$). The predicted FC ($R = 0.92$; $p = 0.02$) and isolated NDOs mixture ($R = 0.93$; $p = 0.02$) showed a similar correlation with the propionate concentration (Table 5.13).

5.5.6.4 Correlation of butyrate concentration (µmol/ml) of mixtures of different isolated FC (n= 5; RS, Soluble DF, Insoluble DF and NDCs) after 24 h incubation vs. predicted FC (n =5) using the predictive equation

The results (Table 5.13) showed there was no significant difference in the concentration of butyrate between the carbohydrate models at 0 hours of fermentation, but there was a substantial butyrate concentration with the increasing incubation time observed at 4 h the for FC mixture of RS, compared with other mixtures. At 24 h, the fermentation of the RS mixture had the highest concentration of butyrate (7.96 ± 0.9 µmol/ml), followed by the soluble DF and the NDOs mixtures and were significantly higher than the insoluble DF mixture.

The isolated FC mixture of RS (Table 5.13) showed an excellent relationship with the butyrate concentration ($R = 0.97$; $p = 0.006$), which was better than the predicted FC ($R = 0.94$; $p = 0.01$). Soluble DF mixtures showed no relationship with butyrate concentration. The isolated mixture of insoluble DF ($R = 0.95$; $p = 0.01$) and the isolated oligosaccharide mixture ($R = 0.97$; $p = 0.004$) showed a higher correlation with the butyrate concentration than predicted FC (Table 5.13).

5.5.6.5 *In vitro* isolated FC mixtures fermentation pH changes

The results for the changes in pH over time (Table 5.14) showed that at 0 h, there were no pH differences observed between all the mixture cultures, and then the pH decreased rapidly up to 4 h during the fermentation of RS, soluble DF, insoluble DF and NDOs mixtures. The pH in culture of insoluble DF mixture did not change appreciably during prolonged incubation, whereas a progressive fall in pH from 0 to 24 hours of fermentation was seen only in cultures containing RS, soluble DF and NDOs mixtures. The final pH (24 hours) was significantly lower in NDOs than insoluble DF mixtures (Table 5.14).

Table 5.14: Changes in pH (mean ± SEM) of human faecal cultures containing, Soluble DF, Insoluble DF and NDOs isolated FC mixtures over 24 h (n=5) after *in vitro* anaerobic incubation

	pH at 0 h	pH at 4 h	pH at 24 h
Isolated mixture of RS	7.14 ± 0.03 [a]	6.05 ± 0.10 [a]	5.95 ± 0.09 [a, b]
Isolated mixture of soluble DF	7.15 ± 0.05 [a]	6.03 ± 0.17 [a, b]	5.93 ± 0.13 [a, b]
Isolated mixture of insoluble DF	7.15 ± 0.04 [a]	6.08 ± 0.09 [a]	6.07 ± 0.07 [b]
Isolated mixture of NDOs	7.14 ± 0.03 [a]	5.88 ± 0.12 [b]	5.80 ± 0.12 [a]

Values with different letters within a column are significantly ($P < 0.05$) different. There are significant ($P < 0.05$) differences between time points within a row except high insoluble NSP model (4 h vs 24 h).

5.6 Discussion

These *in vitro* digestion and fermentation experiments were conducted to validate the relationship between FC and the 24 h urinary excretion of acetate and SCFA, observed in the previous habitual dietary intake and 24 h urinary excretion SCFA study (chapter 4). The results in chapter 4 showed the equations for FC-DF 4 and FC-NSP 4 had a good correlation with 24 h urinary excretion of acetate and SCFA compared to either DF or NSP alone. To the best of our knowledge, this is the first *in vitro* study to estimate FC from single and mixtures of different NDCs with the use of developed equations to predict the amount of SCFA produced in an *in vitro* fermentation of NDC after digestion in an *in vitro* enzymatic digestion model.

Therefore, it is important to perform an *in vitro* model of enzymatic digestion to hydrolyse starch and protein before undergoing *in vitro* fermentation (Aura et al., 1999, Nordlund et al., 2012). In this study an enzymatic *in vitro* digestion was performed to remove digestible starch from the samples and to produce NDCs fractions prior to the *in vitro* colon model. The fructan content of raftiline decreased from 90 to 87 %, which shows the change in the fructan content by the digestion step was small, whereas the content of RS in raw potato starch decreased from 72 to 50 % in this step which is as expected.

In the current study, measurements of SCFA concentration carried out by the two different methods were highly correlated. The SCFA concentration in estimated FC using our developed equations is predicted by mathematical models (Fievez et al., 2005). The agreement of the SCFA concentration measurement methods was assessed using Bland Altman plots. Bland and Altman measures agreement between two quantitative methods by constructing limits of agreement, which calculated by using the mean and the standard deviation of the differences between two methods. This will allow to check the assumptions of normality of differences (Giavarina, 2015). Whereas, Pearson correlation coefficient measures the degree to which two variables are related. However, a high correlation does not mean that there is good agreement between the two methods. The correlation coefficient and regression technique are sometimes inadequate and misleading when assessing agreement, because they evaluate only the linear association of two methods (van Stralen et al., 2008).

The overall bias of SCFA concentration was relatively small and the limits of agreement were wide for all the SCFA concentrations of FC. The most interesting findings of our *in vitro* experiments were that several agreements were observed between the total SCFA and acetate concentrations after *in vitro* fermentation of the single fibres and a different mixture of NDCs, and those predicted the estimated FC using our developed equations. For total SCFA concentration, the fermentation *in vitro* of the mixture of all 4 fibres and the medium FC mixture NDCs had an excellent agreement with predicted FC compared with other NDCs. On the other hand, psyllium had a stronger agreement with predicted FC values when examined against the acetate concentration. This study produced results which corroborate the findings of a large number of the previous work on *in vitro* digestion and fermentation of NDCs to predict the amount of fermentation products *in vivo*. Daniel et al. (1997) used a randomized cross-over study for three weeks which fed six women meals containing low fibre diet, two high fibre diets high or low in protein, an d a barley fibre concentrate. In an *in vitro* fermentation phase, faecal samples were obtained from these women and fibre residues were obtained from the fibre containing foods consumed during the trials. They observed that NSP fermentation in humans was 83.8 ± 0.9 % (low fibre diet), 61.8 ± 3.6 % (high fibre diet high in protein) and 59.2 ± 3.9 % (high fibre diet low in protein). The *in vitro* fermentation was different from fermentation in humans by −4.0 ± 1.6 % (low fibre diet, $p < 0.05$), 4.9 ± 3.7 % (high fibre diet high in protein) and 8.8 ± 3.0 % (high fibre diet low in protein, $p < 0.01$). The authors conclude that fermentation of NSP in humans *in vivo* could be predicted with sufficient accuracy by this *in vitro* fermentation. These findings were also confirmed in a study conducted by the same authors, which found similar results (Wisker et al., 1998).

The SCFA profiles depends on the physiochemical properties of FC in the colon; the human diet is made up of mixtures of NDCs, which might regulate the role SCFA products in the colon (Flint et al., 2012). This current study found that the fermentation pattern of total SCFA concentration of the mixture of all 4 fibres had a higher total SCFA concentration, followed by the raw potato starch and raftiline, which was significantly higher when compared with the culture of wheat barn and psyllium at 24 hours. The lowest SCFA producer was wheat bran, which is composed of > 80 % insoluble DF, which supports our predicted equation that 50 % might be fermented. Chen et al. (1998) reported that 90 % of wheat bran is insoluble fibre, which contributed to an increase faecal output and lower colonic transit time.

Nordlund et al. (2012) investigated fermentability of rye bran and aleurone, wheat bran and aleurone, and oat bran and cell wall concentrate *in vitro*. Their results showed wheat ban produced a lower total SCFA than other fibre (69 mM/ 24 h).

In our experiments, fermentation of raw potato starch, the mixture of all 4 fibres and isolated FC mixture of RS had the highest concentrations of butyrate (16.18 ± 2.3, 13.03 ± 0.6, and 7.96 ± 0.9 respectively), compared with different NDCs (low, medium and high FC) mixtures. This finding supports previous research into this area which links RS fermentation with a high butyrate production. Muir et al. (2004) in a randomized crossover study showed that a diet combining wheat bran and RS increased faecal bulking, reduced transit time, increased butyrate and acetate productions, when compared to wheat bran alone. A randomized cross over study carried out by McOrist et al. (2011) investigated effectiveness of habitual diets supplemented with 25 g of NSP, or 25 g of NSP plus 22 g of RS/day, over 4 weeks in 46 healthy adults, on in increasing faecal butyrate excretion. They concluded that the habitual intake of a high NSP plus RS diet was associated with a higher faecal butyrate concentration than NSP alone, which may result in promoting optimum colorectal health (McOrist et al., 2011). Since the different NDCs (low, medium and high FC) mixtures were obtained from habitual diets of general population, there are several possible explanations for high butyrate and SCFA concentrations when combining RS with other FC substrates. The habitually high fibre consumers are likely to eat a wide variety of fibre types, not all of which may be readily fermentable, for example wheat bran, whereas supplementation studies only added known and relatively well controlled amounts of fermentable NDCs. Another possible explanation is that a greater overall intake of fermentable substrates for example, protein, fibre, and starch combined, which may have overloaded the gut microflora and would lower the SCFA production. Also, individuals differ in the capacity of their large bowel microflora to ferment NDCs (Cummings et al., 1996). This could affect the supply of SCFA products to the large bowel and also an individual's response to dietary change.

The results of these *in vitro* fermentation experiments indicate that propionate formation was favored by the isolated mixture of oligosaccharide DF rather than the isolated mixture of insoluble DF at 24, whereas the high-FC mixture of NDCs had an intermediate value between the two. This supports previous *in vitro* results where PHGG increased propionate production compared to the other fibres, and the work

by Kedia et al. (2009) which showed higher *in vitro* propionate production from oat bran than from FOS. Karppinen et al. (2000) reported a high propionate formation oat bran compared with those from rye and wheat bran. Kim and White (2009) observed a significant propionate production capacity of β-glucan from oat independent of the molecular weight of β-glucan.

The investigation of whether the amount of SCFA produced in an *in vitro* fermentation of FC after use of an *in vitro* enzymatic digestion model could be predicted by the developed equations of FC, showed several agreements between the total SCFA and acetate concentrations after *in vitro* fermentation of FC, and those predicted FC using our developed equation. The mixture of all 4 fibres and the medium-FC mixture NDCs showed an excellent correlation with total SCFA concentration produced and with those predicted FC. Additionally, the predicted FC and psyllium fibre had a strong correlation with acetate concentration. These relationship agreements were supported by the bland Altman plot analysis. Moreover, raw potato starch and raftiline fermentation showed a high agreement with the predicted FC, when examined against the SCFA concentration, especially for total SCFA and acetate concentrations. As > 70 % of raw potato starch is RS (McCleary et al., 2002), and raftiline is also composed of 95 % fructan, the results were a better fit with more SCFA than expected, possibly this is because nearly pure RS was used, which made the RS 100 % in our equation. Therefore, we can conclude that the newly developed predicted equations have been deemed a valid and practical tool to assess SCFA productions for *in vitro* fermentation.

One of the limitations of this study could be the small sample size, but as the results showed the validity of these developed predicted equations of FC, they may be considered as a preliminary practical tool to assess SCFA productions of *in vitro* fermentation in future studies. Another limitation of this study was that we used isolated FC rich diets, and different mixtures of FC obtained from habitual dietary records; using NDC from well-designed interventions and comparing these with the fermentation in humans *in vivo* might give more reliable results. This could include the use of the stable isotope tracers' technique to measure colonic FC fermentation in the colon. Several studies reported that the tracer technique, using stable isotope-labelled biomarkers, could be used to investigate the colonic NDC fermentation in humans, especially as this technique is easy to perform, non-invasive and completely safe (Pouteau et al., 1998, De Preter et al., 2004).

In conclusion, our results from these three *in vitro* digestion and fermentation experiments suggested that there are strong correlations between the amounts of SCFA produced after actual *in vitro* fermentation of single fibre and different mixture of NDC, with the estimated FC from our developed equations. Therefore, these findings support our hypothesis that it is possible to estimate FC using our equations for *in vitro* fermentation of single DF and mixtures of different FC *in vitro*, after an extensive *in vitro* enzymatic digestion model used to remove digested starch prior to the *in vitro* fermentation of NDC samples. Therefore, these equations may be useful in predicting the SCFA produced in the proximal colon in humans, and further studies *in vivo* using stable isotope tracers of SCFA flux are needed for further validation.

6 Chapter Six

General Discussion

6.1 Introduction

The last decade has witnessed a significant increase in research into NDCs for the prevention of diseases (Jones, 2004). Several studies have reported that long term consumption of diets rich in NDCs, such as cereals, fruit and vegetables, may protect against several chronic diseases including CVD (Threapleton et al., 2013b), colorectal cancer (CRC) (Aune et al., 2011a, Park et al., 2005), diabetes mellitus (Carter et al., 2010) and obesity (Lindström et al., 2006). However, in many large cohorts use data from FFQ and 24-h dietary recalls that designed to measure a person's usual dietary fibre intake over a defined period of time. Over the years, researchers have observed that the reported values from FFQs are subject to substantial error of both systematic and random, that can profoundly affect the results of epidemiologic studies (Maki et al., 2014). In addition, participants' dietary intake underreporting appears to increase with increasing BMI, which may affect estimation of dietary intake. Moreover, changes in the food supply and dietary habits over time, incomplete information in food and nutrient databases, and changes in commercial food products due to formula optimization that are not rapidly explained in nutrient databases may also contribute to inaccurate assessments of dietary intakes (Bingham et al., 1995).

Likewise, the World Cancer Research Fund came to a consensus that NDCs 'probably' provide protective effects against CRC incidence when obtained from a high cereal, fruit and vegetable intake (WCRF, 2007). The issue here is that there are some conflicting studies and to allocate a level of 'convincing evidence' the criteria demanded 'no substantial unexplained heterogeneity' in the evidence. This is difficult to achieve if there are inconsistencies in the estimations of NDC intake (what is included as dietary fibre, method of analysis for food tables and definitions) and if there are several mechanisms which may interact in different types of diet. It is also difficult to see any effect if there is limited variation in intake in the cohort which can be a probably in many of the large cohort studies (Jones, 2014a). One of the more varied population cohort studies is the European Prospective Investigation into Cancer and Nutrition Study (EPIC), which is a multi-centre prospective cohort that includes more than 500,000 participants from 10 European countries. This study reported that the risk of colorectal cancer was inversely associated with intakes of total fruit and vegetables and total fibre (Bradbury et al., 2014). The strength of the EPIC study is due to recruiting half a million participants, and to date

more than 60,000 incident cases of cancer have been identified. Also, one of the major strengths of EPIC is the inclusion of study centres in 10 European countries, which provide considerable variation in dietary intakes within the cohort (Ferrari et al., 2004). Furthermore, a recent systematic review and meta-analysis of 42 cohort studies conducted by Liu et al. (2015) proposed that fibre consumption inversely associates with CVD by 23% (HR, 0.77; 95% CI, 0.72-0.81), by 17% (HR, 0.83; 95% CI, 0.74-0.91) for cancer, by 23% (HR, 0.77; 95% CI, 0.73-0.81) for all-cause mortality.

Most of the evidence for the benefits of NDCs has been derived from epidemiological studies, but for many reasons, it has been problematic to fully establish the impact of NDCs on health in epidemiology studies (Jones, 2014a). Firstly, there are a wide range of properties of different NDCs, and combining them all in one category for statistical comparisons may dilute their impact in correlations or multivariate analysis. Secondly, several mechanisms have been suggested to explain the protective effects of NDCs (Jones, 2014a), including increased stool bulk (Karppinen et al., 2000), dilution of carcinogens in the colonic lumen (Tang et al., 2011a), reduced transit time (Lampe et al., 1993a), lowering colonic pH, and bacterial fermentation to SCFA in the colon (Walker et al., 2005). Not all NDCs have the same properties nor exert their effects through the same mechanisms.

Thirdly, one of the major obstacles to epidemiological studies on diet and the risk of chronic disease, is the relative inaccuracy of the methods with which individuals' habitual long term intake of food or nutrients can be measured by structured dietary questionnaires (Jones, 2014a). These methods could cause estimates of relative risk, or other measures of diet disease associations to be weakened (an effect usually referred to as attenuation bias), and reduce the statistical power of epidemiologic studies (Kaaks, 1997, Westenbrink et al., 2013b). The traditional analysis method of epidemiological data depend on food composition databases, which might not have been updated to include NDOs and RS, or to have accurate data on NDC added to food (Westenbrink et al., 2013a, Hollmann et al., 2013).

One major route by which NDCs may act is via fermentation including the production of SCFA. The potential role of SCFA in promoting health has received much attention, and this has included more recently, the role of propionate in satiety (Arora et al., 2011), and the role of SCFA in obesity (Cani et al., 2005). However, it is very

difficult to measure SCFA *in vivo* in humans with any accuracy, so epidemiological studies on the impacts of SCFA are impossible. Most studies use DF or NSP intake to measure SCFA, but not all DF or NSP are equally fermentable (Jones, 2014a)

Moreover, different types of NDCs are fermented to varying extents in the human gut, meaning that the actual amount of FC entering the large intestine is very difficult at present to predict (Laurentin and Edwards, 2005). To the best of our knowledge, currently there is no data in the literature aimed at developing an index which allows estimates of NDCs that escape digestion and absorption, and are fermented fully to SCFA by the colonic bacteria. Therefore, the work in this thesis has focused on developing an index of FC that can be used to estimate, from food diaries, the amount of carbohydrate that escapes digestion and absorption and is fermented to SCFA. Such an index should allow better analysis of epidemiological data to test the hypothesis that NDCs which act via SCFA production have any impact on incidence of chronic disease.

6.2 Summary of findings

The work of this thesis follows the development of a series of potential equations to be used as an index of the amount of FC that reaches the human colon for fermentation. These were tested for suitability for estimating FC from dietary records by comparing their ability to predict SCFA production in *in vitro* models and in human studies as listed below:

1. A series of potential equations for the FC index were developed to estimate FC that entered the human colon and were fermented to SCFA by the bacteria, these were developed by considering in detail the evidence for the digestibility of all ingested carbohydrates and also their likely fermentability. The amount of such carbohydrates in the diet were considered in detail and related to evidence for how much SCFA should be expected from their fermentation from published studies.

2. These equations were then tested using dietary data from a study where urinary SCFA had been previously measured. The results of chapter 4 indicate that there were weak but significant correlation between the 24 h urinary excretion of acetate, and the estimated FC using the equations FC-

DF 4 (based on AOAC DF) and FC-NSP 4. Similarly, the 24 h urinary excretion of SCFA was correlated with estimated FC using these equations unlike NSC or DF values alone. The results of the *in vitro* fermentation of NDCs experiments (chapter 5), showed several agreements between the total SCFA, acetate and propionate concentrations after actual *in vitro* fermentation of NDCs, and those predicted FC using our developed index equations. This was tested using a range of fibres, fibre mixes and combinations of fibres which aimed to have different doses of fermentable carbohydrate to test the robustness of the equations in predicting SCFA production. The mixture of four fibres, and the medium-FC mixture NDCs, showed an excellent correlation with total SCFA concentration produced and with the predicted FC. Furthermore, the predicted FC and psyllium fibre had a strong correlation with acetate and propionate concentrations. The Bland Altman plot analysis also supports these correlations between NDCs fermentation and predicted FC with total SCFA and acetate concentrations.

6.2.1 Developing an index of dietary estimation of FC

Currently there is no validated method to estimate how much of NDCs escapes digestion and absorption in the small intestine (as some are potentially digestible) and how much is fermented in the colon.

Our model equations for estimating FC (chapter 3), were based on evidence from different studies; soluble DF such as pectin is completely fermented (100 %), insoluble DF such as wheat bran is less fermented (10-50 %), NDOs were included as 100 % of NDOs are completely fermented, and finally, the amount of RS was estimated as 5 - 10 % of total starch, but this is assumed to be totally fermented (Table 3.3).

Different models were used in human *in vivo* determination of NDC that escape digestion and enter the colon for fermentation. Direct intubation of the ileum of healthy subjects showed that 3.2 – 9.3% of TS were recovered in the terminal ileum (Stephen et al., 1983, Flourie et al., 1988). The ileostomy model showed that on average < 1 - 12.2% of TS were appeared in ileal effluent of the ileostomies with exception 75% of banana starch was recovered (Chapman et al., 1985, Sandberg et al., 1986, O'Donnell et al., 1989, Wolever et al., 1986). This model was considered

to be a reliable model for studying small intestine digestion. Breath hydrogen measurements suggested that 2.2 - 38% of TS escapes digestion in the small intestine as RS (Levitt et al., 1987, Wolever et al., 1986, Scheppach et al., 1988b, Stephen et al., 1983). The amount of RS in a starchy food is usually less than 5 to10% but can be between 0% and 100% (Silvester et al., 1995). Overall, in the Western diet approximately 10 % of TS might be RS (Chapman et al., 1985, Englyst et al., 1996). Ileostomy patients showed that the mean excretion of inulin and oligofructose at the end of the ileum was approximately 88% and 90% respectively of the ingested dose of inulin and oligofructose recovered from the effluent (Ellegård et al., 1997, Andersson et al., 1999). In addition, the fermentability of NSP is highly influenced by its physiochemical properties. Insoluble fibres (lignin, cellulose, wheat bran and some hemicelluloses) are resistant to colonic microflora fermentation. In humans *in vivo*, Chen et al. (1998) reported that 96% of oat bran was fermented, compared 56 % of wheat bran. Barry et al (1995) reported that only 6-7% of cellulose and maize bran were fermented in *in vitro*. Stevens et al. (1987) stated that less than 50% of wheat bran components were fermented. On the other hand, soluble fibres (pectin, gums, mucilage and psyllium) were almost completely fermented. Barry et al. (1995) showed that more than 97% of pectin and 91% of soybean fibre were fermented.

A major limitation of this approach is that we have not directly measured the absorption of foods ourselves, for example with the use of ileostomy patients, but made decisions based on incomplete published data. However, these are approximate equations and do not claim to give an exact measure of FC in the diet, they are more of an index for epidemiological studies. Further research could be performed to provide more detailed validations based on such models.

In the chapter 4 study, the weak correlation between the 24 h urinary acetate, SCFA and the estimated FC intake suggested that using urinary output may not be an appropriate index to reflect colonic production of SCFA (Layden et al., 2013). Urinary excretion of SCFA was not well validated as it didn’t reflect the colonic production of SCFA as hoped. There are a number of possible reasons that might elucidate the weak correlation between predicted FC intake and 24 h urinary excretion of colonic metabolites. The first reason (as explored in chapter 4) could be that although approximately 95 % of all SCFA (acetate, propionate and butyrate) components produced from NDCs fermentation in the gut by microflora are rapidly

absorbed by the colonocytes (den Besten et al., 2013) most of the butyrate is used by the colonocytes (Roediger, 1980) and 70% - 90% of butyrate is metabolized by the colonocytes (Cummings, 1983).

Second, SCFA are also formed by sources other than NDCs fermentation in the gut such as the fermentation of protein (Ouwehand et al., 2005).

Thirdly acetate is produced by human cells from fatty acid oxidation (but also amino acid and glucose metabolism), (Wolever et al., 1997, Freeland et al., 2010). The major sources of energy in metabolic states with decreased glucose metabolism are ketone bodies which are generated via hepatic acetyl-CoA production during fatty acid oxidation (Pouteau et al., 2003). Hepatic acetate levels were also increased during fatty acid oxidation, and circulating acetate levels increased with the use of acetate as a fuel source in fasting states (Layden et al., 2013).

In addition, acetate, the main SCFA in the circulation is metabolised by human cell as it passes around the body. Nearly 70 % of the acetate formed by colonic bacterial fermentation of NDCs, and endogenous acetate formed in the liver during fatty acid oxidation, is taken up by the liver (Layden et al., 2013). The liver uses acetate as an energy source, but also as a substrate for the synthesis of cholesterol and long-chain fatty acids and as a co-substrate for glutamine and glutamate synthesis. Other tissues including the heart, adipose tissue, kidney, and skeletal muscle metabolize the remainder of the acetate (Bloemen et al., 2009). Moreover, It is suggested that around 30% of propionate concentration in portal blood and hepatic venous blood, is taken up by the liver (Binder, 2010, Bloemen et al., 2009). Peripheral tissues take up the remainder of propionate because peripheral venous blood levels were 23% lower compared with hepatic venous blood levels. Reilly and Rombeau (1993) reported that humans use 50% of the propionate as a substrate for hepatic gluconeogenesis. In general, therefore, it seems that the liver clears a large fraction of propionate from the portal circulation, but absolute values are still unknown.

Therefore, urinary SCFA excretion might provide little information about actual levels of intestinal SCFA metabolism (den Besten et al., 2013). This might explain why urinary excretion of SCFA is not well validated to reflect colonic production of SCFA, and the low level of urinary excretion of acetate in the study participants' urine samples.

In addition to the loss of SCFA before they enter the urine, there are also issues with the dietary records of habitual intake (chapter 4). Participants are likely to eat a wide variety of NDCs, which may not all be readily fermentable, for example wheat bran. However, several interventional studies and supplementation/trial studies have added known and relatively well controlled amounts of fermentable NDCs, which produced a predictable amount SCFA. Muir et al. (2004) observed that diets supplemented WBRS produced a higher faecal concentration of acetate compared with WB alone. Ahmed et al. (2000) found a higher SCFA production, and in particular butyrate production, after consumption of a specific mixture of high RS in the diet as compared with a low RS diet. Moreover, Henningsson et al. (2002) observed that rats fed with specific amounts of high amylose cornstarch with wheat bran produced higher faecal excretion of total SCFA, than rats fed with a single fibre source high amylose cornstarch or wheat barn.

Under-reporting dietary intake could also be one of the reasons that might lead to weak correlation of exogenous acetate concentration in the urine. Under-reporting of food intake is one of the main obstacles inhibiting the collection of accurate habitual dietary intake data to investigate the relationship between diet and health (Conway et al., 2003, Goran and Poehlman, 1992). MacDiarmid and Blundell (1998) stated that under-reporting ranged between 18 % and 54 % in the large nutritional surveys when considering the whole sample, and it could reach up to 70 % in particular subgroups. Females are more likely to under-report their daily dietary intake than men. In our data from all the 122 healthy volunteers (n = 122), 36 % under-reported their dietary intake, and of the 36 % under-reporting their dietary intake, 57 % were female. Within the sub-group of valid participants (n = 78) 53.8 % were female.

In accordance with published data, the last study (chapter 5) showed several agreements between the total SCFA, acetate and propionate concentrations after *in vitro* fermentation of NDCs, with the predicted FC using our developed equation. These might validate the significant relationship between FC and the 24 h urinary excretion of acetate and SCFA, which observed in our previous (chapter 4) study. The mixture of all 4 fibres and the medium-FC mixture NDCs showed an excellent correlation with total SCFA concentration produced, and with the predicted FC. Additionally, the predicted FC and psyllium fibre had a strong correlation with acetate and propionate concentrations. These relationship agreements were

supported by the Bland Altman plot analysis, which showed that the bias between NDCs fermentation and predicted FC with total SCFA and acetate concentrations was relatively small, with wide limits of agreement. Therefore, we can conclude that the newly developed predicted FC index is a valid and practical tool to assess SCFA productions for *in vitro* fermentation. This is in agreement with Daniel et al. (1997) who showed that fermentation of NSP in humans *in vivo* could be predicted with sufficient accuracy by this *in vitro* fermentation. These findings were also confirmed in a study conducted by the same authors, which found similar results (Wisker et al., 1998).

Overall there was significant difference in the *in vitro* faecal fermentation metabolites (including total SCFA, acetate, propionate and butyrate concentrations), in the three *in vitro* fermentation experiment mixtures of different isolated NDCs, single dietary fibre and mixture of all 4 fibres, and the three different NDCs mixtures. In our study the fermentation pattern for the total SCFA concentration of the mixture of all 4 fibres had the highest total SCFA concentration, followed by the raw potato starch and raftiline, which were significantly higher when compared with the culture of wheat bran and psyllium at 24 hours. These findings of the current study are consistent with those of Khan and Edwards (2005) who found that mixture of raftilose and guar gum produced a significantly higher total SCFA concentration than guar gum alone ($p < 0.002$). Moreover, Kaur et al. (2011) observed that the *in vitro* fermentation of benchmark starch-entrapped microspheres resulted in a significantly higher amount of total SCFA, particularly in the 24 to 48 h period, as compared to the fermentation of long-chain inulin, psyllium, alkali-soluble corn bran arabinoxylan, and β-glucan. In the last study (chapter 5), the results showed that wheat bran produced a lower SCFA concentration. The present findings seem to be consistent with that found by Nordlund et al. (2012) which observed that wheat bran produced a lower total SCFA than rye bran, oat bran and aleurone. Wheat bran is composed of > 80 % insoluble DF, which supports our predicted equation that 50 % might be fermented. Chen et al. (1998) reported that 90 % of wheat bran is insoluble fibre, which contributed to an increase faecal output and lower colonic transit time.

Furthermore, one of the important findings in this study was the significantly higher rate of production of propionate in the isolated mixture of oligosaccharide DF, rather than the isolated mixture of NDCs at 24 h. The high-FC mixture of NDCs had an intermediate value between the two other mixtures of NDCs, which might show that

high-FC mixture of NDCs comprised of more rapidly fermented NDCs. The present findings are consistent with other research which found oat bran, rather than FOS, produced higher *in vitro* propionate concentrations (Kedia et al., 2009). Similarly, Karppinen et al. (2000) reported a high propionate formation form oat bran when compared with rye and wheat bran. Additionally, Kim and White (2009) observed a significant propionate production capacity of β-glucan from oat independent of the molecular weight of β-glucan.

Another interesting finding of the last study (chapter 5), was that raw potato starch, the mixture of all 4 fibres, and the isolated FC mixture of RS had the highest concentrations of butyrate, compared with different NDCs (low, medium and high FC) mixtures. These results are consistent with those of other studies and suggests that RS is a good source of butyrate (Cummings and Macfarlane, 1991). Le Leu et al. (2002) reported that combining RS with wheat bran resulted in much higher levels of SCFA, particularly butyrate, observed in the faeces. Moreover, the habitual intake of a high NSP plus RS diet was associated with a higher faecal butyrate concentration than NSP alone, which may result in promoting optimum colorectal health (McOrist et al., 2011). Within this study different NDCs (low, medium and high FC) mixtures of substrates were obtained from habitual diets of the general population, which might explain the high butyrate and SCFA concentrations when combining RS with other FC substrates. The habitually high fibre consumers are likely to eat a wide variety of fibre types, not all of which may be readily fermentable, for example wheat bran. Also, individuals differ in the capacity of their large bowel microflora to ferment RS (Cummings et al., 1996). This could affect the supply of SCFA products to the large bowel and also an individual's response to dietary change.

6.3 Limitations

There are some limitations to these studies; we were unable to validate the equations in our *in vivo* studies using urinary excretion of SCFA products, as more than 95 % of SCFA products are rapidly absorbed by the colonocytes. Also 70 % of the acetate production, which is exogenous acetate formed by colonic bacterial fermentation of NDCs, and endogenous acetate formed in the liver during fatty acid oxidation, is taken up by the liver. This might explain why urinary excretion of SCFA is not well validated to reflect colonic production of SCFA. Moreover, one of the

limitations of the last study (chapter 5), could be the small sample size of the *in vitro* experiments. Using isolated FC rich diets and different mixtures of FC obtained from habitual dietary records could be also another limitation of this study. Using NDCs from well-designed interventions and comparing these with the fermentation in humans *in vivo* might give more reliable results. Nevertheless, it will be necessary to validate the equations more accurately in humans. One approach could be to use stable isotope labelled FC and to monitor the label in breath, plasma and urine. The *in vitro* models were not a perfect match for colonic fermentation as they were batch cultures and did not have any modelling of the absorption of the SCFA, so there may have been some product inhibition especially at low pH. However, these types of models are widely used and have been related to effects in humans by others (Pouteau et al., 2003, Edwards et al., 1992).

6.4 Implications for public health

This thesis demonstrated several relationships between estimated dietary FC, using our equations, and SCFA products *in vivo* and *in vitro*. Interventional studies support the relevance of prebiotic fermentation in obesity management in humans (Cani et al., 2009, Cani et al., 2005). These studies showed that oligofructose fermentation increases satiety and reduced the daily total energy by 5 %. On the other hand, most of health assessment evidence is based on randomized controlled trials examining the relationship between RS, NDOs and the relevant health/disease outcomes. There were no prospective cohort studies examining the relationship between RS and NDOs intake and the relevant health/disease outcomes (SACN, 2015). The traditional analysis method of epidemiological data depends on food composition databases, which might not have been updated to include NDOs and RS or to have accurate data on NDCs added to food (Westenbrink et al., 2013a, Hollmann et al., 2013). These methods could cause estimates of relative risk or other measures of diet disease associations to be weakened (an effect usually referred to as attenuation bias) and reduce the statistical power of epidemiologic studies (Kaaks, 1997). Reanalysis of established data from epidemiological studies using our predictive FC equations, might over-come assessments bias, which might increase the statistical power of the studies to detect the relationships of FC and health, and potentially to show links between SCFA production and health effects.

6.5 Further research

Based on the results of this thesis, using NDCs from well-designed interventions and comparing these with fermentation in humans *in vivo* might give more reliable results. The most reliable method that could be used to measure SCFA in humans' *in vivo* is the isotope dilution method. This involves a labelled SCFA dose, preferably delivered to the colon, or by looking at dilution of the labelled SCFA infused into the plasma by unlabelled SCFA absorbed from the colon. This would provide information on all three major SCFA, and the effects of specific NDC could be related to specific SCFA. Using stable isotope-labeled substrates (primarily ^{13}C) allows us to identify which SCFA metabolites are produced from NDC fermentation by analyzing the presence of ^{13}C-atoms in SCFA metabolites (Breves et al., 1993, Pouteau et al., 2003). This might overcome the problem that FC and protein both contribute to SCFA production (De Preter et al., 2004). This is also might provide reliable information on specific SCFA flux, and a potential explanation of specific SCFA biomarker relationships in disease. For example, the butyrate flux could be assessed in relation to biomarkers of metabolic syndrome, and propionate as a biomarker of satiety. Microorganisms that ferment specific NDC could also be identified by measuring the ^{13}C-incorporation in microbial biomass, for example 16S-rRNA (de Graaf and Venema, 2007).

However, once better validated the FC equations developed in this thesis will be a powerful epidemiological tool for future cohort, case controlled and intervention studies that investigate the role of fermentation in a range of chronic conditions, and will provide more clear results that using dietary fibre alone.

6.6 Conclusions

The data of this thesis demonstrated several relationships between the estimated dietary FC using our equations and SCFA products *in vivo* and *in vitro*. These need to be further validated, possibly by using FC from well-designed interventions and comparing these with fermentation in humans *in vivo.* The most reliable method that could be used to measure SCFA in humans' *in vivo* is the isotope dilution method. Once better validated the FC equations developed in this thesis will be a powerful epidemiological tool for future cohort, case controlled and intervention studies

investigating the role of fermentation in a range of chronic conditions, and will provide more clear results that using DF alone.

7 References

Adlercreutz, H., Hämäläinen, E., Gorbach, S., Goldin, B., Woods, M., Swenson Brunson, L. & Dwyer, J. 1987. Association of diet and sex hormones in relation to breast cancer. *European Journal of Cancer and Clinical Oncology,* 23**,** 1725-1726.

Agostoni, C., Bresson, J., Fairweather-Tait, S., Flynn, A., Golly, I., Korhonen, H., Lagiou, P., Løvik, M., Marchelli, R. & Martin, A. 2010. Scientific opinion on dietary reference values for carbohydrates and dietary fibre. *EFSA journal,* 8.

Ahmed, R., Segal, I. & Hassan, H. 2000. Fermentation of dietary starch in humans. *Am J Gastroenterol,* 95**,** 1017-1020.

Akbaraly, T. N., Ferrie, J. E., Berr, C., Brunner, E. J., Head, J., Marmot, M. G., Singh-Manoux, A., Ritchie, K., Shipley, M. J. & Kivimaki, M. 2011. Alternative Healthy Eating Index and mortality over 18 y of follow-up: results from the Whitehall II cohort. *The American journal of clinical nutrition,* 94**,** 247-253.

Åkerberg, A. K. E., Liljeberg, H. G. M., Granfeldt, Y. E., Drews, A. W. & Björck, I. M. E. 1998. An in vitro method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fiber. *The Journal of Nutrition,* 128**,** 651-660.

Al-Tamimi, E. K., Seib, P. A., Snyder, B. S. & Haub, M. D. 2009. Consumption of cross-linked resistant starch (RS4 XL) on glucose and insulin responses in humans. *Journal of nutrition and metabolism,* 2010.

Alander, M., Mättö, J., Kneifel, W., Johansson, M., Kögler, B., Crittenden, R., Mattila-Sandholm, T. & Saarela, M. 2001. Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of Bifidobacterium lactis Bb-12 in the gastrointestinal tract. *International Dairy Journal,* 11**,** 817-825.

Amrein, T. M., Gränicher, P., Arrigoni, E. & Amadò, R. 2003. In vitro digestibility and colonic fermentability of aleurone isolated from wheat bran. *LWT-Food Science and Technology,* 36**,** 451-460.

Anderson, J. & Bridges, S. 1988. Dietary fiber content of selected foods. *The American journal of clinical nutrition,* 47**,** 440-447.

Anderson, J. W., Baird, P., Davis Jr, R. H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. & Williams, C. L. 2009. Health benefits of dietary fiber. *Nutrition reviews,* 67**,** 188-205.

Anderson, J. W., Story, L., Sieling, B., Chen, W., Petro, M. S. & Story, J. 1984. Hypocholesterolemic effects of oat-bran or bean intake for

hypercholesterolemic men. *The American Journal of Clinical Nutrition,* 40**,** 1146-1155.

Andersson, H., Nävert, B., Bingham, S., Englyst, H. & Cummings, J. 1983. The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. *British Journal of Nutrition,* 50**,** 503-510.

Andersson, H. B., Ellegård, L. H. & Bosaeus, I. G. 1999. Nondigestibility characteristics of inulin and oligofructose in humans. *The Journal of Nutrition,* 129**,** 1428.

Andersson, M., Ellegård, L. & Andersson, H. 2002. Oat bran stimulates bile acid synthesis within 8 h as measured by 7α-hydroxy-4-cholesten-3-one. *The American journal of clinical nutrition,* 76**,** 1111-1116.

Antonopoulos, S. 2002. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation,* 106**,** 3421.

Appleby, P. N., Thorogood, M., Mann, J. I. & Key, T. J. 1999. The Oxford vegetarian study: an overview. *The American journal of clinical nutrition,* 70**,** 525s-531s.

Arora, T., Sharma, R. & Frost, G. 2011. Propionate. Anti-obesity and satiety enhancing factor? *Appetite,* 56**,** 511-515.

Asp, N. G. & Björck, I. 1992. Resistant starch. *Trends in Food Science & Technology,* 3**,** 111-114.

Asp, N. G., Van Amelsvoort, J. & Hautvast, J. 1996. Nutritional implications of resistant starch. *Nutrition Research Reviews,* 9**,** 1-31.

Augeron, C. & Laboisse, C. L. 1984. Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. *Cancer Research,* 44**,** 3961-3969.

Aune, D., Chan, D., Lau, R., Vieira, R., Greenwood, D., Kampman, E. & Norat, T. 2011a. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *Bmj,* 343**,** d6617.

Aune, D., Chan, D. S., Lau, R., Vieira, R., Greenwood, D. C., Kampman, E. & Norat, T. 2011b. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *Bmj,* 343.

Aura, A. M., Härkönen, H., Fabritius, M. & Poutanen, K. 1999. Development of anIn VitroEnzymic Digestion Method for Removal of Starch and Protein and

Assessment of its Performance Using Rye andWheat Breads. *Journal of Cereal Science,* 29**,** 139-152.

Bach Knudsen, K. E. 2001. The nutritional significance of “dietary fibre” analysis. *Animal Feed Science and Technology,* 90**,** 3-20.

Bäckhed, F., Manchester, J. K., Semenkovich, C. F. & Gordon, J. I. 2007. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences,* 104**,** 979-984.

Baer, H. J., Glynn, R. J., Hu, F. B., Hankinson, S. E., Willett, W. C., Colditz, G. A., Stampfer, M. & Rosner, B. 2011. Risk factors for mortality in the nurses' health study: a competing risks analysis. *American journal of epidemiology,* 173**,** 319-329.

Baghurst, K. I., Baghurst, P. A. & Record, S. J. 2001. Dietary Fiber, Nonstarch Polysaccharide, and Resistant Starch Intakes in Australia. *Dietary Fiber in Human Nutrition*.

Baghurst, P. A., Baghurst, K. & Record, S. 1996. Dietary fibre, non-starch polysaccharides and resistant starch: a review. *Food Australia,* 48**,** S3-S35.

Barry, J., Hoebler, C., Macfarlane, G., Macfarlane, S., Mathers, J., Reed, K., Mortensen, P., Nordgaard, I., Rowland, I. & Rumney, C. 1995. Estimation of the fermentability of dietary fibre in vitro: a European interlaboratory study. *British Journal of Nutrition,* 74**,** 303-322.

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J. & Ghatei, M. A. 2002. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature,* 418**,** 650-654.

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M. & Whelton, P. K. 2003. Dietary fiber intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Archives of internal medicine,* 163**,** 1897.

Bazzano, L. A., Song, Y., Bubes, V., Good, C. K., Manson, J. E. & Liu, S. 2005. Dietary intake of whole and refined grain breakfast cereals and weight gain in men. *Obes Res,* 13**,** 1952-60.

Bednar, G. E., Patil, A. R., Murray, S. M., Grieshop, C. M., Merchen, N. R. & Fahey, G. C. 2001. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *The Journal of nutrition,* 131**,** 276-286.

Behall, K. M., Scholfield, D., Yuhaniak, I. & Canary, J. 1989. Diets containing high amylose vs amylopectin starch: effects on metabolic variables in human subjects. *The American journal of clinical nutrition,* 49**,** 337-344.

Behall, K. M., Scholfield, D. J. & Hallfrisch, J. 2006a. Whole-grain diets reduce blood pressure in mildly hypercholesterolemic men and women. *Journal of the American Dietetic Association,* 106**,** 1445-1449.

Behall, K. M., Scholfield, D. J., Hallfrisch, J. G. & Liljeberg-Elmståhl, H. G. 2006b. Consumption of both resistant starch and β-glucan improves postprandial plasma glucose and insulin in women. *Diabetes care,* 29**,** 976-981.

Bernstein, A. M., Rosner, B. A. & Willett, W. C. 2011. Cereal fiber and coronary heart disease: a comparison of modeling approaches for repeated dietary measurements, intermediate outcomes, and long follow-up. *European journal of epidemiology,* 26**,** 877-886.

Binder, H. J. 2010. Role of colonic short-chain fatty acid transport in diarrhea. *Annual Review of Physiology,* 72**,** 297-313.

Bingham, S., Cassidy, A., Cole, T., Welch, A., Runswick, S., Black, A., Thurnham, D., Bates, C., Khaw, K. & Key, T. 1995. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *British Journal of Nutrition,* 73**,** 531-550.

Bingham, S. A., Day, N. E., Luben, R., Ferrari, P., Slimani, N., Norat, T., Clavel-Chapelon, F., Kesse, E., Nieters, A. & Boeing, H. 2003. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *The Lancet,* 361**,** 1496-1501.

Bjursell, M., Admyre, T., Göransson, M., Marley, A. E., Smith, D. M., Oscarsson, J. & Bohlooly-Y, M. 2011. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *American Journal of Physiology-Endocrinology and Metabolism,* 300**,** E211-E220.

Bloemen, J. G., Venema, K., Van De Poll, M. C., Olde Damink, S. W., Buurman, W. A. & Dejong, C. H. 2009. Short chain fatty acids exchange across the gut and liver in humans measured at surgery. *Clinical nutrition,* 28**,** 657-661.

Boehm, G., Stahl, B., Jelinek, J., Knol, J., Miniello, V. & Moro, G. E. 2005. Prebiotic carbohydrates in human milk and formulas. *Acta Paediatrica,* 94**,** 18-21.

Bornet, F., Brouns, F., Tashiro, Y. & Duvillier, V. 2002. Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. *Digestive and Liver Disease,* 34**,** S111-S120.

Bosscher, D., Breynaert, A., Pieters, L. & Hermans, N. 2009. Food-based strategies to modulate the composition of the intestinal microbiota and their associated health effects. *J Physiol Pharmacol,* 60**,** 5-11.

Bouhnik, Y., Raskine, L., Simoneau, G., Paineau, D. & Bornet, F. 2006. The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: a dose-response relationship study in healthy humans. *Nutr J,* 5.

Bourquin, L. D., Titgemeyer, E. C. & Fahey Jr, G. C. 1996. Fermentation of various dietary fiber sources by human fecal bacteria. *Nutrition Research,* 16**,** 1119-1131.

Bourquin, L. D., Titgemeyer, E. C., Garleb, K. A. & Fahey Jr, G. 1992. Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fractionation procedures. *The Journal of nutrition,* 122**,** 1508-1520.

Bradbury, K. E., Appleby, P. N. & Key, T. J. 2014. Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *The American journal of clinical nutrition,* 100**,** 394S-398S.

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

Breves, G., Schulze, E., Sallmann, H. & Gädeken, D. 1993. The application of 13C-labelled short chain fatty acids to measure acetate and propionate production rates in the large intestines. Studies in a pig model. *Zeitschrift fur Gastroenterologie,* 31**,** 179-182.

Bridges, S. R., Anderson, J. W., Deakins, D. A., Dillon, D. W. & Wood, C. L. 1992. Oat bran increases serum acetate of hypercholesterolemic men. *The American journal of clinical nutrition,* 56**,** 455-459.

Brighenti, F., Casiraghi, M. C. & Baggio, C. 1998. Resistant starch in the Italian diet. *British Journal of Nutrition,* 80**,** 333-341.

Brodribb, A. & Groves, C. 1978. Effect of bran particle size on stool weight. *Gut,* 19**,** 60-63.

Brown, L., Rosner, B., Willett, W. W. & Sacks, F. M. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *The American Journal of Clinical Nutrition,* 69**,** 30.

Burkitt, D. P. 1971. Epidemiology of cancer of the colon and rectum. *Cancer,* 28**,** 3-13.

Buttriss, J. & Stokes, C. 2008. Dietary fibre and health: an overview. *Nutrition Bulletin,* 33**,** 186-200.

Buyken, A. E., Flood, V., Empson, M., Rochtchina, E., Barclay, A. W., Brand-Miller, J. & Mitchell, P. 2010. Carbohydrate nutrition and inflammatory disease mortality in older adults. *The American journal of clinical nutrition,* 92**,** 634-643.

Campbell, J. M., Bauer, L. L., Fahey Jr, G. C., Hogarth, A., Wolf, B. W. & Hunter, D. E. 1997. Selected fructooligosaccharide (1-kestose, nystose, and 1F- -

fructofuranosylnystose) composition of foods and feeds. *Journal of agricultural and food chemistry,* 45**,** 3076-3082.

Cani, P. D., Joly, E., Horsmans, Y. & Delzenne, N. M. 2005. Oligofructose promotes satiety in healthy human: a pilot study. *European journal of clinical nutrition,* 60**,** 567-572.

Cani, P. D., Joly, E., Horsmans, Y. & Delzenne, N. M. 2006. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr,* 60**,** 567-572.

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., De Backer, F., Neyrinck, A. M. & Delzenne, N. M. 2009. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *The American journal of clinical nutrition,* 90**,** 1236-1243.

Carr, A. C., Bozonet, S. M., Pullar, J. M., Simcock, J. W. & Vissers, M. 2013. A randomized steady-state bioavailability study of synthetic versus natural (Kiwifruit-Derived) vitamin C. *Nutrients,* 5**,** 3684-3695.

Carr, A. C., Pullar, J. M., Moran, S. & Vissers, M. 2012. Bioavailability of vitamin C from kiwifruit in non-smoking males: Determination of 'healthy'and 'optimal'intakes. *Journal of nutritional science,* 1**,** e14.

Carter, P., Gray, L. J., Troughton, J., Khunti, K. & Davies, M. J. 2010. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *Bmj,* 341.

Cassidy, A., Bingham, S. & Cummings, J. 1994. Starch intake and colorectal cancer risk: an international comparison. *British journal of cancer,* 69**,** 937.

Causey, J. L., Feirtag, J. M., Gallaher, D. D., Tungland, B. C. & Slavin, J. L. 2000. Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research,* 20**,** 191-201.

Champ, M., Langkilde, A. M., Brouns, F., Kettlitz, B. & Le Bail-Collet, Y. 2003. Advances in dietary fibre characterisation. 2. Consumption, chemistry, physiology and measurement of resistant starch, implications for health and food labelling. *Nutrition research reviews.,* 16**,** 143-161.

Chapman, R., Sillery, J., Graham, M. & Saunders, D. 1985. Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. *The American Journal of Clinical Nutrition,* 41**,** 1244.

Charalampopoulos, D., Wang, R., Pandiella, S. & Webb, C. 2002. Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology,* 79**,** 131-141.

Chelikani, P. K., Haver, A. C. & Reidelberger, R. D. 2005. Intravenous infusion of peptide YY (3–36) potently inhibits food intake in rats. *Endocrinology,* 146**,** 879-888.

Chen, H. L., Haack, V. S., Janecky, C. W., Vollendorf, N. W. & Marlett, J. A. 1998. Mechanisms by which wheat bran and oat bran increase stool weight in humans. *The American Journal of Clinical Nutrition,* 68**,** 711-9.

Chen, J., He, J., Wildman, R., Reynolds, K., Streiffer, R. & Whelton, P. 2005. A randomized controlled trial of dietary fiber intake on serum lipids. *European journal of clinical nutrition,* 60**,** 62-68.

Chen, L., Liu, R., Qin, C., Meng, Y., Zhang, J., Wang, Y. & Xu, G. 2010. Sources and intake of resistant starch in the Chinese diet. *Asia Pacific journal of clinical nutrition,* 19.

Chen, W., Anderson, J. W. & Jennings, D. Year. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *In*, 1984. 215.

Cherrington, C. A., Hinton, M., Pearson, G. & Chopra, I. 1991. Short-chain organic acids at pH 5.0 kill Escherichia coli and Salmonella spp. without causing membrane perturbation. *Journal of Applied Microbiology,* 70**,** 161-165.

Chuang, S.-C., Norat, T., Murphy, N., Olsen, A., Tjønneland, A., Overvad, K., Boutron-Ruault, M. C., Perquier, F., Dartois, L. & Kaaks, R. 2012. Fiber intake and total and cause-specific mortality in the European Prospective Investigation into Cancer and Nutrition cohort. *The American journal of clinical nutrition,* 96**,** 164-174.

Clarke, J. M., Topping, D. L., Bird, A. R., Young, G. P. & Cobiac, L. 2008. Effects of high-amylose maize starch and butyrylated high-amylose maize starch on azoxymethane-induced intestinal cancer in rats. *Carcinogenesis,* 29**,** 2190-2194.

Codex Alimentarius 2010. guidelines on nutrition labeling CAC/GL 2–1985 as last amended 2010. *Joint FAO/WHO Food Standards Programme, Secretariat of the CODEX Alimentarius Commission, Rome: FAO*.

Committee, A. D. F. D. 2001. Definition of dietary fiber: Report of the Dietary Fiber Definition Committee to the Board of Directors of the American Association of Cereal Chemists. *Cereal Foods World,* 46**,** 112 - 126.

Conway, J. M., Ingwersen, L. A., Vinyard, B. T. & Moshfegh, A. J. 2003. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *The American journal of clinical nutrition,* 77**,** 1171-1178.

Cook, S. & Sellin, J. 1998. Review article: short chain fatty acids in health and disease. *Alimentary pharmacology & therapeutics,* 12**,** 499.

Coudray, C., Bellanger, J., Castiglia-Delavaud, C., Remesy, C., Vermorel, M. & Rayssignuier, Y. 1997. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *European Journal of Clinical Nutrition,* 51**,** 375-380.

Craig, S. a. S., Holden, J. F. & Khaled, M. Y. 2000. Determination of polydextrose as dietary fiber in foods. *Journal of AOAC International,* 83**,** 1006-1012.

Crowe, F., Key, T., Appleby, P., Overvad, K., Schmidt, E. B., Egeberg, R., Tjønneland, A., Kaaks, R., Teucher, B. & Boeing, H. 2012. Dietary fibre intake and ischaemic heart disease mortality: the European Prospective Investigation into Cancer and Nutrition-Heart study. *European Journal of Clinical Nutrition,* 66**,** 950-956.

Csordas, A. 1996. Butyrate, aspirin and colorectal cancer. *European journal of cancer prevention,* 5**,** 221-231.

Cui, W., Wood, P., Blackwell, B. & Nikiforuk, J. 2000. Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan—comparison with other cereal β-D-glucans. *Carbohydrate Polymers,* 41**,** 249-258.

Cummings, J. 1983. Colonic absorption: the importance of short chain fatty acids in man. *Scandinavian journal of gastroenterology. Supplement,* 93**,** 89-99.

Cummings, J., Banwell, J., Segal, I., Coleman, N., Englyst, H. & Macfarlane, G. 1990. The amount and composition of large bowel contents in man. *Gastroenterology,* 98**,** 2.

Cummings, J., Branch, W., Jenkins, D., Southgate, D., Houston, H. & James, W. 1978. Colonic response to dietary fibre from carrot, cabbage, apple, bran, and guar gum. *The Lancet,* 311**,** 5-9.

Cummings, J. & Englyst, H. 1991. Measurement of starch fermentation in the human large intestine. *Can J Physiol Pharmacol,* 69**,** 121-129.

Cummings, J., Hill, M., Bone, E., Branch, W. & Jenkins, D. 1979. The effect of meat protein and dietary fiber on colonic function and metabolism; II. Bacterial metabolites in feces and urine. *American Journal of Clinical Nutrition,* 32.

Cummings, J. & Macfarlane, G. 1991. The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Microbiology,* 70**,** 443-459.

Cummings, J., Pomare, E., Branch, W., Naylor, C. & Macfarlane, G. 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut,* 28**,** 1221-1227.

Cummings, J. H. 1981. Short chain fatty acids in the human colon. *Gut,* 22**,** 763.

Cummings, J. H. 1984. Microbial Digestion of Complex Carbohydrates in Man. *Proceedings of the Nutrition Society,* 43**,** 35-44.

Cummings, J. H. 1997. *The large intestine in nutrition and disease*, Institut Danone Brussels, Belgium.

Cummings, J. H. 2001. The effect of dietary fiber on fecal weight and composition. *CRC handbook of dietary fiber in human nutrition,* 3**,** 183-252.

Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A. & Englyst, H. N. 1996. Digestion and physiological properties of resistant starch in the human large bowel. *British Journal of Nutrition,* 75**,** 733-747.

Cummings, J. H., Macfarlane, G. T. & Englyst, H. N. 2001. Prebiotic digestion and fermentation. *The American Journal of Clinical Nutrition,* 73**,** 415S-420S.

Cummings, J. H. & Stephen, A. M. 2007. Carbohydrate terminology and classification. *Eur J Clin Nutr,* 61**,** S5-S18.

Daniel, M., Wisker, E., Rave, G. & Feldheim, W. 1997. Fermentation in human subjects of nonstarch polysaccharides in mixed diets, but not in a barley fiber concentrate, could be predicted by in vitro fermentation using human fecal inocula. *The Journal of nutrition,* 127**,** 1981-1988.

Dauchet, L., Amouyel, P., Hercberg, S. & Dallongeville, J. 2006. Fruit and Vegetable Consumption and Risk of Coronary Heart Disease: A Meta-Analysis of Cohort Studies. *The Journal of Nutrition,* 136**,** 2588-2593.

Davies, G., Crowder, M., Reid, B. & Dickerson, J. 1986. Bowel function measurements of individuals with different eating patterns. *Gut,* 27**,** 164-169.

De Graaf, A. A. & Venema, K. 2007. Gaining insight into microbial physiology in the large intestine: a special role for stable isotopes. *Advances in microbial physiology,* 53**,** 73-314.

De Menezes, E. W., Giuntini, E. B., Dan, M. C. T., Sardá, F. a. H. & Lajolo, F. M. 2013. Codex dietary fibre definition–Justification for inclusion of carbohydrates from 3 to 9 degrees of polymerisation. *Food Chemistry,* 140**,** 581-585.

De Preter, V., Geboes, K., Verbrugghe, K., De Vuyst, L., Vanhoutte, T., Huys, G., Swings, J., Pot, B. & Verbeke, K. 2004. The in vivo use of the stable isotope-labelled biomarkers lactose-[15N] ureide and [2H4] tyrosine to assess the effects of pro-and prebiotics on the intestinal flora of healthy human volunteers. *British Journal of Nutrition,* 92**,** 439-446.

Degen, L. & Phillips, S. 1996. Variability of gastrointestinal transit in healthy women and men. *Gut,* 39**,** 299-305.

Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J. & Bakker, B. M. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research,* 54**,** 2325-2340.

Dikeman, C. L. & Fahey Jr, G. C. 2006. Viscosity as related to dietary fiber: A review. *Critical Reviews in Food Science and Nutrition,* 46**,** 649-663.

Dongowski, G., Lorenz, A. & Proll, J. 2002. The Degree of Methylation Influences the Degradation of Pectin in the Intestinal Tract of Rats and In Vitro. *The Journal of Nutrition,* 132**,** 1935-1944.

Doublier, J.-L. & Wood, P. J. 1995. Rheological Properties of Aqueous Solutions of (1--> 3)(1--> 4)-bD-Glucan from Oats (Avena sativa L.). *Cereal chemistry,* 72**,** 335-340.

Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E. & Flint, H. J. 2002. Acetate utilization and butyryl coenzyme A (CoA): acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Applied and environmental microbiology,* 68**,** 5186-5190.

Duncan, S. H., Holtrop, G., Lobley, G. E., Calder, A. G., Stewart, C. S. & Flint, H. J. 2004a. Contribution of acetate to butyrate formation by human faecal bacteria. *British Journal of Nutrition,* 91**,** 915-923.

Duncan, S. H., Louis, P. & Flint, H. J. 2004b. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environmental Microbiology,* 70**,** 5810-5817.

Duncan, S. H., Louis, P., Thomson, J. M. & Flint, H. J. 2009. The role of pH in determining the species composition of the human colonic microbiota. *Environmental microbiology,* 11**,** 2112-2122.

Dunn, S., Datta, A., Kallis, S., Law, E., Myers, C. E. & Whelan, K. 2011. Validation of a food frequency questionnaire to measure intakes of inulin and oligofructose. *European journal of clinical nutrition,* 65**,** 402-408.

Dysseler, P. & Hoffem, D. Year. Estimation of resistant starch intake in Europe. *In:* Proceedings of the concluding plenary meeting of EURESTA, 1994. Wageningen The Netherlands, 84-86.

Eastwood, M. & Hamilton, D. 1968. Studies on the adsorption of bile salts to non-absorbed components of diet. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism,* 152**,** 165-173.

Eastwood, M. A., Brydon, W. G., Baird, J., Elton, R., Helliwell, S., Smith, J. & Pritchard, J. 1984. Fecal weight and composition, serum lipids, and diet among subjects aged 18 to 80 years not seeking health care. *The American Journal of Clinical Nutrition,* 40**,** 628-634.

Ebringerová, A., Hromádková, Z. & Heinze, T. 2005. Hemicellulose. *Polysaccharides i.* Springer.

Edwards, C., Bowen, J. & Eastwood, M. 1990. Effect of isolated complex carbohydrates on cecal and fecal short chain fatty acids in stool output in the rat. Royal Society of Chemistry, London.

Edwards, C., Gibson, G., Champ, M., Jensen, B., Mathers, J., Nagengast, F., Rumney, C. & Quehl, A. 1996. In vitro method for quantification of the fermentation of starch by human faecal bacteria. *Journal of the Science of Food and Agriculture,* 71**,** 209-217.

Edwards, C. & Rowland, I. 1992. Bacterial fermentation in the colon and its measurement. *Dietary fiber: a component of food. Springer, London, United Kingdom***,** 119-136.

Edwards, C. A., Adiotomre, J. & Eastwood, M. A. 1992. Dietary fibre: The use of in-vitro and rat models to predict action on stool output in man. *Journal of the Science of Food and Agriculture,* 59**,** 257-260.

Ellegård, L. & Andersson, H. 2007. Oat bran rapidly increases bile acid excretion and bile acid synthesis: an ileostomy study. *European Journal of Clinical Nutrition,* 61**,** 938-945.

Ellegård, L., Andersson, H. & Bosaeus, I. 1997. Inulin and oligofructose do not influence the absorption of cholesterol, or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. *European journal of clinical nutrition,* 51**,** 1.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. 2011. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry,* 124**,** 411-421.

Elmstahl, H. L. 2002. Resistant starch content in a selection of starchy foods on the Swedish market. *European journal of clinical nutrition,* 56**,** 500-505.

Englyst, H. 1989. Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology,* 23**,** 27-42.

Englyst, H., Bingham, S., Runswick, S., Collinson, E. & Cummings, J. 1989. Dietary fibre (non-starch polysaccharides) in cereal products. *Journal of Human Nutrition and Dietetics,* 2**,** 253-271.

Englyst, H. & Cummings, J. 1985. Digestion of the polysaccharides of some cereal foods in the human small intestine. *The American Journal of Clinical Nutrition,* 42**,** 778.

Englyst, H. & Cummings, J. 1987. Digestion of polysaccharides of potato in the small intestine of man. *The American Journal of Clinical Nutrition,* 45**,** 423.

Englyst, H. & Cummings, J. 1990. Non-starch polysaccharides (dietary fiber) and resistant starch. *Advances in experimental medicine and biology,* 270**,** 205.

Englyst, H. N., Bingham, S. A., Runswick, S. A., Collinson, E. & Cummings, J. H. 1988. Dietary fibre (non-starch polysaccharides) in fruit, vegetables and nuts. *Journal of Human Nutrition and Dietetics,* 1**,** 247-286.

Englyst, H. N., Kingman, S. & Cummings, J. 1992. Classification and measurement of nutritionally important starch fractions. *European journal of clinical nutrition,* 46**,** S33.

Englyst, H. N., Kingman, S. M., Hudson, G. J. & Cummings, J. H. 1996. Measurement of resistant starch in vitro and in vivo. *British journal of nutrition,* 75**,** 749-755.

Englyst, H. N. & Macfarlane, G. T. 1986. Breakdown of resistant and readily digestible starch by human gut bacteria. *Journal of the Science of Food and Agriculture,* 37**,** 699-706.

Englyst, K. N., Liu, S. & Englyst, H. N. 2007. Nutritional characterization and measurement of dietary carbohydrates. *Eur J Clin Nutr,* 61**,** S19-S39.

Eshak, E., Iso, H., Date, C., Kikuchi, S., Watanabe, Y., Wada, Y., Wakai, K., Tamakoshi, A. & Group:, J. S. 2010a. Dietary fiber intake is associated with reduced risk of mortality from cardiovascular disease among Japanese men and women. *J Nutr,* 140**,** 1445 - 1453.

Eshak, E. S., Iso, H., Date, C., Kikuchi, S., Watanabe, Y., Wada, Y., Wakai, K. & Tamakoshi, A. 2010b. Dietary fiber Intake is associated with reduced risk of mortality from cardiovascular disease among Japanese men and women. *The Journal of nutrition,* 140**,** 1445.

Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Basora-Gallisá, J., Ruiz-Gutierrez, V., Covas, M. I., Fiol, M., Gómez-Gracia, E., Lopez-Sabater, M. C. & Escoda, R. 2009. Effects of dietary fibre intake on risk factors for cardiovascular disease in subjects at high risk. *Journal of epidemiology and community health,* 63**,** 582.

Evenepoel, P., Claus, D., Geypens, B., Hiele, M., Geboes, K., Rutgeerts, P. & Ghoos, Y. 1999. Amount and fate of egg protein escaping assimilation in the small intestine of humans. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 277**,** G935-G943.

Faisant, N., Planchot, V., Kozlowski, F., Pacouret, M. P., Colonna, P. & Champ, M. 1995. Resistant starch determination adapted to products containing high level of resistant starch. *Sciences des Aliments,* 15**,** 83-89.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. & Parkin, D. M. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer,* 127**,** 2893-2917.

Fernandes, J., Wang, A., Su, W., Rozenbloom, S. R., Taibi, A., Comelli, E. M. & Wolever, T. M. 2013. Age, Dietary Fiber, Breath Methane, and Fecal Short Chain Fatty Acids Are Interrelated in Archaea-Positive Humans. *The Journal of nutrition,* 143**,** 1269-1275.

Ferrari, P., Kaaks, R., Fahey, M. T., Slimani, N., Day, N. E., Pera, G., Boshuizen, H. C., Roddam, A., Boeing, H., Nagel, G., Thiebaut, A., Orfanos, P., Krogh, V., Braaten, T. & Riboli, E. 2004. Within- and Between-Cohort Variation in Measured Macronutrient Intakes, Taking Account of Measurement Errors, in the European Prospective Investigation into Cancer and Nutrition Study. *American Journal of Epidemiology,* 160**,** 814-822.

Fievez, V., Babayemi, O. & Demeyer, D. 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. *Animal Feed Science and Technology,* 123**,** 197-210.

Fischer, M. H., Yu, N., Gray, G. R., Ralph, J., Anderson, L. & Marlett, J. A. 2004. The gel-forming polysaccharide of psyllium husk (Plantago ovata Forsk). *Carbohydrate Research,* 339**,** 2009-2017.

Fisher, M. & Yousef, I. 1973. Sex differences in the bile acid composition of human bile: studies in patients with and without gallstones. *Canadian Medical Association Journal,* 109**,** 190.

Flint, H. J., Scott, K. P., Louis, P. & Duncan, S. H. 2012. The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology and Hepatology,* 9**,** 577-589.

Floch, M. H. 2011. Intestinal microecology in health and wellness. *Journal of clinical gastroenterology,* 45**,** S108-S110.

Flourie, B., Leblond, A., Florent, C., Rautureau, M., Bisalli, A. & Rambaud, J. 1988. Starch malabsorption and breath gas excretion in healthy humans consuming low-and high-starch diets. *GASTROENTEROLOGY,* 95**,** 356.

Ford, A. C., Talley, N. J., Spiegel, B. M., Foxx-Orenstein, A. E., Schiller, L., Quigley, E. M. & Moayyedi, P. 2008. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. *Bmj,* 337.

Freeland, K. R., Wilson, C. & Wolever, T. 2010. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *British Journal of Nutrition,* 103**,** 82-90.

Friedenreich, C. M., Brant, R. F. & Riboli, E. 1994. Influence of methodologic factors in a pooled analysis of 13 case-control studies of colorectal cancer and dietary fiber. *Epidemiology,* 5**,** 66-79.

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M. & Zhang, S. 2014a. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature communications,* 5.

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J. R., Gibson, G., Viardot, A., Morrison, D., Louise Thomas, E. & Bell, J. D. 2014b. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature communications,* 5**,** 3611.

Fuchs, C. S., Giovannucci, E. L., Colditz, G. A., Hunter, D. J., Stampfer, M. J., Rosner, B., Speizer, F. E. & Willett, W. C. 1999. Dietary fiber and the risk of colorectal cancer and adenoma in women. *New England Journal of Medicine,* 340**,** 169-176.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M., Sánchez-Zapata, E. & Pérez-Álvarez, J. 2010. Resistant starch as functional ingredient: A review. *Food Research International,* 43**,** 931-942.

Fung, K. Y., Cosgrove, L., Lockett, T., Head, R. & Topping, D. L. 2012. A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *British Journal of Nutrition,* 108**,** 820-831.

Gallaher, D. D., Hassel, C. A., Lee, K. J. & Gallaher, C. M. 1993. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. *Journal of Nutrition,* 123**,** 244-252.

Gamet, L., Daviaud, D., Denis-Pouxviel, C., Remesy, C. & Murat, J. C. 1992. Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. *International journal of cancer,* 52**,** 286-289.

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T. & Ye, J. 2009. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes,* 58**,** 1509-1517.

Ge, H., Li, X., Weiszmann, J., Wang, P., Baribault, H., Chen, J.-L., Tian, H. & Li, Y. 2008. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology,* 149**,** 4519-4526.

Gear, J., Brodribb, A., Ware, A. & Mannt, J. 1981. Fibre and bowel transit times. *British Journal of Nutrition,* 45**,** 77-82.

Gee, J., Wortley, G., Price, K. & Johnson, I. Year. Use of an in vitro model to assess factors influencing the effect of legume saponins on the intestinal epithelium. *In:* COST 98. Proceedings of the sixth scientific workshop in Rome, 1999. 8-10.

Geypens, B., Claus, D., Evenepoel, P., Hiele, M., Maes, B., Peeters, M., Rutgeerts, P. & Ghoos, Y. 1997. Influence of dietary protein supplements on the formation of bacterial metabolites in the colon. *Gut,* 41**,** 70-76.

Giavarina, D. 2015. Understanding Bland Altman analysis. *Biochemia medica,* 25**,** 141-151.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A. & Roberfroid, M. B. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev,* 17**,** 259-275.

Gibson, G. R. & Roberfroid, M. B. 1995. Dietary modulation of the human colonie microbiota: introducing the concept of prebiotics. *Journal of Nutrition,* 125**,** 1401-1412.

Goñi, I., García-Diz, L., Mañas, E. & Saura-Calixto, F. 1996. Analysis of resistant starch: a method for foods and food products. *Food Chemistry,* 56**,** 445-449.

Goran, M. I. & Poehlman, E. T. 1992. Total energy expenditure and energy requirements in healthy elderly persons. *Metabolism,* 41**,** 744-753.

Grabitske, H. A. & Slavin, J. L. 2009. Gastrointestinal effects of low-digestible carbohydrates. *Critical reviews in food science and nutrition,* 49**,** 327-360.

Gråsten, S., Liukkonen, K.-H., Chrevatidis, A., El-Nezami, H., Poutanen, K. & Mykkänen, H. 2003. Effects of wheat pentosan and inulin on the metabolic activity of fecal microbiota and on bowel function in healthy humans. *Nutrition Research,* 23**,** 1503-1514.

Grootaert, C., Delcour, J. A., Courtin, C. M., Broekaert, W. F., Verstraete, W. & Van De Wiele, T. 2007. Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends in Food Science & Technology,* 18**,** 64-71.

Guillon, F. & Champ, M. 2000a. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International,* 33**,** 233-245.

Guillon, F. & Champ, M. 2000b. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International,* 33**,** 233-245.

Gurr, M. & Asp, N. 1994. Dietary Fiber. ILSI Europe Concise Monograph Series. *International Life Sciences Institute Press, Brussels***,** 15.

Guyton, A. & Hall, J. 1996a. Digestion and absorption in the gastrointestinal tract. *Textbook of medical physiology,* 10**,** 754-763.

Guyton, A. & Hall, J. 1996b. Transport and mixing of food in the alimentary tract. *Textbook of medical physiology, 9th edn. WB Saunders Company, Philadelphia***,** 803-813.

Hague, A., Elder, D. J., Hicks, D. J. & Paraskeva, C. 1995. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *International journal of cancer,* 60**,** 400-406.

Hallert, C., Björck, I., Nyman, M., Pousette, A., Grännö, C. & Svensson, H. 2003. Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. *Inflammatory bowel diseases,* 9**,** 116-121.

Hamer, H. M., De Preter, V., Windey, K. & Verbeke, K. 2012. *Functional analysis of colonic bacterial metabolism: relevant to health?*

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J. & Brummer, R. J. 2008. Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics,* 27**,** 104-119.

Han, K.-H., Fukushima, M., Kato, T., Kojima, M., Ohba, K., Shimada, K.-I., Sekikawa, M. & Nakano, M. 2003. Enzyme-resistant fractions of beans lowered serum cholesterol and increased sterol excretions and hepatic mRNA levels in rats. *Lipids,* 38**,** 919-924.

Haralampu, S. 2000. Resistant starch—a review of the physical properties and biological impact of RS< sub> 3</sub>. *Carbohydrate Polymers,* 41**,** 285-292.

Heller, S., Hackler, L., Rivers, J., Soest, P. V., Roe, D., Lewis, B. & Robertson, J. 1980. Dietary fiber: the effect of particle size of wheat bran on colonic function in young adult men. *American Journal of Clinical Nutrition,* 33**,** 1734-1744.

Henningsson, Å. M., Björck, I. M. & Nyman, E. M. G. 2002. Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats. *The Journal of nutrition,* 132**,** 3098-3104.

Henry, R. J. 1985. A comparison of the non-starch carbohydrates in cereal grains. *Journal of the Science of Food and Agriculture,* 36**,** 1243-1253.

Henry, R. J. 1987. Pentosan and (1 → 3),(1 → 4)-β-Glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. *Journal of Cereal Science,* 6**,** 253-258.

Higgins, J. A. 2004. Resistant starch: metabolic effects and potential health benefits. *Journal of AOAC International,* 87**,** 761-768.

Hill, M. 1995. Bacterial fermentation of complex carbohydrate in the human colon. *European journal of cancer prevention,* 4**,** 353-358.

Hogarth, A., Hunter, D. E., Jacobs, W. A., Garleb, K. A. & Wolf, B. W. 2000. Ion chromatographic determination of three fructooligosaccharide oligomers in prepared and preserved foods. *Journal of agricultural and food chemistry,* 48**,** 5326-5330.

Hollmann, J., Themeier, H., Neese, U. & Lindhauer, M. G. 2013. Dietary fibre fractions in cereal foods measured by a new integrated AOAC method. *Food Chemistry,* 140**,** 586-589.

Holt, P. R., Atillasoy, E., Lindenbaum, J., Ho, S. B., Lupton, J. R., Mcmahon, D. & Moss, S. F. 1996. Effects of acarbose on fecal nutrients, colonic pH, and short-chain fatty acids and rectal proliferative indices. *Metabolism,* 45**,** 1179-1187.

Hong, Y.-H., Nishimura, Y., Hishikawa, D., Tsuzuki, H., Miyahara, H., Gotoh, C., Choi, K.-C., Feng, D. D., Chen, C. & Lee, H.-G. 2005. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology,* 146**,** 5092-5099.

Hosseini, E., Grootaert, C., Verstraete, W. & Van De Wiele, T. 2011. Propionate as a health-promoting microbial metabolite in the human gut. *Nutrition Reviews,* 69**,** 245-258.

Hughes, R. & Rowland, I. 2001. Stimulation of apoptosis by two prebiotic chicory fructans in the rat colon. *Carcinogenesis,* 22**,** 43-47.

Hylla, S., Gostner, A., Dusel, G., Anger, H., Bartram, H.-P., Christl, S. U., Kasper, H. & Scheppach, W. 1998. Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. *The American journal of clinical nutrition,* 67**,** 136-142.

Iso, H., Naito, Y., Sato, S., Kitamura, A., Okamura, T., Sankai, T., Shimamoto, T., Iida, M. & Komachi, Y. 2001. Serum triglycerides and risk of coronary heart disease among Japanese men and women. *American journal of epidemiology,* 153**,** 490.

Izydorczyk, M. S. & Biliaderis, C. G. 1995. Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers,* 28**,** 33-48.

Jaganath, I. B., Mullen, W., Lean, M. E., Edwards, C. A. & Crozier, A. 2009. In vitro catabolism of rutin by human fecal bacteria and the antioxidant capacity of its catabolites. *Free Radical Biology and Medicine,* 47**,** 1180-1189.

Jenkins, D. J. A., Kendall, C. W. C., Vuksan, V., Vidgen, E., Parker, T., Faulkner, D., Mehling, C. C., Garsetti, M., Testolin, G. & Cunnane, S. C. 2002. Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *The American journal of clinical nutrition,* 75**,** 834.

Jenkins, D. J. A., Vuksan, V., Kendall, C. W. C., Würsch, P., Jeffcoat, R., Waring, S., Mehling, C. C., Vidgen, E., Augustin, L. S. A. & Wong, E. 1998. Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. *Journal of the American College of Nutrition,* 17**,** 609-616.

Jensen, J. K., Sørensen, S. O., Harholt, J., Geshi, N., Sakuragi, Y., Møller, I., Zandleven, J., Bernal, A. J., Jensen, N. B. & Sørensen, C. 2008. Identification of a xylogalacturonan xylosyltransferase involved in pectin biosynthesis in Arabidopsis. *The Plant Cell,* 20**,** 1289-1302.

Jiménez-Escrig, A. & Sánchez-Muniz, F. J. 2000. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research,* 20**,** 585-598.

Jones, J. 2004. Dietary fiber intake, disease prevention, and health promotion: an overview with emphasis on evidence from epidemiology. *Bioactive Carbohydrates for Food and Feed***,** 143 - 164.

Jones, J. 2014a. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutrition journal,* 13**,** 34.

Jones, J. M. 2014b. CODEX-aligned dietary fiber definitions help to bridge the ‘fiber gap’. *Nutrition journal,* 13**,** 34.

Kaaks, R. J. 1997. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. *The American journal of clinical nutrition,* 65**,** 1232S-1239S.

Karaki, S.-I., Tazoe, H., Hayashi, H., Kashiwabara, H., Tooyama, K., Suzuki, Y. & Kuwahara, A. 2008. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *Journal of Molecular Histology,* 39**,** 135-142.

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

Karra, E. & Batterham, R. L. 2010. The role of gut hormones in the regulation of body weight and energy homeostasis. *Molecular and cellular endocrinology,* 316**,** 120-128.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A. & Kimura, I. 2015. Dietary Gut Microbial Metabolites, Short-chain Fatty Acids, and Host Metabolic Regulation. *Nutrients,* 7**,** 2839-2849.

Kato, I., Akhmedkhanov, A., Koenig, K., Toniolo, P. G., Shore, R. E. & Riboli, E. 1997. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study.

Kaur, A., Rose, D. J., Rumpagaporn, P., Patterson, J. A. & Hamaker, B. R. 2011. In Vitro Batch Fecal Fermentation Comparison of Gas and Short-Chain Fatty Acid Production Using “Slowly Fermentable” Dietary Fibers. *Journal of food science,* 76**,** H137-H142.

Kaur, N. & Gupta, A. K. 2002. Applications of inulin and oligofructose in health and nutrition. *Journal of biosciences,* 27**,** 703-714.

Kaushik, S., Wang, J. J., Wong, T. Y., Flood, V., Barclay, A., Brand-Miller, J. & Mitchell, P. 2009. Glycemic index, retinal vascular caliber, and stroke mortality. *Stroke,* 40**,** 206-212.

Kedia, G., Vázquez, J. A., Charalampopoulos, D. & Pandiella, S. S. 2009. In vitro fermentation of oat bran obtained by debranning with a mixed culture of human fecal bacteria. *Current microbiology,* 58**,** 338-342.

Kelsay, J. L. & Clark, W. 1984. Fiber intakes, stool frequency, and stool weights of subjects consuming self-selected diets. *The American Journal of Clinical Nutrition,* 40**,** 1357-1360.

Kendall, C. W. C., Esfahani, A. & Jenkins, D. J. A. 2010. The link between dietary fibre and human health. *Food Hydrocolloids,* 24**,** 42-48.

Keogh, G. F., Cooper, G. J., Mulvey, T. B., Mcardle, B. H., Coles, G. D., Monro, J. A. & Poppitt, S. D. 2003. Randomized controlled crossover study of the effect of a highly β-glucan–enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *The American journal of clinical nutrition,* 78**,** 711-718.

Khan, K. M. & Edwards, C. A. 2005. In vitro fermentation characteristics of a mixture of Raftilose and guar gum by human faecal bacteria. *European journal of nutrition,* 44**,** 371-376.

Kim, H. J. & White, P. J. 2009. In vitro bile-acid binding and fermentation of high, medium, and low molecular weight β-glucan. *Journal of agricultural and food chemistry,* 58**,** 628-634.

Kim, Y.-H., Park, J.-W., Lee, J.-Y. & Kwon, T. K. 2004. Sodium butyrate sensitizes TRAIL-mediated apoptosis by induction of transcription from the DR5 gene promoter through Sp1 sites in colon cancer cells. *Carcinogenesis,* 25**,** 1813-1820.

Kimura, I., Inoue, D., Hirano, K. & Tsujimoto, G. 2014. The SCFA receptor GPR43 and energy metabolism. *Frontiers in endocrinology,* 5.

King, D. E., Egan, B. M., Woolson, R. F., Mainous Iii, A. G., Al-Solaiman, Y. & Jesri, A. 2007. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Archives of internal medicine,* 167**,** 502.

Kirby, R. W., Anderson, J. W., Sieling, B., Rees, E. D., Chen, W., Miller, R. E. & Kay, R. M. 1981. Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. *The American journal of clinical nutrition,* 34**,** 824-829.

Knowles, S. E., Jarrett, I. G., Filsell, O. H. & Ballard, F. J. 1974. Production and utilization of acetate in mammals. *Biochem. J,* 142**,** 401-411.

Knudsen, B. K. & Hessov, I. 1995. Recovery of inulin from Jerusalem artichoke (Helianthus tuberosus L.) in the small intestine of man. *British Journal of Nutrition,* 74**,** 101-113.

Kokubo, Y., Iso, H., Saito, I., Yamagishi, K., Ishihara, J., Inoue, M. & Tsugane, S. 2011. Dietary fiber intake and risk of cardiovascular disease in the Japanese population: the Japan Public Health Center-based study cohort. *European journal of clinical nutrition*.

Kovatcheva-Datchary, P. & Arora, T. 2013. Nutrition, the gut microbiome and the metabolic syndrome. *Best Practice & Research Clinical Gastroenterology,* 27**,** 59-72.

Kritchevsky, D. & Story, J. 1986. Influence of dietary fiber on cholesterol metabolism in experimental animals. *Handbook of Dietary Fiber in Human Nutrition. JA Spiller, editor. CRC Press, Boca Raton, FL***,** 129-142.

Kritchevsky, D. & Story, J. A. 1974. Binding of bile salts in vitro by nonnutritive fiber. *The Journal of nutrition,* 104**,** 458-462.

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G. & Becker, K. 2011. Dietary Roles of Non-Starch Polysachharides in Human Nutrition: A Review. *Critical Reviews in Food Science and Nutrition,* 52**,** 899-935.

Lairon, D., Arnault, N., Bertrais, S., Planells, R., Clero, E., Hercberg, S. & Boutron-Ruault, M. C. 2005. Dietary fiber intake and risk factors for cardiovascular disease in French adults. *The American journal of clinical nutrition,* 82**,** 1185.

Lampe, J., Fredstrom, S., Slavin, J. & Potter, J. 1993a. Sex differences in colonic function: a randomised trial. *Gut,* 34**,** 531-536.

Lampe, J., Wetsch, R., Thompson, W. & Slavin, J. 1993b. Gastrointestinal effects of sugarbeet fiber and wheat bran in healthy men. *European journal of clinical nutrition,* 47**,** 543.

Lattimer, J. M. & Haub, M. D. 2010. Effects of dietary fiber and its components on metabolic health. *Nutrients,* 2**,** 1266-1289.

Laurentin, A. & Edwards, C. 2005. Resistant starch and oligosaccharides. *GUIDE TO NUTRITIONAL SUPPLEMENTS***,** 84.

Laurentin, A. & Edwards, C. A. 2004. Differential fermentation of glucose-based carbohydrates in vitro by human faecal bacteria. *European journal of nutrition,* 43**,** 183-189.

Layden, B. T., Angueira, A. R., Brodsky, M., Durai, V. & Lowe Jr, W. L. 2013. Short chain fatty acids and their receptors: new metabolic targets. *Translational Research,* 161**,** 131-140.

Layden, B. T., Yalamanchi, S. K., Wolever, T., Dunaif, A. & Lowe Jr, W. L. 2011. Negative association of acetate with visceral adipose tissue and insulin levels. *Diabetes, metabolic syndrome and obesity: targets and therapy,* 5**,** 49-55.

Le Leu, R. K., Hu, Y., Brown, I. L. & Young, G. P. 2009. Effect of high amylose maize starches on colonic fermentation and apoptotic response to DNA-damage in the colon of rats. *Nutr Metab (Lond),* 6**,** 11.

Le Leu, R. K., Hu, Y. & Young, G. P. 2002. Effects of resistant starch and nonstarch polysaccharides on colonic luminal environment and genotoxin-induced apoptosis in the rat. *Carcinogenesis,* 23**,** 713-719.

Lee, S., Prosky, L. & De Vries, J. 1992. Determination of total, soluble, and insoluble dietary fiber in foods-enzymatic-gravimetric method, MES-TRIS buffer: collaborative study. *Journal of AOAC international,* 75**,** 395-416.

Letexier, D., Diraison, F. & Beylot, M. 2003. Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. *The American Journal of Clinical Nutrition,* 77**,** 559-564.

Leu, R., Hu, Y. & Young, G. 2002. Effects of resistant starch and nonstarch polysaccharides on colonic luminal environment and genotoxin-induced apoptosis in the rat. *Carcinogenesis,* 23**,** 713-719.

Levitt, M., Hirsh, P., Fetzer, C., Sheahan, M. & Levine, A. 1987. H2 excretion after ingestion of complex carbohydrates. *GASTROENTEROLOGY,* 92**,** 383.

Lia, A., Hallmans, G., Sandberg, A.-S., Sundberg, B., Aman, P. & Andersson, H. 1995. Oat beta-glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. *The American journal of clinical nutrition,* 62**,** 1245-1251.

Liener, I. 1996. Effects of processing on antinutritional factors in legumes: the soybean case. *Archivos latinoamericanos de nutrición,* 44**,** 48S-54S.

Lin, H. V., Frassetto, A., Kowalik Jr, E. J., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., Hubert, J. A., Szeto, D., Yao, X. & Forrest, G. 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PloS one,* 7**,** e35240.

Lin, J., Zhang, S. M., Cook, N. R., Rexrode, K. M., Liu, S., Manson, J. E., Lee, I.-M. & Buring, J. E. 2005. Dietary intakes of fruit, vegetables, and fiber, and risk of colorectal cancer in a prospective cohort of women (United States). *Cancer causes & control,* 16**,** 225-233.

Lin, Y., Vonk, R. J., Slooff, M. J., Kuipers, F. & Smit, M. J. 1995. Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. *British Journal of Nutrition,* 74**,** 197-207.

Lina, B. a. R., Jonker, D. & Kozianowski, G. 2002. Isomaltulose (Palatinose®): a review of biological and toxicological studies. *Food and Chemical Toxicology,* 40**,** 1375-1381.

Lindström, J., Peltonen, M., Eriksson, J. G., Louheranta, A., Fogelholm, M., Uusitupa, M. & Tuomilehto, J. 2006. High-fibre, low-fat diet predicts long-term weight loss and decreased type 2 diabetes risk: the Finnish Diabetes Prevention Study. *Diabetologia,* 49**,** 912-920.

Lipkin, M., Reddy, B., Newmark, H. & Lamprecht, S. A. 1999. Dietary factors in human colorectal cancer. *Annual review of nutrition,* 19**,** 545-586.

Liu, L., Wang, S. & Liu, J. 2015. Fiber consumption and all-cause, cardiovascular, and cancer mortalities: A systematic review and meta-analysis of cohort studies. *Molecular nutrition & food research,* 59**,** 139-146.

Liu, S., Buring, J. E., Sesso, H. D., Rimm, E. B., Willett, W. C. & Manson, J. a. E. 2002. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. *Journal of the American College of Cardiology,* 39**,** 49-56.

Liu, S., Willett, W. C., Stampfer, M. J., Hu, F. B., Franz, M., Sampson, L., Hennekens, C. H. & Manson, J. E. 2000. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr,* 71**,** 1455-1461.

Lopez, H. W., Levrat-Verny, M.-A., Coudray, C., Besson, C., Krespine, V., Messager, A., Demigné, C. & Rémésy, C. 2001. Class 2 resistant starches lower plasma and liver lipids and improve mineral retention in rats. *The Journal of nutrition,* 131**,** 1283-1289.

Lopez, H. W., Levrat, M.-A., Guy, C., Messager, A., Demigné, C. & Rémésy, C. 1999. Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. *The Journal of nutritional biochemistry,* 10**,** 500-509.

Louis, P. & Flint, H. J. 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS microbiology letters,* 294**,** 1-8.

Lu, Z. X., Walker, K. Z., Muir, J. G., Mascara, T. & O'dea, K. 2000. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *The American Journal of Clinical Nutrition,* 71**,** 1123-1128.

Lunn, J. & Buttriss, J. 2007. Carbohydrates and dietary fibre. *Nutrition Bulletin,* 32**,** 21-64.

Lupton, J. R. 2004. Microbial degradation products influence colon cancer risk: the butyrate controversy. *The Journal of nutrition,* 134**,** 479-482.

Lupton, J. R. & Turner, N. D. 1999. Potential protective mechanisms of wheat bran fiber. *The American journal of medicine,* 106**,** 24-27.

Macdiarmid, J. & Blundell, J. 1998. Assessing dietary intake: who, what and why of under-reporting. *Nutrition Research Reviews,* 11**,** 231-253.

Macfarlane, G., Cummings, J., Macfarlane, S. & Gibson, G. 1989. Influence of retention time on degradation of pancreatic enzymes by human colonic bacteria grown in a 3-stage continuous culture system. *Journal of applied bacteriology,* 67**,** 521-527.

Macfarlane, G., Steed, H. & Macfarlane, S. 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology,* 104**,** 305-344.

Maes, C. & Delcour, J. 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science,* 35**,** 315-326.

Mai, V., Flood, A., Peters, U., Lacey, J. V., Schairer, C. & Schatzkin, A. 2003. Dietary fibre and risk of colorectal cancer in the Breast Cancer Detection Demonstration Project (BCDDP) follow-up cohort. *International journal of epidemiology,* 32**,** 234-239.

Maki, K. C., Pelkman, C. L., Finocchiaro, E. T., Kelley, K. M., Lawless, A. L., Schild, A. L. & Rains, T. M. 2012. Resistant Starch from High-Amylose Maize Increases Insulin Sensitivity in Overweight and Obese Men. *The Journal of Nutrition,* 142**,** 717-723.

Maki, K. C., Slavin, J. L., Rains, T. M. & Kris-Etherton, P. M. 2014. Limitations of Observational Evidence: Implications for Evidence-Based Dietary Recommendations. *Advances in Nutrition: An International Review Journal,* 5**,** 7-15.

Marklund, M., Landberg, R., Åman, P. & Kamal-Eldin, A. 2010. Determination of alkylresorcinol metabolites in human urine by gas chromatography–mass spectrometry. *Journal of Chromatography B,* 878**,** 888-894.

Marlett, J. A. & Fischer, M. H. 2003. The active fraction of psyllium seed husk. *Proceedings of the Nutrition Society,* 62**,** 207-209.

Marlett, J. A., Kajs, T. M. & Fischer, M. H. 2000. An unfermented gel component of psyllium seed husk promotes laxation as a lubricant in humans. *The American journal of clinical nutrition,* 72**,** 784-789.

Mcburney, M., Thompson, L., Cuff, D. & Jenkins, D. 1988. Comparison of ileal effluents, dietary fibers, and whole foods in predicting the physiological importance of colonic fermentation. *The American journal of gastroenterology,* 83**,** 536.

Mccance, R. & Widdowson, E. 1949. Phytic acid. *British Journal of Nutrition,* 2**,** 401-403.

Mccarty, M. F. 2005. Nutraceutical resources for diabetes prevention - An update. *Medical Hypotheses,* 64**,** 151-158.

Mccleary, B. V., Mcnally, M. & Rossiter, P. 2002. Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: collaborative study. *Journal of AOAC International,* 85**,** 1103-1111.

Mccleary, B. V. & Monaghan, D. A. 2002. Measurement of resistant starch. *Journal of AOAC international,* 85**,** 665-675.

Mccleary, B. V., Murphy, A. & Mugford, D. C. 2000. Measurement of total fructan in foods by enzymatic/spectrophotometric method: collaborative study. *Journal of AOAC International,* 83**,** 356-364.

Mcorist, A. L., Miller, R. B., Bird, A. R., Keogh, J. B., Noakes, M., Topping, D. L. & Conlon, M. A. 2011. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *The Journal of nutrition,* 141**,** 883-889.

Medina, V., Edmonds, B., Young, G. P., James, R., Appleton, S. & Zalewski, P. D. 1997. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer Research,* 57**,** 3697-3707.

Metcalf, A., Phillips, S., Zinsmeister, A., Maccarty, R., Beart, R. & Wolff, B. 1987. Simplified assessment of segmental colonic transit. *GASTROENTEROLOGY,* 92**,** 40.

Meyer, K. A., Kushi, L. H., Jacobs, D. R., Slavin, J., Sellers, T. A. & Folsom, A. R. 2000. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *The American journal of clinical nutrition,* 71**,** 921-930.

Michels, K. B., Fuchs, C. S., Giovannucci, E., Colditz, G. A., Hunter, D. J., Stampfer, M. J. & Willett, W. C. 2005. Fiber intake and incidence of colorectal cancer among 76,947 women and 47,279 men. *Cancer Epidemiology Biomarkers & Prevention,* 14**,** 842-849.

Mikulíková, D., Masár, Š. & Kraic, J. 2008. Biodiversity of Legume Health-promoting Starch. *Starch-Stärke,* 60**,** 426-432.

Miller, J. B., Bull, S., Miller, J. & Mcveagh, P. 1994. The Oligosaccharide Composition of Human Milk: Temporal and Individual Variations in Monosaccharide Components. *Journal of Pediatric Gastroenterology and Nutrition,* 19**,** 371-376.

Miller, T. L., Weaver, G. & Wolin, M. 1984. Methanogens and anaerobes in a colon segment isolated from the normal fecal stream. *Applied and Environmental Microbiology,* 48**,** 449-450.

Miller, T. L. & Wolin, M. 1979. Fermentations by saccharolytic intestinal bacteria. *The American Journal of Clinical Nutrition,* 32**,** 164.

Miller, T. L. & Wolin, M. J. 1996. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Applied and Environmental Microbiology,* 62**,** 1589-92.

Mohnen, D. 2008. Pectin structure and biosynthesis. *Current opinion in plant biology,* 11**,** 266-277.

Molis, C., Flourié, B., Ouarne, F., Gailing, M. F., Lartigue, S., Guibert, A., Bornet, F. & Galmiche, J. P. 1996. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. *The American Journal of Clinical Nutrition,* 64**,** 324-8.

Morais, M. B., Feste, A., Miller, R. G. & Lifschitz, C. H. 1996. Effect of resistant and digestible starch on intestinal absorption of calcium, iron, and zinc in infant pigs. *Pediatric research,* 39**,** 872-876.

Morita, T., Kasaoka, S., Hase, K. & Kiriyama, S. 1999. Psyllium shifts the fermentation site of high-amylose cornstarch toward the distal colon and increases fecal butyrate concentration in rats. *The Journal of Nutrition,* 129**,** 2081-2087.

Moshfegh, A. J., Friday, J. E., Goldman, J. P. & Ahuja, J. K. C. 1999. Presence of inulin and oligofructose in the diets of Americans. *The Journal of Nutrition,* 129**,** 1407.

Mozaffarian, D., Kumanyika, S. K., Lemaitre, R. N., Olson, J. L., Burke, G. L. & Siscovick, D. S. 2003. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. *JAMA: the journal of the American Medical Association,* 289**,** 1659.

Mudgil, D. & Barak, S. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *International journal of biological macromolecules,* 61**,** 1-6.

Muir, J. G., Birkett, A., Brown, I., Jones, G. & O'dea, K. 1995. Food processing and maize variety affects amounts of starch escaping digestion in the small intestine. *The American journal of clinical nutrition,* 61**,** 82-89.

Muir, J. G. & O'dea, K. 1992. Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. *The American Journal of Clinical Nutrition,* 56**,** 123-7.

Muir, J. G. & O'dea, K. 1993. Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *The American journal of clinical nutrition,* 57**,** 540-546.

Muir, J. G., Yeow, E. G., Keogh, J., Pizzey, C., Bird, A. R., Sharpe, K., O'dea, K. & Macrae, F. A. 2004. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. *The American Journal of Clinical Nutrition,* 79**,** 1020-1028.

Murphy, M. M., Douglass, J. S. & Birkett, A. 2008. Resistant Starch Intakes in the United States. *Journal of the American Dietetic Association,* 108**,** 67-78.

Mussatto, S. I. & Mancilha, I. M. 2007. Non-digestible oligosaccharides: A review. *Carbohydrate Polymers,* 68**,** 587-597.

Musso, G., Gambino, R. & Cassader, M. 2011. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annual review of medicine,* 62**,** 361-380.

Mutwil, M., Debolt, S. & Persson, S. 2008. Cellulose synthesis: a complex complex. *Current opinion in plant biology,* 11**,** 252-257.

Naumann, E., Van Rees, A. B., Önning, G., Öste, R., Wydra, M. & Mensink, R. P. 2006. β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations. *The American journal of clinical nutrition,* 83**,** 601-605.

Nishina, P. M. & Freedland, R. A. 1990. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *The Journal of Nutrition,* 120**,** 668-673.

Nomura, A. M., Hankin, J. H., Henderson, B. E., Wilkens, L. R., Murphy, S. P., Pike, M. C., Le Marchand, L., Stram, D. O., Monroe, K. R. & Kolonel, L. N. 2007. Dietary fiber and colorectal cancer risk: the multiethnic cohort study. *Cancer causes & control,* 18**,** 753-764.

Nordlund, E., Aura, A.-M., Mattila, I., KöSsö, T., Rouau, X. & Poutanen, K. 2012. Formation of phenolic microbial metabolites and short-chain fatty acids from rye, wheat, and oat bran and their fractions in the metabolical in vitro colon model. *Journal of agricultural and food chemistry,* 60**,** 8134-8145.

Nugent, A. 2005. Health properties of resistant starch. *Nutrition Bulletin,* 30**,** 27-54.

O'donnell, L., Emmett, P. M. & Heaton, K. W. 1989. Size of flour particles and its relation to glycaemia, insulinaemia, and colonic disease. *British Medical Journal,* 298**,** 1616.

O'keefe, S. J. D., Kidd, M., Espitalier-Noel, G. & Owira, P. 1999. Rarity of colon cancer in Africans is associated with low animal product consumption, not fiber. *Am J Gastroenterol,* 94**,** 1373-1380.

Othman, R. A., Moghadasian, M. H. & Jones, P. J. 2011. Cholesterol-lowering effects of oat β-glucan. *Nutrition Reviews,* 69**,** 299-309.

Ouwehand, A. C., Derrien, M., De Vos, W., Tiihonen, K. & Rautonen, N. 2005. Prebiotics and other microbial substrates for gut functionality. *Current opinion in biotechnology,* 16**,** 212-217.

Pal, S. & Radavelli-Bagatini, S. 2012. Effects of psyllium on metabolic syndrome risk factors. *Obesity Reviews,* 13**,** 1034-1047.

Park, Y., Brinton, L. A., Subar, A. F., Hollenbeck, A. & Schatzkin, A. 2009. Dietary fiber intake and risk of breast cancer in postmenopausal women: the National Institutes of Health–AARP Diet and Health Study. *The American journal of clinical nutrition,* 90**,** 664-671.

Park, Y., Hunter, D. J., Spiegelman, D., Bergkvist, L., Berrino, F., Van Den Brandt, P. A., Buring, J. E., Colditz, G. A., Freudenheim, J. L. & Fuchs, C. S. 2005. Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. *Jama,* 294**,** 2849-2857.

Park, Y., Subar, A. F., Hollenbeck, A. & Schatzkin, A. 2011. Dietary fiber intake and mortality in the NIH-AARP diet and health study. *Archives of internal medicine,* 171**,** 1061-1068.

Pereira, M. A., O'reilly, E., Augustsson, K., Fraser, G. E., Goldbourt, U., Heitmann, B. L., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens,

J., Virtamo, J., Willett, W. C. & Ascherio, A. 2004. Dietary Fiber and Risk of Coronary Heart Disease: A Pooled Analysis of Cohort Studies. *Arch Intern Med,* 164**,** 370-376.

Phillips, G. O. & Cui, S. W. 2011. An introduction: Evolution and finalisation of the regulatory definition of dietary fibre. *Food Hydrocolloids,* 25**,** 139-143.

Phillips, J., Muir, J. G., Birkett, A., Lu, Z. X., Jones, G. P., O'dea, K. & Young, G. P. 1995. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *The American Journal of Clinical Nutrition,* 62**,** 121-30.

Pieper, R., Kröger, S., Richter, J. F., Wang, J., Martin, L., Bindelle, J., Htoo, J. K., Von Smolinski, D., Vahjen, W., Zentek, J. & Van Kessel, A. G. 2012. Fermentable Fiber Ameliorates Fermentable Protein-Induced Changes in Microbial Ecology, but Not the Mucosal Response, in the Colon of Piglets. *The Journal of Nutrition,* 142**,** 661-667.

Pietinen, P., Malila, N., Virtanen, M., Hartman, T. J., Tangrea, J. A., Albanes, D. & Virtamo, J. 1999. Diet and risk of colorectal cancer in a cohort of Finnish men. *Cancer causes & control,* 10**,** 387-396.

Pietinen, P., Rimm, E. B., Korhonen, P., Hartman, A. M., Willett, W. C., Albanes, D. & Virtamo, J. 1996. Intake of dietary fiber and risk of coronary heart disease in a cohort of Finnish men: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Circulation,* 94**,** 2720.

Porikos, K. P. & Van Itallie, T. B. 1983. Diet-induced changes in serum transaminase and triglyceride levels in healthy adult men: role of sucrose and excess calories. *The American journal of medicine,* 75**,** 624-630.

Poulsen, M., Jensen, B. B. & Engberg, R. M. 2012. The effect of pectin, corn and wheat starch, inulin and pH on in vitro production of methane, short chain fatty acids and on the microbial community composition in rumen fluid. *Anaerobe,* 18**,** 83-90.

Pouteau, E., Nguyen, P., Ballèvre, O. & Krempf, M. 2003. Production rates and metabolism of short-chain fatty acids in the colon and whole body using stable isotopes. *Proceedings of the Nutrition Society,* 62**,** 87-93.

Pouteau, E., Vahedi, K., Messing, B., Flourié, B., Nguyen, P., Darmaun, D. & Krempf, M. 1998. Production rate of acetate during colonic fermentation of lactulose: a stable-isotope study in humans. *The American journal of clinical nutrition,* 68**,** 1276-1283.

Prosky, L. & Hoebregs, H. 1999. Methods to determine food inulin and oligofructose. *The Journal of Nutrition,* 129**,** 1418S-1423s.

Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S. & Flint, H. J. 2002. The microbiology of butyrate formation in the human colon. *FEMS microbiology letters,* 217**,** 133-139.

Psaltopoulou, T., Ilias, I. & Alevizaki, M. 2010. The role of diet and lifestyle in primary, secondary, and tertiary diabetes prevention: a review of meta-analyses. *The review of diabetic studies: RDS,* 7**,** 26.

Raben, A., Tagliabue, A., Christensen, N. J., Madsen, J., Holst, J. J. & Astrup, A. 1994. Resistant starch: the effect on postprandial glycemia, hormonal response, and satiety. *The American Journal of Clinical Nutrition,* 60**,** 544-551.

Ragsdale, S. W. & Pierce, E. 2008. Acetogenesis and the Wood–Ljungdahl pathway of CO< sub> 2</sub> fixation. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics,* 1784**,** 1873-1898.

Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B. & Wolever, T. M. S. 2014. Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *Int J Obes*.

Rao, S., Read, N., Brown, C., Bruce, C. & Holdsworth, C. 1987. Studies on the mechanism of bowel disturbance in ulcerative colitis. *GASTROENTEROLOGY,* 93**,** 934.

Reader, D., Johnson, M., Hollander, P. & Franz, M. 1997. Response of resistant starch in a food bar vs. two commercially available bars in persons with type II diabetes mellitus. *Diabetes,* 46**,** 254A.

Reader, D. M., O'connell, B. S., Johnson, M. L. & Franz, M. 2002. Glycemic and insulinemic response of subjects with type 2 diabetes after consumption of three energy bars. *Journal of the American Dietetic Association,* 102**,** 1139-1142.

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., Leitch, C. M., Scott, K. P., Flint, H. J. & Louis, P. 2014. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *The ISME journal,* 8**,** 1323-1335.

Reilly, K. & Rombeau, J. 1993. Metabolism and potential clinical applications of short-chain fatty acids. *Clinical nutrition,* 12**,** S97-S105.

Reiser, S., Powell, A., Scholfield, D., Panda, P., Ellwood, K. & Canary, J. 1989. Blood lipids, lipoproteins, apoproteins, and uric acid in men fed diets containing fructose or high-amylose cornstarch. *The American journal of clinical nutrition,* 49**,** 832-839.

Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M. J. & Willett, W. C. 1996. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA: the journal of the American Medical Association,* 275**,** 447.

Ring, S. G. & Selvendran, R. R. 1980. Isolation and analysis of cell wall material from beeswing wheat bran (< i> Triticum aestivum</i>). *Phytochemistry,* 19**,** 1723-1730.

Roberfroid, M., Gibson, G. R., Hoyles, L., Mccartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H. & Stahl, B. 2010. Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition,* 104**,** S1-S63.

Roberfroid, M. B. 2005. Introducing inulin-type fructans. *British Journal of Nutrition,* 93**,** 13-26.

Roediger, W. 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut,* 21**,** 793-798.

Ross, A. B., Pere-Trépat, E., Montoliu, I., Martin, F.-P. J., Collino, S., Moco, S., Godin, J.-P., Cléroux, M., Guy, P. A. & Breton, I. 2013. A Whole-Grain–Rich Diet Reduces Urinary Excretion of Markers of Protein Catabolism and Gut Microbiota Metabolism in Healthy Men after One Week. *The Journal of nutrition,* 143**,** 766-773.

Roy, C. C., Kien, C. L., Bouthillier, L. & Levy, E. 2006. Short-chain fatty acids: ready for prime time? *Nutrition in clinical practice,* 21**,** 351.

Ruepert, L., Quartero, A. O., De Wit, N. J., Van Der Heijden, G. J., Rubin, G. & Muris, J. W. 2011. Bulking agents, antispasmodics and antidepressants for the treatment of irritable bowel syndrome. *The Cochrane Library*.

Russo, F., Chimienti, G., Riezzo, G., Pepe, G., Petrosillo, G., Chiloiro, M. & Marconi, E. 2008. Inulin-enriched pasta affects lipid profile and Lp (a) concentrations in Italian young healthy male volunteers. *European journal of nutrition,* 47**,** 453-459.

Sacn 2015. Carbohydrates and Health. London: Scientific Advisory Committee on Nutrition.

Sajilata, M., Singhal, R. S. & Kulkarni, P. R. 2006. Resistant starch–a review. *Comprehensive reviews in food science and food safety,* 5**,** 1-17.

Sakakibara, I., Fujino, T., Ishii, M., Tanaka, T., Shimosawa, T., Miura, S., Zhang, W., Tokutake, Y., Yamamoto, J. & Awano, M. 2009. Fasting-induced hypothermia and reduced energy production in mice lacking acetyl-CoA synthetase 2. *Cell metabolism,* 9**,** 191-202.

Sandberg, A.-S. & Andersson, H. 1988. Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. *The Journal of Nutrition,* 118**,** 469-473.

Sandberg, A., Ahderinne, R., Andersson, H., Hallgren, B. & Hulten, L. 1983. The effect of citrus pectin on the absorption of nutrients in the small intestine. *Human nutrition. Clinical nutrition,* 37**,** 171.

Sandberg, A. S., Andersson, H., Hallgren, B., Hasselblad, K., Isaksson, B. & Hultbn, L. 1981. Experimental model for in vivo determination of dietary fibre and its effect on the absorption of nutrients in the small intestine. *British journal of nutrition,* 45**,** 283-294.

Sandberg, A. S., Andersson, H., Kivistö, B. & Sandström, B. 1986. Extrusion cooking of a high-fibre cereal product. *British journal of nutrition,* 55**,** 245-254.

Sangeetha, P. T., Ramesh, M. N. & Prapulla, S. G. 2005. Recent trends in the microbial production, analysis and application of Fructooligosaccharides. *Trends in Food Science & Technology,* 16**,** 442-457.

Sanjoaquin, M. A., Appleby, P. N., Thorogood, M., Mann, J. I. & Key, T. J. 2004. Nutrition, lifestyle and colorectal cancer incidence: a prospective investigation of 10[thinsp]998 vegetarians and non-vegetarians in the United Kingdom. *Br J Cancer,* 90**,** 118-121.

Sanz, T., Salvador, A. & Fiszman, S. 2008. Evaluation of four types of resistant starch in muffin baking performance and relationship with batter rheology. *European Food Research and Technology,* 227**,** 813-819.

Sarwar, N., Danesh, J., Eiriksdottir, G., Sigurdsson, G., Wareham, N., Bingham, S., Boekholdt, S. M., Khaw, K. T. & Gudnason, V. 2007. Triglycerides and the risk of coronary heart disease: 10 158 incident cases among 262 525 participants in 29 Western prospective studies. *Circulation,* 115**,** 450.

Savage, D. C. 1986. Gastrointestinal Microflora in Mammalian Nutrition. *Annual Review of Nutrition,* 6**,** 155-178.

Scharlau, D., Borowicki, A., Habermann, N., Hofmann, T., Klenow, S., Miene, C., Munjal, U., Stein, K. & Glei, M. 2009. Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutation Research/Reviews in Mutation Research,* 682**,** 39-53.

Schatzkin, A., Park, Y., Leitzmann, M. F., Hollenbeck, A. R. & Cross, A. J. 2008. Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. *Gastroenterology,* 135**,** 1163-1167.

Scheller, H. V. & Ulvskov, P. 2010. Hemicelluloses. *Plant Biology,* 61**,** 263.

Scheppach, W., Fabian, C., Ahrens, F., Spengler, M. & Kasper, H. 1988a. Effect of starch malabsorption on colonic function and metabolism in humans. *Gastroenterology,* 95**,** 1549-1555.

Scheppach, W., Fabian, C., Sachs, M. & Kasper, H. 1988b. The effect of starch malabsorption on fecal short-chain fatty acid excretion in man. *Scandinavian journal of gastroenterology,* 23**,** 755-759.

Scheppach, W., Sommer, H., Kirchner, T., Paganelli, G.-M., Bartram, P., Christl, S., Richter, F., Dusel, G. & Kasper, H. 1992. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology,* 103**,** 51-56.

Scheppan W, Bach M, Bartram P & Kasper H 1989. The malabsorption of potato starch is modified by freezing and reheating. *GASTROENTEROLOGY,* 96**,** A447.

Schofield, W. 1984. Predicting basal metabolic rate, new standards and review of previous work. *Human nutrition. Clinical nutrition,* 39**,** 5-41.

Schweizer, T., Andersson, H., Langkilde, A., Reimann, S. & Torsdottir, I. 1990. Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes. II. Starch, dietary fibre and sugars. *European Journal of Clinical Nutrition,* 44**,** 567-575.

Schwiertz, A., Taras, D., Schäfer, K., Beijer, S., Bos, N. A., Donus, C. & Hardt, P. D. 2010. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity,* 18**,** 190-195.

Seidell, J. C. 2005. Epidemiology of obesity. *Seminars in Vascular Medicine,* 5**,** 3-14.

Seifert, S. & Watzl, B. 2007. Inulin and Oligofructose: Review of Experimental Data on Immune Modulation. *The Journal of nutrition,* 137**,** 2563S-2567S.

Sekirov, I., Russell, S. L., Antunes, L. C. M. & Finlay, B. B. 2010. Gut microbiota in health and disease. *Physiological reviews,* 90**,** 859-904.

Sharma, A., Yadav, B. S. & Ritika 2008. Resistant starch: physiological roles and food applications. *Food Reviews International,* 24**,** 193-234.

Sharrett, A. R., Ballantyne, C., Coady, S., Heiss, G., Sorlie, P., Catellier, D. & Patsch, W. 2001. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein (a), apolipoproteins AI and B, and HDL density subfractions. *Circulation,* 104**,** 1108-1113.

Silvester, K. R., Englyst, H. N. & Cummings, J. H. 1995. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent [published erratum appears in Am J Clin Nutr 1996 Mar; 63 (3): 407]. *The American Journal of Clinical Nutrition,* 62**,** 403-411.

Singh, M., Sharma, R. & Banerjee, U. C. 2002. Biotechnological applications of cyclodextrins. *Biotechnology Advances,* 20**,** 341-359.

Singh, N. P. & Lai, H. C. 2005. Synergistic Cytotoxicity of Artemisinin and Sodium Butyrate on Human Cancer Cells. *Anticancer Research,* 25**,** 4325-4331.

Slavin, J. 2004. Whole grains and human health. *Nutrition research reviews,* 17**,** 99-110.

Slavin, J., Stewart, M., Timm, D., Grabitske, H., Hospattankar, A., Van Der Kamp, J., Jones, J., Mccleary, B. & Topping, D. 2010. Fermentation patterns and short chain fatty acid profiles of wheat dextrin and other functional fibres. *Dietary Fibre: New Frontiers for Food and Health***,** 177.

Sleeth, M. L., Thompson, E. L., Ford, H. E., Zac-Varghese, S. E. K. & Frost, G. 2010. Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutrition Research Reviews,* 23**,** 135-145.

Smolina, K., Wright, F. L., Rayner, M. & Goldacre, M. J. 2012. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: linked national database study. *BMJ: British Medical Journal,* 344.

Soergel, K. 1994. Colonic fermentation: metabolic and clinical implications. *The clinical investigator,* 72**,** 742-748.

Sola, R., Godas, G., Ribalta, J., Vallvé, J.-C., Girona, J., Anguera, A., Ostos, M., Recalde, D., Salazar, J. & Caslake, M. 2007a. Effects of soluble fiber (Plantago ovata husk) on plasma lipids, lipoproteins, and apolipoproteins in men with ischemic heart disease. *The American journal of clinical nutrition,* 85**,** 1157-1163.

Sola, R., Godas, G., Ribalta, J., Vallvé, J. C., Girona, J., Anguera, A., Ostos, M. A., Recalde, D., Salazar, J. & Caslake, M. 2007b. Effects of soluble fiber (Plantago ovata husk) on plasma lipids, lipoproteins, and apolipoproteins in men with ischemic heart disease. *The American journal of clinical nutrition,* 85**,** 1157.

Southgate, D., Branch, W., Hill, M., Drasar, B., Walters, R., Davies, P. & Baird, I. M. 1976. Metabolic responses to dietary supplements of bran. *Metabolism,* 25**,** 1129-1135.

Steffen, L. M., Jacobs, D. R. & Murtaugh, M. A. 2003. Whole grain intake is associated with lower body mass and greater insulin sensitivity among adolescents. *Am J Epidemiol* 158**,** 243-50.

Stephen, A., Haddad, A. & Phillips, S. 1983. Passage of carbohydrate into the colon: direct measurements in humans. *GASTROENTEROLOGY,* 85**,** 589-595.

Stephen, A. M. 1991. Starch and dietary fibre: their physiological and epidemiological interrelationships. *Canadian Journal of Physiology and Pharmacology,* 69**,** 116-120.

Stephen, A. M. & Cummings, J. 1979. Water-holding by dietary fibre in vitro and its relationship to faecal output in man. *Gut,* 20**,** 722-729.

Stephen, A. M. & Cummings, J. 1980. The microbial contribution to human faecal mass. *Journal of Medical Microbiology,* 13**,** 45-56.

Stephen, A. M., Sieber, G. M., Gerster, Y. A. & Morgan, D. R. 1995. Intake of carbohydrate and its components--international comparisons, trends over time, and effects of changing to low-fat diets. *The American journal of clinical nutrition,* 62**,** 851S-867S.

Stephen, A. M., Wiggins, H. & Cummings, J. 1987. Effect of changing transit time on colonic microbial metabolism in man. *Gut,* 28**,** 601-609.

Stevens, J., Levitsky, D. A., Vansoest, P. J., Robertson, J. B., Kalkwarf, H. J. & Roe, D. A. 1987. Effect of psyllium gum and wheat bran on spontaneous energy intake. *The American journal of clinical nutrition,* 46**,** 812-817.

Stevenson, L., Phillips, F., O'sullivan, K. & Walton, J. 2012. Wheat bran: its composition and benefits to health, a European perspective. *International journal of food sciences and nutrition,* 63**,** 1001-1013.

Stone, B. & Fincher, G. B. 2004. Chemistry of Non-Starch Polysaccharides from Cereal Grains.

Story, J. A., Furumoto, E. J. & Buhman, K. K. 1997. Dietary fiber and bile acid metabolism—an update. *Dietary fiber in health and disease.* Springer.

Streppel, M. T., Ocké, M. C., Boshuizen, H. C., Kok, F. J. & Kromhout, D. 2008. Dietary fiber intake in relation to coronary heart disease and all-cause mortality over 40 y: the Zutphen Study. *The American journal of clinical nutrition,* 88**,** 1119.

Suzuki, T., Yoshida, S. & Hara, H. 2008. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. *British Journal of Nutrition,* 100**,** 297-305.

Tan, J., Mckenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R. & Macia, L. 2013. The role of short-chain fatty acids in health and disease. *Advances in immunology,* 121**,** 91-119.

Tang, Y., Chen, Y., Jiang, H., Robbins, G. T. & Nie, D. 2011a. G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. *International Journal of Cancer,* 128**,** 847-856.

Tang, Y., Chen, Y., Jiang, H., Robbins, G. T. & Nie, D. 2011b. G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. *International Journal of Cancer,* 128**,** 847-856.

Terry, P., Hu, F. B., Hansen, H. & Wolk, A. 2001. Prospective study of major dietary patterns and colorectal cancer risk in women. *American journal of epidemiology,* 154**,** 1143-1149.

Thakur, B. R., Singh, R. K., Handa, A. K. & Rao, M. 1997. Chemistry and uses of pectin—a review. *Critical Reviews in Food Science & Nutrition,* 37**,** 47-73.

Thangaraju, M., Cresci, G. A., Liu, K., Ananth, S., Gnanaprakasam, J. P., Browning, D. D., Mellinger, J. D., Smith, S. B., Digby, G. J., Lambert, N. A., Prasad, P. D. & Ganapathy, V. 2009. GPR109A Is a G-protein–Coupled Receptor for the Bacterial Fermentation Product Butyrate and Functions as a Tumor Suppressor in Colon. *Cancer Research,* 69**,** 2826-2832.

Tharanathan, R. & Mahadevamma, S. 2003. Grain legumes—a boon to human nutrition. *Trends in Food Science & Technology,* 14**,** 507-518.

Theuwissen, E. & Mensink, R. P. 2008a. Water-soluble dietary fibers and cardiovascular disease. *Physiology and Behavior,* 94**,** 285-292.

Theuwissen, E. & Mensink, R. P. 2008b. Water-soluble dietary fibers and cardiovascular disease. *Physiology & Behavior,* 94**,** 285-292.

Threapleton, D. E., Greenwood, D. C., Burley, V. J., Aldwairji, M. & Cade, J. E. 2013a. Dietary fibre and cardiovascular disease mortality in the UK Women's Cohort Study. *European journal of epidemiology,* 28**,** 335-346.

Threapleton, D. E., Greenwood, D. C., Evans, C. E., Cleghorn, C. L., Nykjaer, C., Woodhead, C., Cade, J. E., Gale, C. P. & Burley, V. J. 2013b. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ: British Medical Journal,* 347.

Todd, S., Woodward, M., Tunstall-Pedoe, H. & Bolton-Smith, C. 1999. Dietary Antioxidant vitamins and Fiber in the Etiology of Cardiovasucalr Disease and All-Causes Mortality: Resulsts from the Scottish Heart Health Study. *American journal of epidemiology,* 150**,** 1073.

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F. & Gribble, F. M. 2012. Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein–Coupled Receptor FFAR2. *Diabetes,* 61**,** 364-371.

Topping, D. L. & Clifton, P. M. 2001. Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. *Physiological Reviews,* 81**,** 1031-1064.

Trinidad, T. P., Wolever, T. & Thompson, L. U. 1996. Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans. *The American journal of clinical nutrition,* 63**,** 574-578.

Trowell, H. 1972. Crude fibre, dietary fibre and atherosclerosis. *Atherosclerosis,* 16**,** 138.

Trowell, H. 1976. Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. *The American Journal of Clinical Nutrition,* 29**,** 417-427.

Tucker, L. A. & Thomas, K. S. 2009. Increasing total fiber intake reduces risk of weight and fat gains in women. *The Journal of nutrition,* 139**,** 576-581.

Tufvesson, F., Wahlgren, M. & Eliasson, A. C. 2003. Formation of Amylose-Lipid Complexes and Effects of Temperature Treatment. Part 2. Fatty Acids. *Starch-Stärke,* 55**,** 138-149.

Tungland, B. & Meyer, D. 2002. Nondigestible Oligo-and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food. *Comprehensive Reviews in food science and food safety,* 1**,** 90-109.

Unal, B., Critchley, J. A. & Capewell, S. 2004. Explaining the decline in coronary heart disease mortality in England and Wales between 1981 and 2000. *Circulation,* 109**,** 1101-1107.

Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H. & Smits, G. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science & Nutrition,* 35**,** 525-552.

Van Nuenen, M. H., Diederick Meyer, P. & Venema, K. 2003. The effect of various inulins and Clostridium difficile on the metabolic activity of the human colonic microbiota in vitro. *Microbial Ecology in Health and Disease,* 15**,** 137-144.

Van Stralen, K. J., Jager, K. J., Zoccali, C. & Dekker, F. W. 2008. Agreement between methods. *Kidney international,* 74**,** 1116-1120.

Vázquez, M. J., Alonso, J. L., DomíNguez, H. & Parajó, J. C. 2000. Xylooligosaccharides: manufacture and applications. *Trends in Food Science & Technology,* 11**,** 387-393.

Venema, K. 2010. Role of gut microbiota in the control of energy and carbohydrate metabolism. *Current Opinion in Clinical Nutrition & Metabolic Care,* 13**,** 432-438.

Venter, C., Vorster, H. & Cummings, J. 1990a. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *American Journal of Gastroenterology,* 85**,** 549-553.

Venter, C. S., Vorster, H. H. & Cummings, J. H. 1990b. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *American journal of Gastroenterology,* 85.

Vernia, P., Annese, V., Bresci, G., D'albasio, G., D'incà, R., Giaccari, S., Ingrosso, M., Mansi, C., Riegler, G., Valpiani, D., Caprilli, R. & Gisc 2003. Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: results of a multicentre trial. *European Journal of Clinical Investigation,* 33**,** 244-248.

Voragen, A. G. J. 1998. Technological aspects of functional food-related carbohydrates. *Trends in Food Science & Technology,* 9**,** 328-335.

Vulevic, J., Juric, A., Tzortzis, G. & Gibson, G. R. 2013. A Mixture of trans-Galactooligosaccharides Reduces Markers of Metabolic Syndrome and Modulates the Fecal Microbiota and Immune Function of Overweight Adults. *The Journal of Nutrition,* 143**,** 324-331.

Walker, A. R., Richardson, B. D., Walker, B. F. & Woolford, A. 1973. Appendicitis, fibre intake and bowel behaviour in ethnic groups in South Africa. *Postgraduate medical journal,* 49**,** 243-249.

Walker, A. W., Duncan, S. H., Leitch, E. C. M., Child, M. W. & Flint, H. J. 2005. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Applied and Environmental Microbiology,* 71**,** 3692-3700.

Wallström, P., Sonestedt, E., Hlebowicz, J., Ericson, U., Drake, I., Persson, M., Gullberg, B., Hedblad, B. & Wirfält, E. 2012. Dietary fiber and saturated fat intake associations with cardiovascular disease differ by sex in the Malmö Diet and Cancer Cohort: a prospective study. *PloS one,* 7**,** e31637.

Ward, H., Keogh, R., Lentjes, M., Luben, R., Wareham, N. & Khaw, K. 2012. Fibre intake in relation to serum total cholesterol levels and CHD risk: a comparison of dietary assessment methods. *European Journal of Clinical Nutrition,* 66**,** 296-304.

Wcrf 2007. *Food, nutrition and the prevention of cancer: a global perspective*, American Institute for Cancer Research.

Weaver, L. T., Ewing, G. & Taylor, L. C. 1988. The bowel habit of milk-fed infants. *Journal of Pediatric Gastroenterology and Nutrition,* 7**,** 568-571.

Westenbrink, S., Brunt, K. & Van Der Kamp, J.-W. 2013a. Dietary fibre: challenges in production and use of food composition data. *Food Chemistry,* 140**,** 562-567.

Westenbrink, S., Brunt, K. & Van Der Kamp, J. 2013b. Dietary fibre: challenges in production and use of food composition data. *Food Chem,* 140**,** 562 - 567.

Williams, R. D., Olmsted, W. H., Hamann, C., Fiorito, J. & Duckles, D. 1936. The Effect of Cellulose, Hemicellulose and Lignin on the Weight of the Stool: A Contribution to the Study of Laxation in Man Two Figures. *The Journal of nutrition,* 11**,** 433-449.

Willis, H. J., Eldridge, A. L., Beiseigel, J., Thomas, W. & Slavin, J. L. 2009. Greater satiety response with resistant starch and corn bran in human subjects. *Nutrition Research,* 29**,** 100-105.

Wisker, E., Daniel, M., Rave, G. & Feldheim, W. 1998. Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and in vitro. *British Journal of Nutrition,* 80**,** 253-261.

Wolever, T., Brighenti, F., Royall, D., Jenkins, A. & Jenkins, D. 1989. Effect of rectal infusion of short chain fatty acids in human subjects. *The American journal of gastroenterology,* 84**,** 1027.

Wolever, T., Cohen, Z., Thompson, L., Thorne, M., Jenkins, M., Prokipchuk, E. & Jenkins, D. 1986. Ileal loss of available carbohydrate in man: comparison of a breath hydrogen method with direct measurement using a human ileostomy model. *The American journal of gastroenterology,* 81**,** 115.

Wolever, T., Josse, R. G., Leiter, L. A. & Chiasson, J.-L. 1997. Time of day and glucose tolerance status affect serum short-chain fatty concentrations in humans. *Metabolism,* 46**,** 805-811.

Wolever, T., Spadafora, P. & Eshuis, H. 1991. Interaction between colonic acetate and propionate in humans. *The American Journal of Clinical Nutrition,* 53**,** 681.

Wolever, T., Spadafora, P. J., Cunnane, S. C. & Pencharz, P. B. 1995. Propionate inhibits incorporation of colonic [1, 2-13C] acetate into plasma lipids in humans. *The American journal of clinical nutrition,* 61**,** 1241-1247.

Wolin, M. J. 1981. Fermentation in the rumen and human large intestine. *Science,* 213**,** 1463-1468.

Wolin, M. J. & Miller, T. L. Year. Interactions of microbial populations in cellulose fermentation. *In:* Fed. Proc, 1983. 109-113.

Wolk, A., Manson, J. a. E., Stampfer, M. J., Colditz, G. A., Hu, F. B., Speizer, F. E., Hennekens, C. H. & Willett, W. C. 1999. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. *JAMA: the journal of the American Medical Association,* 281**,** 1998.

Wong, J. M. W., De Souza, R., Kendall, C. W. C., Emam, A. & Jenkins, D. J. A. 2006. Colonic health: fermentation and short chain fatty acids. *Journal of clinical gastroenterology,* 40**,** 235.

Wong, J. M. W. & Jenkins, D. J. A. 2007. Carbohydrate digestibility and metabolic effects. *The Journal of Nutrition,* 137**,** 2539S-2546S.

Wood, P. J. 1997. Functional foods for health. *Cereals.* Springer.

Wood, P. J. 2007. Cereal β-glucans in diet and health. *Journal of Cereal Science,* 46**,** 230-238.

Wu, K., Bowman, R., Welch, A. A., Luben, R. N., Wareham, N., Khaw, K. T. & Bingham, S. A. 2007. Apolipoprotein E polymorphisms, dietary fat and fibre, and serum lipids: the EPIC Norfolk study. *European heart journal,* 28**,** 2930.

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

Xu, J. & Gordon, J. I. 2003. Honor thy symbionts. *Proceedings of the National Academy of Sciences,* 100**,** 10452-10459.

Younes, H., Levrat, M.-A., Demigné, C. & Rémésy, C. 1995. Resistant starch is more effective than cholestyramine as a lipid-lowering agent in the rat. *Lipids,* 30**,** 847-853.

Young, G. P., Hu, Y., Le Leu, R. K. & Nyskohus, L. 2005. Dietary fibre and colorectal cancer: a model for environment–gene interactions. *Molecular nutrition & food research,* 49**,** 571-584.

Zhou, J., Martin, R. J., Tulley, R. T., Raggio, A. M., Mccutcheon, K. L., Shen, L., Danna, S. C., Tripathy, S., Hegsted, M. & Keenan, M. J. 2008. Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *American Journal of Physiology-Endocrinology and Metabolism,* 295**,** E1160-E1166.

Zoetendal, E. G., Akkermans, A. D., Akkermans-Van Vliet, W. M., De Visser, J. a. G. & De Vos, W. M. 2001. The host genotype affects the bacterial community in the human gastronintestinal tract. *Microbial Ecology in Health and Disease,* 13**,** 129-134.

8 Appendices

- **Dietary fibre content (Non-starch polysaccharides) in cereals (adapted from Englyst et al., 1989).**
- **Dietary fibre content (Non-starch polysaccharides) in cereals (adapted from Englyst et al., 1988).**
- **Dietary fibre (soluble, insoluble and total DF g/100 g as eaten) contents of 70 high consumption foods (adapted from Li et al., 2002).**
- **Fructooligosaccharide composition of fruits (adapted from Campbell et al., 1997).**
- **Fructooligosaccharide Composition of vegetables (adapted from Campbell et al., 1997).**
- **Fructooligosaccharide Composition of fruits, vegetables and cereals (adapted from (Hogarth et al., 2000).**
- **Inulin and oligofructose content of foods (adapted from Moshfegh et al., 1999 and Van Loo et al., 1995)**

Dietary fibre content (Non-starch polysaccharides) in cereals (adapted from Englyst et al., 1989).

Flour, grain and starches		Total g/100 g Fresh Weight	Flour, grain and starches		Total g/100 g Fresh Weight
	Soluble NSP	1.6		Soluble NSP	0.1
Plain White Flour, Homepride	Insoluble NSP	1.6	Cornflour	Insoluble NSP	t
	Total NSP	3.2		Total NSP	0.1
	Soluble NSP	1.4		Soluble NSP	0.3
Plain White Flour, McDougalls	Insoluble NSP	1.7	Cornmeal	Insoluble NSP	1.4
	Total NSP	3.1		Total NSP	1.7
	Soluble NSP	2.2		Soluble NSP	0.8
Country Cookbook Plain	Insoluble NSP	4.7	Maize Meal	Insoluble NSP	4.1
	Total NSP	6.9		Total NSP	4.9
	Soluble NSP	1.9		Soluble NSP	0.2
Harvest Gold Brown Flour	Insoluble NSP	3.9	Millet	Insoluble NSP	1.7
	Total NSP	5.8		Total NSP	1.9
	Soluble NSP	1.5		Soluble NSP	4.3
Wholewheat Flour, Jordans	Insoluble NSP	6.8	Oatmeal, course	Insoluble NSP	2.7
	Total NSP	8.3		Total NSP	7.0
	Soluble NSP	2.5		Soluble NSP	3.7
Wholewheat Flour, Allinsons	Insoluble NSP	7.2	Oatmeal, medium ground	Insoluble NSP	2.9
	Total NSP	9.7		Total NSP	6.6
	Soluble NSP	0.1		Soluble NSP	7.6
Arrowroot	Insoluble NSP	t	Oatbran & Oatgerm	Insoluble NSP	4.8
	Total NSP	0.1		Total NSP	12.4
	Soluble NSP	4.0		Soluble NSP	0.5
Barley, flake	Insoluble NSP	10.8	Popcorn	Insoluble NSP	9.3
	Total NSP	14.8		Total NSP	9.8
	Soluble NSP	3.4		Soluble NSP	t
Barley, Peart	Insoluble NSP	6.8	Rice, brown	Insoluble NSP	1.9
	Total NSP	10.2		Total NSP	1.9

Flour, grain and starches		Total g/100 g Fresh Weight	Flour, grain and starches		Total g/100 g Fresh Weight
		1.0		Soluble NSP	2.1
Buckwheat	Insoluble NSP	1.1	Sunflower Seeds	Insoluble NSP	3.0
	Total NSP	2.1		Total NSP	5.1
	Soluble NSP	-		Soluble NSP	0.1
Rice, White, Long Grain	Insoluble NSP	0.4	Tapioca	Insoluble NSP	0.3
	Total NSP	0.4		Total NSP	0.4
	Soluble NSP	-		Soluble NSP	2.1
Rice, White, Round Grain	Insoluble NSP	0.5	Wheat Grain	Insoluble NSP	7.7
	Total NSP	0.5		Total NSP	9.8
Rye Flakes	Soluble NSP	4.0	Wheat Bran, Arjuna	Soluble NSP	3.8
	Insoluble NSP	7.2		Insoluble NSP	32.2
	Total NSP	11.2		Total NSP	36.0
Rye Flour	Soluble NSP	3.9	Wheat Bran, Allinsons	Soluble NSP	2.9
	Insoluble NSP	7.8		Insoluble NSP	34.3
	Total NSP	11.7		Total NSP	37.2
Rye Grain	Soluble NSP	4.0	Bran, Boots' Natural Unprocessed	Soluble NSP	4.6
	Insoluble NSP	7.4		Insoluble NSP	32.7
	Total NSP	11.4		Total NSP	37.3
Sago	Soluble NSP	0.3	Wheat-flakes	Soluble NSP	2.0
	Insoluble NSP	0.4		Insoluble NSP	7.4
	Total NSP	0.7		Total NSP	9.4
Semolina	Soluble NSP	1.0	Wheat-germ	Soluble NSP	3.2
	Insoluble NSP	1.1		Insoluble NSP	12.4
	Total NSP	2.1		Total NSP	15.6
Sesame Seeds	Soluble NSP	1.9			
	Insoluble NSP	5.7			
	Total NSP	7.6			

Bread		Total g/100 g Fresh Weight	Bread		Total g/100 g Fresh Weight
White Bread, Mothers Pride	Soluble NSP	0.9	Rye Bread, Local	Soluble NSP	2.2
	Insoluble NSP	0.7		Insoluble NSP	2.2
	Total NSP	1.6		Total NSP	4.4
White Bread, Slimcea	Soluble NSP	1.1	Rye Bread, Imported	Soluble NSP	3.7
	Insoluble NSP	0.7		Insoluble NSP	3.6
	Total NSP	1.8		Total NSP	7.3
White Bread, Sunblest	Soluble NSP	0.9	Pumpernickel, Imported	Soluble NSP	3.9
	Insoluble NSP	0.6		Insoluble NSP	3.6
	Total NSP	1.5		Total NSP	7.5
Brown Bread, Sunblest	Soluble NSP	1.1	**PASTA**		
	Insoluble NSP	2.5	White Macaroni, raw	Soluble NSP	1.6
	Total NSP	3.6		Insoluble NSP	1.5
Brown Bread, Windmill Country	Soluble NSP	1.2		Total NSP	3.1
	Insoluble NSP	2.1	White Macaroni, cooked	Soluble NSP	0.5
	Total NSP	3.3		Insoluble NSP	0.4
Wholemeal Bread, Allinsons	Soluble NSP	1.2		Total NSP	0.9
	Insoluble NSP	4.0	Whole-wheat Macaroni, raw	Soluble NSP	2.1
	Total NSP	5.2		Insoluble NSP	6.2
Wholemeal Bread, Sainsburys	Soluble NSP	1.6		Total NSP	8.3
	Insoluble NSP	3.2	Whole-wheat Macaroni, cooked	Soluble NSP	0.6
	Total NSP	4.8		Insoluble NSP	2.2
Wholemeal Bread, Windmill	Soluble NSP	1.2		Total NSP	2.8
	Insoluble NSP	3.7	White Spaghetti, raw	Soluble NSP	1.5
	Total NSP	4.9		Insoluble NSP	1.4
Hovis Wheatgerm	Soluble NSP	0.8		Total NSP	2.9
	Insoluble NSP	1.4	White Spaghetti, cooked	Soluble NSP	0.6
	Total NSP	2.2		Insoluble NSP	0.6
Hovis Wheatgerm, Sainsburys	Soluble NSP	1.3		Total NSP	1.2
	Insoluble NSP	1.7	Whole-wheat Spaghetti, raw	Soluble NSP	2.0
	Total NSP	3.0		Insoluble NSP	6.4
Hovis Stoneground	Soluble NSP	0.9		Total NSP	8.4
	Insoluble NSP	3.3	Whole-wheat Spaghetti, cooked	Soluble NSP	0.8

	Total NSP	4.2		Insoluble NSP	2.7
Granary Loaf, Local	Soluble NSP	2.1		Total NSP	3.5
	Insoluble NSP	2.2			
	Total NSP	4.3			

Breakfast cereals		Total g/100 g Fresh Weight	Breakfast cereals		Total g/100 g Fresh Weight
All Bran, Kelloggs	Soluble NSP	4.1	Meusli, Jordans' Special	Soluble NSP	1.7
	Insoluble NSP	20.4		Insoluble NSP	4.5
	Total NSP	24.5		Total NSP	6.2
Bran Flakes, Kelloggs	Soluble NSP	3.0	Meusli, Jordans' Fruit and Nut	Soluble NSP	1.6
	Insoluble NSP	8.3		Insoluble NSP	4.2
	Total NSP	11.3		Total NSP	5.8
Coco Pops, Kelloggs	Soluble NSP	0.2	Original Crunchy, Jordan's Natural	Soluble NSP	2.7
	Insoluble NSP	0.4		Insoluble NSP	4.6
	Total NSP	0.6		Total NSP	7.3
Cornflakes, Kelloggs	Soluble NSP	0.4	Original Crunchy, Jordan's Bran	Soluble NSP	1.5
	Insoluble NSP	0.5		Insoluble NSP	5.6
	Total NSP	0.9		Total NSP	7.1
Kelloggs Country Store	Soluble NSP	1.8	Original Crunchy, Jordan's Honey,	Soluble NSP	1.6
	Insoluble NSP	4.4		Insoluble NSP	5.0
	Total NSP	6.2		Total NSP	6.6
Crunchy Nut Cornflakes, Kelloggs	Soluble NSP	0.3	Original Crunchy, Jordan's Original	Soluble NSP	3.3
	Insoluble NSP	0.5		Insoluble NSP	2.7
	Total NSP	0.8		Total NSP	6.0
Frosties, Kellogg’s	Soluble NSP	0.2	Quaker Oats	Soluble NSP	4.5
	Insoluble NSP	0.3		Insoluble NSP	3.2
	Total NSP	0.5		Total NSP	7.7
Fruit 'n' Fibre, Kelloggs	Soluble NSP	2.6	Porridge Oats, Scotts	Soluble NSP	3.6
	Insoluble NSP	4.4		Insoluble NSP	2.8
	Total NSP	7.0		Total NSP	6.4
Honey Smacks, Kelloggs	Soluble NSP	1.6	Ready Bret, Kelloggs	Soluble NSP	3.1
	Insoluble NSP	3.1		Insoluble NSP	4.1
	Total NSP	4.7		Total NSP	7.2

Breakfast cereals		Total g/100 g Fresh Weight	CRISPBREADS		Total g/100 g Fresh Weight
Rice Krispies, Kelloggs	Soluble NSP	0.1	Allinsons Wholemeal	Soluble NSP	3.1
	Insoluble NSP	0.4		Insoluble NSP	6.0
	Total NSP	0.5		Total NSP	9.1
Ricicles, Kelloggs	Soluble NSP	t	Cracottes, Original Wheat Slice	Soluble NSP	2.6
	Insoluble NSP	0.3		Insoluble NSP	1.2
	Total NSP	0.3		Total NSP	3.8
Shredded Wheat, Nabisco	Soluble NSP	2.0	Cracottes, Brown Wheat	Soluble NSP	3.3
	Insoluble NSP	7.8		Insoluble NSP	5.8
	Total NSP	9.8		Total NSP	9.1
Special K	Soluble NSP	0.8	Hovis Traditional Wheat Slice	Soluble NSP	3.3
	Insoluble NSP	1.2		Insoluble NSP	4.9
	Total NSP	2.0		Total NSP	8.2
Start, Kelloggs	Soluble NSP	1.8	Rye King, Brown	Soluble NSP	5.4
	Insoluble NSP	3.9		Insoluble NSP	6.2
	Total NSP	5.7		Total NSP	11.6
Sugar Puffs, Kelloggs	Soluble NSP	1.6	Ryvita, Original	Soluble NSP	3.9
	Insoluble NSP	1.6		Insoluble NSP	7.8
	Total NSP	3.2		Total NSP	11.7
Sultana Bran, Kelloggs	Soluble NSP	4.3	Ryvita, Brown	Soluble NSP	5.1
	Insoluble NSP	5.7		Insoluble NSP	8.5
	Total NSP	10.0		Total NSP	13.6
Weetabix	Soluble NSP	3.1	Ryvita, High Fibre	Soluble NSP	4.4
	Insoluble NSP	6.6		Insoluble NSP	13.5
	Total NSP	9.7		Total NSP	17.9

BISCUITS		Total g/100 g Fresh Weight
Cheddars, McVities	Soluble NSP	1.3
	Insoluble NSP	0.7
	Total NSP	2.0
Chocolate Homewheat, McVities	Soluble NSP	1.1
	Insoluble NSP	1.1
	Total NSP	2.2
Cream Crackers, Jacobs	Soluble NSP	1.4
	Insoluble NSP	0.8
	Total NSP	2.2
Custard Creams, Peak Frean	Soluble NSP	0.9
	Insoluble NSP	0.5
	Total NSP	1.4
Digestive, McVities	Soluble NSP	1.1
	Insoluble NSP	1.1
	Total NSP	2.2
Farmhouse Wheat Crackers, Jacobs	Soluble NSP	1.9
	Insoluble NSP	1.9
	Total NSP	3.8
Fig Rolls	Soluble NSP	0.8
	Insoluble NSP	2.0
	Total NSP	2.8
Garibaldi	Soluble NSP	1.1
	Insoluble NSP	1.0
	Total NSP	2.1
Ginger Nuts, McVities	Soluble NSP	0.9
	Insoluble NSP	0.5
	Total NSP	1.4
Matzos	Soluble NSP	1.6
	Insoluble NSP	1.4
	Total NSP	3.0
Nice, Peak Frean	Soluble NSP	1.2
	Insoluble NSP	0.9
Oat Cakes	Soluble NSP	4.0
	Insoluble NSP	3.3
	Total NSP	7.3
Penguin biscuits	Soluble NSP	0.6
	Insoluble NSP	0.7
	Total NSP	1.3
Rich Tea, McVities	Soluble NSP	1.1
	Insoluble NSP	0.6
	Total NSP	1.7
Shortcake, Peak Frean	Soluble NSP	0.8
	Insoluble NSP	0.7
	Total NSP	1.5
Wafer Biscuits	Soluble NSP	0.8
	Insoluble NSP	0.8
	Total NSP	1.6
Water Biscuits	Soluble NSP	1.8
	Insoluble NSP	1.3
	Total NSP	3.1
Sauces		
HP Sauce	Soluble NSP	0.3
	Insoluble NSP	0.4
	Total NSP	0.7
Tomato Ketchup	Soluble NSP	0.3
	Insoluble NSP	0.5
	Total NSP	0.8
Tomato Ketchup, Heinz	Soluble NSP	0.5
	Insoluble NSP	0.6
	Total NSP	1.1
Branston Pickle	Soluble NSP	0.5
	Insoluble NSP	0.7
	Total NSP	1.2

	Total NSP	2.1

Dietary fibre content (Non-starch polysaccharides) in cereals (adapted from Englyst et al., 1988).

Vegetables		Total g/100 g Fresh Weight	Vegetables		Total g/100 g Fresh Weight
Asparagus, cooked, bases of stalks	Soluble NSP	0.8	Beans, haricot, dried, raw	Soluble NSP	7.9
	Insoluble NSP	0.9		Insoluble NSP	9.1
	Total NSP	1.7		Total NSP	17.0
Aubergine, raw	Soluble NSP	1.0	Beans, haricot, dried, cooked	Soluble NSP	3.7
	Insoluble NSP	1.0		Insoluble NSP	4.6
	Total NSP	2.0		Total NSP	8.3
Bean sprouts, raw as purchased	Soluble NSP	0.6	Beans, red kidney, dried, raw	Soluble NSP	6.9
	Insoluble NSP	0.9		Insoluble NSP	8.8
	Total NSP	1.5		Total NSP	15.7
Beans, baked, tinned 5 brans in	Soluble NSP	2.1	Beans, red kidney, dried, cooked	Soluble NSP	3.2
	Insoluble NSP	1.4		Insoluble NSP	3.5
	Total NSP	3.5		Total NSP	6.7
Beans, broad, cooked	Soluble NSP	1.2	Beans, mung, dried	Soluble NSP	2.8
	Insoluble NSP	4.2		Insoluble NSP	7.2
	Total NSP	5.4		Total NSP	10.0
Beans, butter, dried, raw	Soluble NSP	6.3	Beans, mung, dried, cooked	Soluble NSP	0.7
	Insoluble NSP	9.7		Insoluble NSP	2.3
	Total NSP	16.0		Total NSP	3.0
Beans, butter, dried, cooked	Soluble NSP	2.9	Beans, runner, cooked	Soluble NSP	0.9
	Insoluble NSP	4.0		Insoluble NSP	1.4
	Total NSP	6.9		Total NSP	2.3
Beans, french, cooked	Soluble NSP	1.3	Beans, soya, dried	Soluble NSP	6.8
	Insoluble NSP	1.8		Insoluble NSP	8.9
	Total NSP	3.1		Total NSP	15.7
Beans, frozen sliced, cooked	Soluble NSP	1.3	Beans, soya bran	Soluble NSP	14.1
	Insoluble NSP	1.8		Insoluble NSP	54.9
	Total NSP	3.1		Total NSP	69.0

Vegetables		Total g/100 g Fresh Weight	Vegetables		Total g/100 g Fresh Weight
Beans, soya bran (gluten free)	Soluble NSP	9.8	Cabbage, Winter Admiral Hawke, cooked	Soluble NSP	0.9
	Insoluble NSP	41.6		Insoluble NSP	1.1
	Total NSP	51.4		Total NSP	2.0
Beans, soya flour	Soluble NSP	5.2	Cabbage, Winter January King, raw	Soluble NSP	1.4
	Insoluble NSP	6.0		Insoluble NSP	1.5
	Total NSP	11.2		Total NSP	2.9
Beetroot, cooked	Soluble NSP	1.2	Cabbage, Winter January King, cooked	Soluble NSP	1.2
	Insoluble NSP	1.3		Insoluble NSP	1.5
	Total NSP	2.5		Total NSP	2.7
Broccoli, sprouting, cooked, boiled 10 min	Soluble NSP	1.5	Cabbage, Winter Savoy raw	Soluble NSP	2.0
	Insoluble NSP	1.5		Insoluble NSP	1.7
	Total NSP	3.0		Total NSP	3.7
Cabbage, summer, early summer, raw	Soluble NSP	1.5	Cabbage, Winter Savoy, cooked	Soluble NSP	1.6
	Insoluble NSP	1.6		Insoluble NSP	1.6
	Total NSP	3.1		Total NSP	3.2
Cabbage, summer, early summer, cooked	Soluble NSP	0.9	Cabbage, Red, raw	Soluble NSP	1.8
	Insoluble NSP	1.1		Insoluble NSP	2.0
	Total NSP	2.0		Total NSP	3.8
Cabbage, Summer Golden Acre, cooked	Soluble NSP	0.8	Cababge, Red, cooked	Soluble NSP	1.5
	Insoluble NSP	0.8		Insoluble NSP	1.8
	Total NSP	1.6		Total NSP	3.3
Cabbage, Summer Greyhound, cooked	Soluble NSP	0.7	Calabrese, cooked	Soluble NSP	1.2
	Insoluble NSP	0.7		Insoluble NSP	1.5
	Total NSP	1.4		Total NSP	2.7
Cabbage, Summer Emerald Cross, cooked	Soluble NSP	0.9	Carrots, raw, Flesh only	Soluble NSP	1.4
	Insoluble NSP	1.1		Insoluble NSP	1.0
	Total NSP	2.0		Total NSP	2.4
Vegetables		**Total g/100 g Fresh Weight**	**Vegetables**		**Total g/100 g Fresh Weight**
Carrots, cooked, Flesh only, sliced,	Soluble NSP	1.4	Cress, raw	Soluble NSP	0.5
	Insoluble NSP	1.1		Insoluble NSP	0.6

	Total NSP	2.5		Total NSP	1.1
Carrots, tinned, Drained contents	Soluble NSP	1.0	Cress, water, raw	Soluble NSP	0.8
	Insoluble NSP	0.9		Insoluble NSP	1.0
	Total NSP	1.9		Total NSP	1.8
Cauliflower, raw	Soluble NSP	0.9	Cucumber, raw,	Soluble NSP	0.2
	Insoluble NSP	0.9		Insoluble NSP	0.3
	Total NSP	1.8		Total NSP	0.5
Cauliflower, cooked	Soluble NSP	0.7	Cucumber, raw,	Soluble NSP	0.2
	Insoluble NSP	0.9		Insoluble NSP	0.3
	Total NSP	1.6		Total NSP	0.5
Celeriac, raw, Flesh only	Soluble NSP	2.4	Endives, raw	Soluble NSP	0.9
	Insoluble NSP	1.3		Insoluble NSP	1.1
	Total NSP	3.7		Total NSP	2.0
Celery, raw, Stems only	Soluble NSP	0.6	Fennel, raw	Soluble NSP	1.1
	Insoluble NSP	0.7		Insoluble NSP	1.3
	Total NSP	1.3		Total NSP	2.4
Chicory, raw	Soluble NSP	0.5	Leeks, cooked	Soluble NSP	1.2
	Insoluble NSP	0.7		Insoluble NSP	1.1
	Total NSP	1.2		Total NSP	2.3
Courgette, raw	Soluble NSP	0.5	Lentils, Red, dried	Soluble NSP	1.3
	Insoluble NSP	0.6		Insoluble NSP	3.6
	Total NSP	1.1		Total NSP	4.9
Courgette, cooked,	Soluble NSP	0.6	Lentils, Red, dried, cooked	Soluble NSP	0.6
	Insoluble NSP	0.6		Insoluble NSP	1.3
	Total NSP	1.2		Total NSP	1.9

Vegetables		**Total g/100 g Fresh Weight**	**Vegetables**		**Total g/100 g Fresh Weight**
Lettuce, round, raw	Soluble NSP	0.6	Parsnip, cooked	Soluble NSP	2.3
	Insoluble NSP	0.6		Insoluble NSP	1.7
	Total NSP	1.2		Total NSP	4.0
Lettuce, Iceberg, raw	Soluble NSP	0.4	Peas, Chick, dried	Soluble NSP	3.3
	Insoluble NSP	0.5		Insoluble NSP	7.4
	Total NSP	0.9		Total NSP	10.7
Marrow, cooked	Soluble NSP	0.3	Peas, Chick, dried, cooked	Soluble NSP	1.6

	Insoluble NSP	0.7		Insoluble NSP	3.2
	Total NSP	1.0		Total NSP	4.8
Mushrooms, raw	Soluble NSP	0.2	Peas, dried	Soluble NSP	3.6
	Insoluble NSP	0.9		Insoluble NSP	9.4
	Total NSP	1.1		Total NSP	13.0
Okra, raw	Soluble NSP	2.9	Peas, dried, cooked	Soluble NSP	1.6
	Insoluble NSP	1.9		Insoluble NSP	3.9
	Total NSP	4.8		Total NSP	5.5
Onion, raw	Soluble NSP	1.1	Peas, fresh, cooked	Soluble NSP	0.8
	Insoluble NSP	0.9		Insoluble NSP	2.1
	Total NSP	2.0		Total NSP	2.9
Onion, cooked	Soluble NSP	1.0	Peas, frozen, cooked	Soluble NSP	1.6
	Insoluble NSP	0.8		Insoluble NSP	3.6
	Total NSP	1.8		Total NSP	5.2
Onion, salad, raw	Soluble NSP	0.9	Peas, Garden, tinned	Soluble NSP	1.1
	Insoluble NSP	0.6		Insoluble NSP	2.9
	Total NSP	1.7		Total NSP	4.0
Parsley, raw	Soluble NSP	2.8	Peas, processed, tinned	Soluble NSP	1.0
	Insoluble NSP	2.2		Insoluble NSP	2.4
	Total NSP	5.0		Total NSP	3.4

Vegetables		Total g/100 g Fresh Weight	Vegetables		Total g/100 g Fresh Weight
Pea hulls, dried, test sample	Soluble NSP	11.8	Radish, raw	Soluble NSP	0.4
	Insoluble NSP	55.8		Insoluble NSP	0.6
	Total NSP	67.6		Total NSP	1.0
Peas, split, dried	Soluble NSP	2.2	Spinach, cooked leaves	Soluble NSP	0.6
	Insoluble NSP	4.1		Insoluble NSP	1.0
	Total NSP	6.3		Total NSP	1.6
Peas, split, dried, cooked	Soluble NSP	1.1	Spring greens, cooked leaves	Soluble NSP	1.8
	Insoluble NSP	1.6		Insoluble NSP	1.8
	Total NSP	2.7		Total NSP	3.6
Pepper, green, raw	Soluble NSP	0.7	Sprouts, brussels, raw	Soluble NSP	3.0
	Insoluble NSP	0.9		Insoluble NSP	2.6
	Total NSP	1.6		Total NSP	5.6

Plantain, cooked	Soluble NSP	0.5	Sprouts, brussels	Soluble NSP	2.5
	Insoluble NSP	0.7		Insoluble NSP	2.3
	Total NSP	1.2		Total NSP	4.8
Potato, new, cooked	Soluble NSP	0.6	Swede, cooked	Soluble NSP	1.6
	Insoluble NSP	0.5		Insoluble NSP	1.8
	Total NSP	1.1		Total NSP	3.4
Potato, old, cooked	Soluble NSP	0.7	Sweetcorn kernels, cooked	Soluble NSP	0.1
	Insoluble NSP	0.5		Insoluble NSP	1.3
	Total NSP	1.2		Total NSP	1.4
Potato, sweet, cooked	Soluble NSP	1.1	Sweetcorn on the cob, cooked	Soluble NSP	0.5
	Insoluble NSP	1.3		Insoluble NSP	2.2
	Total NSP	2.4		Total NSP	2.7
Potato Crisps	Soluble NSP	2.7	Tomato, fresh, raw	Soluble NSP	0.4
	Insoluble NSP	2.2		Insoluble NSP	0.7
	Total NSP	4.9		Total NSP	1.1

Vegetables		**Total g/100 g Fresh Weight**	**Fruits**		**Total g/100 g Fresh Weight**
Tomato, tinned	Soluble NSP	0.4	Apples, Bramley	Soluble NSP	0.6
	Insoluble NSP	0.6		Insoluble NSP	1.0
	Total NSP	1.0		Total NSP	1.6
Turnip, cooked	Soluble NSP	2.2	Apples, Bramley	Soluble NSP	0.7
	Insoluble NSP	3.5		Insoluble NSP	0.9
	Total NSP	5.7		Total NSP	1.6
Yam, raw	Soluble NSP	0.5	Apples, Cox	Soluble NSP	0.9
	Insoluble NSP	0.8		Insoluble NSP	1.1
	Total NSP	1.3		Total NSP	2.0
Yam, cooked	Soluble NSP	0.5	Apples, Cox	Soluble NSP	0.7
	Insoluble NSP	0.6		Insoluble NSP	1.0
	Total NSP	1.1		Total NSP	1.7

Fruits		Total g/100 g Fresh Weight
Apples, Golden Delicious	Soluble NSP	0.7
	Insoluble NSP	1.0
	Total NSP	1.7
Apples, Golden Delicious	Soluble NSP	0.6
	Insoluble NSP	0.9
	Total NSP	1.5
Apples, Granny Smith	Soluble NSP	0.7
	Insoluble NSP	1.0
	Total NSP	1.7
Apples, Granny Smith	Soluble NSP	0.6
	Insoluble NSP	0.9
	Total NSP	1.5
Apples, red	Soluble NSP	0.9
	Insoluble NSP	1.0
	Total NSP	1.9
Apples, red	Soluble NSP	0.6
	Insoluble NSP	1.0
	Total NSP	1.6
Apricots, fresh	Soluble NSP	1.3
	Insoluble NSP	1.0
	Total NSP	2.3
Apricots, tinned	Soluble NSP	0.6
	Insoluble NSP	0.7
	Total NSP	1.3
Apricots, dried	Soluble NSP	4.4
	Insoluble NSP	2.9
	Total NSP	7.3

Fruits		Total g/100 g Fresh Weight
Avocado pears, raw	Soluble NSP	1.8
	Insoluble NSP	2.6
	Total NSP	4.4
Bananas, raw	Soluble NSP	0.7
	Insoluble NSP	0.4
	Total NSP	1.1
Blackberries, raw	Soluble NSP	1.0
	Insoluble NSP	2.1
	Total NSP	3.1
Cherries (Black), raw	Soluble NSP	0.7
	Insoluble NSP	0.5
	Total NSP	1.2
Clementines, raw	Soluble NSP	0.8
	Insoluble NSP	0.5
	Total NSP	1.3
Dates, dried	Soluble NSP	1.2
	Insoluble NSP	2.8
	Total NSP	4.0
Figs, raw	Soluble NSP	1.7
	Insoluble NSP	1.1
	Total NSP	2.8
Figs, dried,	Soluble NSP	4.0
	Insoluble NSP	3.5
	Total NSP	7.5
Fruit pie filling, tinned, Whole can	Soluble NSP	0.5
	Insoluble NSP	0.5
	Total NSP	1.0
Fruit cocktail, tinned	Soluble NSP	0.6
	Insoluble NSP	0.9
	Total NSP	1.5

Cranberries, raw	Soluble NSP	1.1	Gooseberries, raw	Soluble NSP	0.9
	Insoluble NSP	1.9		Insoluble NSP	1.5
	Total NSP	3.0		Total NSP	2.4
Currants (red), raw	Soluble NSP	0.7	Grapefruit, raw	Soluble NSP	0.9
	Insoluble NSP	2.7		Insoluble NSP	0.4
	Total NSP	3.4		Total NSP	1.3
Currants (black), raw	Soluble NSP	1.6	Grapefruit, tinned	Soluble NSP	0.7
	Insoluble NSP	2.0		Insoluble NSP	0.4
	Total NSP	3.6		Total NSP	1.1
Currants (dried grapes), raw	Soluble NSP	1.0	Grapes (white), raw	Soluble NSP	0.4
	Insoluble NSP	0.9		Insoluble NSP	0.3
	Total NSP	1.9		Total NSP	0.7

Fruits		Total g/100 g Fresh Weight	Fruits		Total g/100 g Fresh Weight
Grapes (black), raw	Soluble NSP	0.3	Mango, tinned	Soluble NSP	0.6
	Insoluble NSP	0.3		Insoluble NSP	0.5
	Total NSP	0.6		Total NSP	1.1
Greengages, raw	Soluble NSP	1.3	Melon, Canteloupe, raw	Soluble NSP	0.2
	Insoluble NSP	0.8		Insoluble NSP	0.4
	Total NSP	2.1		Total NSP	0.6
Guava, tinned	Soluble NSP	0.9	Melon, Gallia, raw	Soluble NSP	0.2
	Insoluble NSP	3.9		Insoluble NSP	0.3
	Total NSP	4.8		Total NSP	0.5
Kiwi fruit, raw	Soluble NSP	0.8	Melon, Honeydew, raw	Soluble NSP	0.2
	Insoluble NSP	0.9		Insoluble NSP	0.3
	Total NSP	1.7		Total NSP	0.5
Lemon juice, fresh	Soluble NSP	t	Melon, Water, raw	Soluble NSP	0.2
	Insoluble NSP	0.1		Insoluble NSP	0.1
	Total NSP	0.1		Total NSP	0.3
Loganberries, tinned	Soluble NSP	0.5	Nectarines, raw	Soluble NSP	0.6
	Insoluble NSP	2.4		Insoluble NSP	0.6
	Total NSP	2.9		Total NSP	1.2
Lychees, tinned	Soluble NSP	0.7	Olives, tinned	Soluble NSP	0.3
	Insoluble NSP	0.3		Insoluble NSP	2.6

	Total NSP	1.0		Total NSP	2.9
Mandarins, tinned	Soluble NSP	0.2	Oranges, raw	Soluble NSP	1.4
	Insoluble NSP	0.2		Insoluble NSP	0.7
	Total NSP	0.4		Total NSP	2.1
Mango, raw	Soluble NSP	1.6	Orange juice	Soluble NSP	0.1
	Insoluble NSP	1.0		Insoluble NSP	t
	Total NSP	2.6		Total NSP	0.1

Fruits		Total g/100 g Fresh Weight	Fruits		Total g/100 g Fresh Weight
Passion fruit, raw	Soluble NSP	0.5	Pears, tinned	Soluble NSP	0.5
	Insoluble NSP	0.1		Insoluble NSP	1.3
	Total NSP	0.6		Total NSP	1.8
Paw paw, tinned	Soluble NSP	0.5	Pineapples, raw	Soluble NSP	0.1
	Insoluble NSP	0.7		Insoluble NSP	1.1
	Total NSP	1.2		Total NSP	1.2
Peaches, raw	Soluble NSP	0.8	Pineapples, tinned	Soluble NSP	0.1
	Insoluble NSP	0.7		Insoluble NSP	1.0
	Total NSP	1.5		Total NSP	1.1
Peaches, tinned	Soluble NSP	0.5	Plums, Sicoval	Soluble NSP	1.0
	Insoluble NSP	0.5		Insoluble NSP	0.6
	Total NSP	1.0		Total NSP	1.6
Peaces, dried	Soluble NSP	3.8	Plums, Victoria	Soluble NSP	1.2
	Insoluble NSP	3.5		Insoluble NSP	0.6
	Total NSP	7.3		Total NSP	1.8
Pears, Comice,	Soluble NSP	0.6	Pomengranate, raw	Soluble NSP	0.7
	Insoluble NSP	1.4		Insoluble NSP	2.7
	Total NSP	2.0		Total NSP	3.4
Pears, Comice,	Soluble NSP	0.6	Prunes, dried	Soluble NSP	4.7
	Insoluble NSP	0.9		Insoluble NSP	2.2
	Total NSP	1.5		Total NSP	6.9
Pears, Conference	Soluble NSP	0.8	Prunes, cooked, stewed (no sugar), no juice	Soluble NSP	1.7
	Insoluble NSP	1.6		Insoluble NSP	0.9
	Total NSP	2.4		Total NSP	2.6
Pears, Conference	Soluble NSP	0.6	Pumpkin, raw	Soluble NSP	0.4

Insoluble NSP	1.2	Insoluble NSP	0.6
Total NSP	1.8	Total NSP	1.0

Fruits		Total g/100 g Fresh Weight
Raisins, dried	Soluble NSP	1.1
	Insoluble NSP	1.0
	Total NSP	2.1
Raspberries, raw	Soluble NSP	0.7
	Insoluble NSP	1.8
	Total NSP	2.5
Raspberries, tinned	Soluble NSP	0.5
	Insoluble NSP	2.3
	Total NSP	2.8
Rhubarb, raw	Soluble NSP	0.6
	Insoluble NSP	1.0
	Total NSP	1.6
Satsumas, raw	Soluble NSP	1.1
	Insoluble NSP	0.5
	Total NSP	1.6
Strawberries, raw	Soluble NSP	0.6
	Insoluble NSP	0.8
	Total NSP	1.4
Strawberries, tinned	Soluble NSP	0.5
	Insoluble NSP	0.8

Sultanas, dried	Total NSP	1.3
	Soluble NSP	1.0
	Insoluble NSP	1.2
	Total NSP	2.2

Dietary fibre (soluble, insoluble and total DF g/100 g as eaten) contents of 70 high consumption foods (adapted from Li et al., 2002).

Baked products	**Soluble DF**	**Insoluble DF**	**Total DF**
Bagel, plain, frozen	1.17	1.29	2.46
Bread, white, reduced calorie, soft	0.01	8.46	9.47
Bread, white, reduced calorie, firm	1.03	8.64	9.67
Bread, rye, w/ caraway seeds	1.09	1.98	3.07
Bread, rye, seedless	1.62	2.84	4.46
Bread, wheat, soft	1.26	2.13	3.38
Bread, wheat, firm	1.56	4.63	6.19
Bread, white, soft	1.02	0.53	1.54
Bread, white, firm	1.30	1.36	2.66
Bread, whole wheat, soft	1.26	4.76	6.01
Bread, whole wheat, firm	1.51	5.21	6.71
Hamburger/hotdog rolls	0.56	1.44	1.99
Tortilla, corn	1.11	4.39	5.50
Tortilla, flour (wheat)	1.51	0.85	2.37
Cereal grains and pasta	**Soluble DF**	**Insoluble DF**	**Total DF**
Corn meal, yellow, degermed	0.62	3.32	3.94
Cornstarch, wholesale	1.00	0.08	1.08
Brown rice, long grain, cooked	0.44	2.89	3.33
Flour, all purpose, bleached	1.54	1.50	3.04
Grits, quick, cooked	0.12	1.14	1.26

Grits, instant, cooked	0.07	1.48	1.55
Oatmeal, instant, cooked	1.45	1.14	2.58
Oatmeal, regular, cooked	0.42	1.23	1.65
Spaghetti, cooked	0.54	1.33	2.06
White rice, long grain, cooked	-	0.34	0.34
Fruits	**Soluble DF**	**Insoluble DF**	**Total DF**
Apple (Red delicious), raw, ripe w/ skin	0.67	1.54	2.21
Avocado (California, Haas), raw, ripe	2.03	3.51	5.53
Avocado (Florida, Fuertes), raw, ripe	1.25	5.48	6.72
Bananas, raw, ripe	0.58	1.21	1.79
Grapefruit, raw, white, ripe	0.58	0.32	0.89
Grapes (Thompson seedless), raw, ripe	0.24	0.36	0.60
Guava, raw, ripe	1.54	11.81	12.72
Mango, raw, ripe	0.69	1.08	1.76
Nectarine, raw, ripe, w/ skin	0.98	1.06	2.04
Oranges (Navel), raw, ripe	1.37	0.99	2.35
Orange juice, retail, from concentrate	0.28	0.03	0.31
Peaches, raw, ripe, w/ skin	1.31	1.54	2.85
Peaches, raw, ripe, w/o skin	0.84	1.16	2.00
Pears, raw, ripe, w/ skin	0.92	2.25	3.16
Pineapple (smooth Cayenne), raw, ripe	0.04	1.42	1.46
Plum, raw, ripe, w/ skin	1.12	1.76	2.87
Prunes, pitted	4.50	3.63	8.13
Raisins, seedless	0.90	2.17	3.07
Watermelon, raw, ripe	0.13	0.27	0.40
Legumes	**Soluble DF**	**Insoluble DF**	**Total DF**
Beans, canned, w/ pork and tomato sauce	1.38	4.02	5.40
Chick peas, canned, drained	0.41	5.79	6.19

Cowpeas, canned, drained	0.43	4.11	4.53
Lentils, dry, cooked, drained	0.44	5.42	5.86
Pinto beans, canned, drained	0.99	5.66	6.65
Red kidney beans, can, drained	1.36	5.77	7.13
Split peas, dry, cooked, drained	0.09	10.56	10.65
Vegetables, cooked	**Soluble DF**	**Insoluble DF**	**Total DF**
Beans, green, fresh, microwaved	1.38	2.93	4.31
Broccoli, fresh microwaved	1.85	2.81	4.66
Carrots, fresh, microwaved	1.58	2.29	3.87
Corn, yellow, from cob, grocery store	0.13	4.12	4.25
Corn, yellow, from cob, farm market	0.25	2.63	2.87
Lima beans, immature, froz., microwaved	1.02	4.21	5.23
Peas, green, froz., microwaved	0.94	2.61	3.54
Potato, french fries, fast food	0.67	3.44	4.11
Potato, white, baked, w/ skin	0.61	1.70	2.31
Potato, white, boiled, w/o skin	0.99	1.06	2.05
Vegetables, raw	**Soluble DF**	**Insoluble DF**	**Total DF**
Broccoli, raw	0.44	3.06	3.50
Cabbage, green, raw	0.46	1.79	2.24
Carrots, raw	0.49	2.39	2.88
Cauliflower, raw	0.47	2.15	2.62
Cucumber, raw, with peel	0.20	0.94	1.14
Lettuce, iceberg, raw	0.10	0.88	0.98
Onion, mature, raw	0.71	1.22	1.93
Pepper, sweet, green, raw	0.53	0.99	1.52
Tomatoes, red, ripe, raw	0.15	1.19	1.34
Spinach, raw	0.77	2.43	3.20

Fructooligosaccharide composition of fruits (adapted from Campbell et al., 1997).

Fruits	TOTAL (mg/g as is)
apple, Red Delicious	0.1
apple, Golden Delicious	0.0
apple, Granny Smith	0.1
apple, Jonagold	0.1
apple, Rome	0.0
banana	1.4
banana, green	0.7
banana, red	0.5
banana, ripe	2.0
blackberry	0.2
blueberry	0.0
cantaloupe	0.0
gooseberry	0.1
grapes, black	0.2
grapes, Thompson	0.0
muskmelon	0.1
orange, navel	0.3
peach	0.4
pear, bosc	0.1
pear, d'Anjou	0.2
plantain	0.4
plum, red	0.2
raspberry, red	0.2
rhubarb	0.0
strawberry	tr
watermelon	0.2

Fructooligosaccharide Composition of vegetables (adapted from Campbell et al., 1997).

Vegetables	TOTAL (mg/g as is)	INGREDIENT	TOTAL (mg/g as is)
acorn squash	0.4	**grains**	
artichoke, globe	2.4	barley	1.7

asparagus	0.0	corn	0.0
bean, green	0.0	hominy	tr
bean, kidney	0.1	milo	0.0
beet, red	0.0	oats	0.3
carrot, Bunny Luv	0.3	rice, brown	0.0
carrot, Dole	0.2	rice, white	0.0
celery	0.0	rye	3.8
chicory root, raw	3.9	soybean	tr
chicory root, roasted	4.2	wheat	1.3
Chinese chive	0.0	**forages**	
daikon	0.0	alfalfa	0.0
eggplant	0.0	Brome-grass	tr
endive	0.0	clover hay	0.4
garlic	3.9	oat straw	0.7
garlic powder	1.6	Orchard-grass	0.2
ginger root	0.0	timothy hay	1.0
Jerusalem artichoke	58.4	wheat straw	0.0
kiwi	0.0	**other**	
leek	0.9	alfalfa meal	2.1
lettuce	0.5	beet pulp	0.1
onion, red	1.4	brewer's rice	0.0
onion, Welch	1.1	canola meal	0.0
onion, white	3.1	corn distiller’s solution	tr
onion, yellow	2.6	corn gluten feed	0.1
onion powder	45.0	corn gluten meal	0.3
peas	0.1	oat groats	0.1
peas, snap	1.1	peanut hulls	2.2
peas, snow	0.6	rice bran	0.1
potato, Idaho	0.0	rice hulls	0.0
potato, sweet	0.2	seaweed	0.0
radish, red	0.1	soybean hulls	0.1
shallot	8.5	soybean meal	0.0
taro root	0.0	wheat bran	3.5
tomato	0.0	wheat germ	4.2
tomato, cherry	0.0	wheat middling’s	4.6
tomato, Roma	0.0		
yam	0.2		

zucchini	0.0

Fructooligosaccharide Composition of fruits, vegetables and cereals (adapted from (Hogarth et al., 2000).

PRODUCT A. Fruit Products	Total	PRODUCT C. Sugars, Grains, and Flours	Total
apples	<0.02	barley flour	0.43
apple juice	0.05	barley, quick cook	0.19
apple juice	0.04	brown sugar, dark	0.36
apple juice	<0.02	brown sugar, dark	0.12
applesauce	<0.02	honey	0.06
applesauce, stage 1	<0.02	honey	<0.02
bananas, stage 1	0.13	molasses, blackstrap	0.43
bananas, stage 1	0.04	oatmeal	<0.02
bananas, stage 1	0.04	oat bran	<0.02
banana chips	<0.02	oat bran cereal	<0.02
grapes, seedless	<0.02	rice cereal	
grapes, seedless, liquid	<0.02	rye flour	0.96
Concord grape juice, can	<0.02	rye flour, dark	1.17
Concord grape juice, jar	<0.02	wheat bran	0.53
Concord grape juice, juice-makers	<0.02	wheat flour	0.15
white grape juice	<0.02	***D. Miscellaneous Products***	
tomato juice	<0.02	formula, infant	1.78
tomato paste	0.25	Garlic Plus FOS, tablets	25.70
tomato puree	<0.02	Garliphants	0.88
tomato sauce	<0.02	Sportalyte	<0.02
		Sweetener, Japanese	99.90

PRODUCT Vegetable Products	Total
artichoke hearts	0.13
artichoke hearts, liquid	15.10
artichoke hearts	0.13

artichoke hearts, liquid	14.90
artichoke hearts, marinade	7.00
artichoke hearts	<0.02
asparagus spears	<0.02
asparagus spears, liquid	<0.02
asparagus spears	<0.02
asparagus spears, liquid	<0.02
garlic powder	0.22
onions, liquid	6.13
onions, whole	0.04
onions, dry, minced	4.40
onions, dry, minced	3.94
pumpkin	<0.02
squash, stage 1	<0.02
squash, stage 3	<0.02
sweet potatoes	0.03
sweet potatoes, syrup	1.70
sweet potatoes, stage 1	<0.02
yams	<0.02
yams, candied, syrup	0.59
yams candied	<0.02

Inulin and oligofructose content of foods (adapted from Moshfegh et al., 1999 and Van Loo et al., 1995)

Food item	Inulin		Oligofructose	
	g/100g			
	Range	Midpoint	Range	Midpoint
Banana				
Raw	0.3-0.7	0.5	0.3-0.7	0.5
Raw-dried	0.9-2.0	1.4	0.9-2.0	1.4
Canned	0.1-0.3	0.2	0.1-0.3	0.2
Asparagus				
Raw	2.0-3.0	2.5	2.0-3.0	2.5
Boiled	1.4-2.0	1.7	1.4-2.0	1.7
Chicory root	35.7-47.6	41.6	19.6-26.2	22.9
Dandelion greens				

	Raw	12.0-15.0	13.5	9.6-12.0	10.8
	Cooked	8.1-10.1	9.1	6.5-8.1	7.3
Garlic					
	Raw	9.0-16.0	12.5	3.6-6.4	5.0
	Dried	20.3-36.1	28.2	8.1-14.5	11.3
Globe artichoke		2.0-6.8	4.4	0.2-0.7	0.4
Jerusalem artichoke		16.0-20.0	18.0	12.0-15.0	13.5
Leeks					
	Raw	3.0-10.0	6.5	2.4-8.0	5.2
Onions					
	Raw	1.1-7.5	4.3	1.1-7.5	4.3
	Raw-dried	4.7-31.9	18.3	4.7-31.9	18.3
	Cooked	0.8-5.3	3.0	0.8-5.3	3.0
Wheat					
	Bran-raw	1.0-4.0	2.5	1.0-4.0	2.5
	Flour-baked	1.0-3.8	2.4	1.0-3.8	2.4
	Flour-boiled	0.2-.06	0.4	0.2-.06	0.4
Barley					
	Raw	0.5-1.0	0.8	0.5-1.0	0.8
	Cooked	0.1-0.2	0.2	0.1-0.2	0.2
Rye					
	Baked	0.5-0.9	0.7	0.5-0.9	0.7