DIETARY FIBRE AND THE EFFECT ON DIGESTIVE FUNCTION, ENERGY INTAKE AND MAJOR CONSEQUENCES FOR HUMAN NUTRITION.

KOSTFIBERE OG DERES INNVIRKNING PÅ FORDØYELSE, ENERGIINNTAK OG BETYDELIGE KONSEKVENSER FOR HUMAN ERNÆRING.

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“We live not upon what we eat, but upon what we digest”

- Wilbur Olin Atwater

Acknowledgements

"Let food be thy medicine and medicine be thy food" – Hippocrates.

This quote by the father of medicine in addition to the slogan "you are what you eat" is what initially got me interested in food science and nutrition. The desire for more knowledge of how our body is affected by the nutrients we consume lead me to Ås. After five years of late study sessions filled with laughter, frustration and joy, the journey must now come to an end. When this I said, I would like to thank my supervisor Birger Svihus, for excellent guidance and help along the way! You inspire and activate the thinking of most people I would like to think! Dzung Bao Diep and Kari Olsen for lending me the laboratory. Özgün Candan for kind words, guidance and help in stressful times. Marianne Haug Lunde for encouraging phone calls and good help with interpretation of data. A sincere thanks to mamma for proofreading and correcting my spelling in addition to being my ever-supporting friend. A sincere and warm thank you to Shani, one of my supportive rocks during this year!!! I can always count on and ask for your advice! Pappa and Magne for help and support during difficult times. Finally, a warm thanks to my dearest girlfriends, and last but not least Bjarne! Thank you so much for standing to listen to my indefinite talk about diet and health!

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Abstract

Knowledge of dietary fibres´ chemical composition and impact on human health has increased significantly over the past decades, and fibres are today acknowledged for being composed of all plant polysaccharides other than starch. Non-starch polysaccharides (NSP) consist of a complex diversity of chemical structures, which possess a great variety of physiochemical effects. This thesis comprises an extensive literature review of the nutritional outcomes of increased dietary fibre intake. The hypocholesterolaemic effect of soluble dietary fibre has been demonstrated by several studies. Furthermore, the anti-nutritional properties of fibres of different solubility have proved to exhibit multiple health effects, including effectively decreasing the nutritional value of diets. Therefore, the development of energy systems and energy conversion factors of the macronutrients were reviewed in addition to the energy humans are capable of obtaining from anaerobic fermentation of dietary fibre. The results of the reviewed studies supported the assumption that the energy conversion factor of 8kJ/g dietary fibre tends to overestimate the calorific value of NSP-rich diets.

In addition to the literature review, the fermentability and digestibility of neutral detergent fibre from pea hull fibre was analysed based on data collected from the work conducted by Ragnhild Tokvam Aas. The results indicated pea hull fibre to be of lower fermentability and digestibility than fibre from wholegrain wheat, which indicates a lower energy value. Furthermore, the data from Marianne Haug Lunde's study were analysed for the effects consumption of pea hull fibre enriched bread elicits on blood parameters. The results indicated no significant differences. Other studies conducted with pea hull fibre have resulted in such effects. The study design was thus unfit to determine the effects of replacing habitual bread with pea hull fibre enriched bread.

Sammendrag.

Kunnskapen om kostfibers kjemiske sammensetning og effekt på menneskers helse har økt betydelig i løpet av de senere tiårene og det er i dag veletablert viten at kostfiber består av alle plantebaserte polysakkarider annet enn stivelse. Ikke-stivelse polysakkarider (NSP) består av et kompleks mangfold av kjemiske strukturer som resulterer i et stort utvalg av fysio-kjemiske effekter. Denne avhandlingen består av en omfattende litteraturgjennomgang av de ernæringsmessige effektene av økt kostfiberinntak. Den hypokolesterolemiske effekten av løselig fiber har blitt demonstrert av mange studier. I tillegg har de antinutritive egenskapene til fiber av forskjellig løselighet vist atskillige helseeffekter, hvorav nedsatt energiverdi av mat er en av de mest vesentlige. Derfor ble utviklingen av energisystemer samt energiomregningsfaktorene til makronæringsstoffene drøftet i tillegg til energien mennesker er i stand til å innhente fra anaerob fermentering av kostfiber. Resultatene av de drøftede studiene støtter påstanden at energiomregningsfaktoren på 8kJ/g kostfiber har en tendens til å overestimere energiverdien av NSP-rike dietter.

I tillegg til litteraturgjennomgangen ble fermenteringskapasiteten, samt fordøyeligheten av ”neutral detergent fibre” (NDF) fra erteskallfiber analysert ved å bearbeide prøvemateriale fra avhandlingen til Ragnhild Tokvam Aas. Resultatet indikerte at erteskallfiber hadde lavere fermenterings- og fordøyelseskapasitet enn fiber fra fullkornhvete, som igjen indikerer en lavere energiverdi. Videre ble den effekten brød beriket med erteskallfiber uttrykker på blodparametere studert ved bearbeiding av dataene fra Marianne Haug Lundes studie. Resultatene indikerte ingen signifikante forskjeller. Andre studier utført med erteskallfiber har resultert i signifikante effekter. Forsøksdesignet av dette studiet var dermed uegnet til å avgjøre effekten av å erstatte vanlig brød med erteskallfiber beriket brød.

Abbreviation list.

AACC – The American Association of Cereal Chemists.

ADF – Acid detergent fibre.

AOAC – Association of Official Analytical Chemists.

ATP – Adenosine triphosphate.

BMI – Body mass index.

CCK – Cholecystokinin.

CHD – Coronary heart disease.

CMC - Carboxymethyl cellulose.

DE – Digestible energy.

EFSA – The European Food Safety Authority.

EU – The European Union.

FAO – Food and Agriculture Organisation.

GC – Gas chromatography.

GE – Gross energy value.

GI – Glycaemic index.

GL – Glycaemic load.

GLP-1 – Glucagon-like peptide 1.

HDL – (high density lipoprotein) cholesterol.

HPLC – High-performance liquid chromatography.

IR – Infrared.

LDL – (low density lipoprotein) cholesterol.

ME – Metabolisable energy.

MEOS – Microsomal ethanol oxidizing system.

NADH – Nicotine adenine dinucleotide.

NDF – Neutral detergent fibre.

NME – Net metabolisable energy.

NPN – Non-protein nitrogen.

NR-NCD – Nutrient-related non-communicable diseases.

NSP – Non-starch polysaccharides.

PPG – Postprandial blood glucose.

PPM – Parts per million.

PYY – peptide YY.

SCFA – Short chain fatty acids.

T2DM – Type 2 Diabetes Mellitus.

UMB – The Norwegian University of Life Sciences.

USDA – The United States Department of Agriculture.

UV – Ultraviolet.

WHO – World Health Organisation.

Contents.

Tables and figures list.

1. Introduction

Diet is an important factor with regards to health and disease and the slogan *"You are what you eat"* has more truth to it than people might think. The global prevalence of obesity, type two diabetes and coronary heart disease is increasing in industrialized as well as in developing countries. Ironically, the global quantity of people suffering from hunger equals the quantity suffering from obesity, and annually more people die of overeating than starvation (WHO, 2000). The availability and increased consumption of processed food has made life style diseases a severe burden to the international health budget. Globally, overweight and obesity is increasing and if dietary trends do not change there is an estimated 33% rise in obesity in the American population by the year 2030. Approximately 20% of the Norwegian population suffers from obesity, which is equal to 1 in 5 subjects (Helsedirektoratet, 2011b). This trend towards rising occurrences of lifestyle diseases and poor health of increasingly younger populations is thought to be one of the greater health challenges of the 21th century (Nolan et al., 2011, Finkelstein et al., 2012).

Determining the nutritional value of food is thus an important factor in the prevention and management of life style diseases. The main reason for development of life style diseases is imbalance between energy intake and expenditure, when the intake exceeds the expenditure people gain weight. Calorie dense diets along with inactive life styles has led to an epidemic of obesity, in addition to a drastic increase in the prevalence of type two diabetes. Furthermore coronary heart disease is ranked as the leading cause of death worldwide and is highly associated with diet and physical activity.
One way that has proven effective in lowering the nutritional value of food is addition of dietary fibre. Dietary fibres are not digested by humans and may therefore decrease the calorific value of food in addition to exhibiting multiple health benefits as regards cardiovascular parameters (Baer et al., 1997). Blunting of postprandial blood glucose, in addition to decreasing the insulin response are some of the positive effects that may be achieved by including dietary fibre in ones diet (Anderson et al., 2004). Multiple clinical trials have demonstrated the cholesterol lowering effect of soluble dietary fibre, which additionally possesses anti-nutritional properties that may help weight regulation (Theuwissen and Mensink, 2008, Kristensen and Jensen, 2011).

Since diet is regarded as a modifiable factor, which may influence health, the purpose of this thesis is thus to describe the physiochemical properties of different fibre sources and their effect on human health. In order to understand the importance of food energy, the development of energy systems and conversion factors will be discussed, followed by dietary fibres effect on energy availability and thus the accuracy of the conversion factor of 8kJ/g dietary fibre.

In addition to the extensive literature analysis, experiments have been conducted with respect to pea hull fibres effect when consumed by healthy, human subjects. In relation to the central role of bread as a carbohydrate source in the Norwegian diet, the effect of adding pea hull fibre was studied in two crossover studies. This part of the thesis has its origin in the work conducted by Ragnhild Tokvam Aas and Marianne Haug Lunde. The specific effects elicited by pea hull fibre, collected from yellow peas (*Pisum sativum* L.), will be reviewed with regard to colonic fermentation, NDF-digestibility and effect on blood parameters in healthy, human subjects.

2. Dietary fibre.

2.1 The chemical structures of non-starch polysaccharides and the development of the chemical definition.

Dietary fibre is composed of all plant polysaccharides other than starch, and may be described as non-starch polysaccharides (NSP) with lignin and resistant starch being the major exceptions to the rule (Kumar et al., 2012). Additionally to NSP, the term "complex carbohydrates" is often used to describe dietary fibre. Polysaccharides of NSPs appearing most commonly include cellulose, pectin, β-glucans, heteroxylans and xyloglucans, which are linked by glycosidic bonds through β-linkages to form complex structures (Kumar et al., 2012). Dietary fibres escape digestion in the small intestine of humans and other monogastric species, resisting hydrolysis of our endogenous enzymes, which are capable of hydrolysing α - (1→4) glycosidic bonds (Whitcomb and Lowe, 2007). However, NSPs may undergo further degradation by the colonic microflora through anaerobic fermentation. Unlike us, the colonic microflora possess digestive enzymes, which may further degrade structural fibre and result in short chain fatty acids and other end products (Roberfroid, 1993).

Components classified as dietary fibre include polysaccharides, oligosaccharides, lignin and other associated plant tissues such as starch that escapes digestion (resistant starch) (Whistler and BeMiller, 1997). The monosaccharides (sugar units) that these molecules consist of make up the backbone and provide different characteristics to each fibre source. Figure 2.1.1 and 2.1.2 lists the different D-aldoses and D–ketoses, with glucose, mannose, galactose and fructose being the most common monosaccharaides in biological systems.

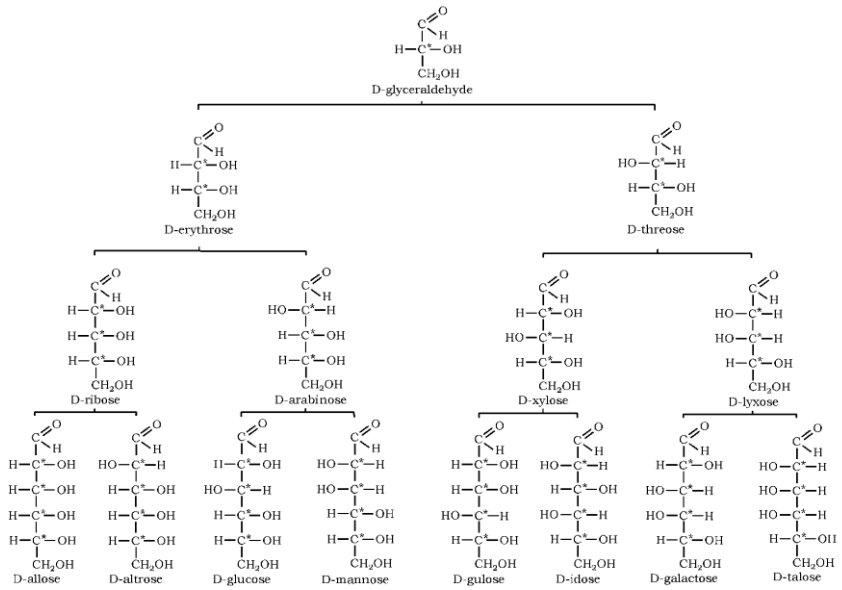

Figure 2.1.1 The stereo chemical relationship (Fisher Projections) among the D-aldoses with three to six carbon atoms (McDonald et al., 2002).

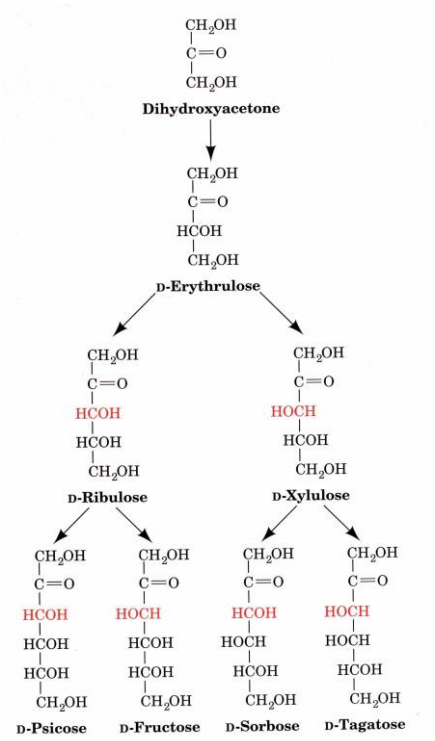

Figure 2.1.2 The stereo chemical relationship (Fisher Projections) among the D-ketoses with three to six carbon atoms (McDonald et al., 2002).

The monosaccharides are linked together to form oligosaccharides (*oligo*- Greek meaning few), which contain two to ten sugar units, while polysaccharides contain more than twenty monosaccharide units. Polysaccharides, also known as glycans, are polymers of monosaccharide units classified into two groups: Homoglycans, which contain one type of monosaccharide units, and heteroglycans, which consist of mixtures of monosaccharaides and derived products (McDonald et al., 2002). Homoglycans include among others starch, glycogen, cellulose, inulin and glucosamine, while heteroglycans include pectin substances, arabinoxylans, exudate gums and many more (McDonald et al., 2002).
Dietary fibre may be divided into soluble and insoluble fibre, which is determined by its water binding capacity. Interactions with water or other solvents occur due to structural, physical and chemical properties of the NSPs chains and side chains. Soluble dietary fibre binds readily to water through polar and hydrophobic interactions and hydrogen bonding to form a fibre-solvent complex, which delays gastric emptying (Chaplin, 2003). As its name implies, insoluble dietary fibre does not bind to water and pass through the gastrointestinal tract relatively intact, mainly contributing to decreased intestinal transit time.

Cellulose is one of the most studied insoluble dietary fibre fractions, and is the abundant component in plant cell walls. Hence, high concentrations are found in the outer layer of grains, generally labelled bran. Cellulose is a linear, insoluble homoglycan consisting of repeated (1→4)- β-D-glucopyranose units with hydrogen bonds linking the chains (Smidsrød and Moe, 1995). Cellulose (Figure 2.1.3) mainly provides strength and structure to plant cell walls, through formation of crystal-like fibres, thereby chemically being considered a highly inert molecule (Sonia and Dasan, 2013). The β-linkage between the glucose units makes it indigestible to humans resulting in the main physiological function being increased faecal bulk and bowl movements (Whistler and BeMiller, 1997, Smidsrød and Moe, 1995).

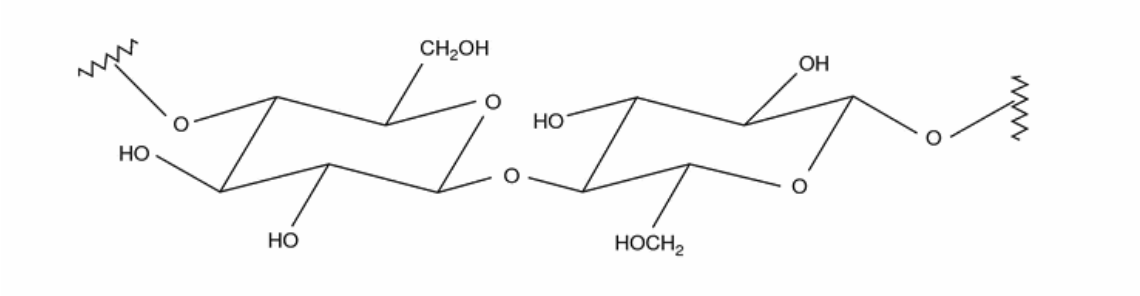

Figure 2.1.3 Fragment of a cellulose chain. Illustrating the β-linkage between the glucose monomers (O'SULLIVAN, 1997).

Cellulose from different plants is all the same at the molecular level. However, they differ in crystallinity as cellulose contains both crystalline (ordered) and amorphous (less ordered) regions (Park et al., 2010). The latter is easily degraded by cellulases (degradable enzymes), while crystalline cellulose is more inert (Park et al., 2010). Each monomer bears three hydroxyl groups, and it is evident that the ability of these groups to form inter-sheet hydrogen bonds between the cellulose chains, will highly impact the crystallization of the structure (Siqueira et al., 2010). Native cellulose is the most abundant structure found in nature and is composed of two allomorphs denominated I_α and I_β (Festucci-Buselli et al., 2007). The hydrogen bonds in the two crystalline allomorphs differ in bond length (Figure 2.1.4). I_β is more stable than I_α due to longer hydrogen bonds, the degree of crystallinity in cellulose is thus determined by I_β/ I_α ratio (Nishiyama et al., 2003).

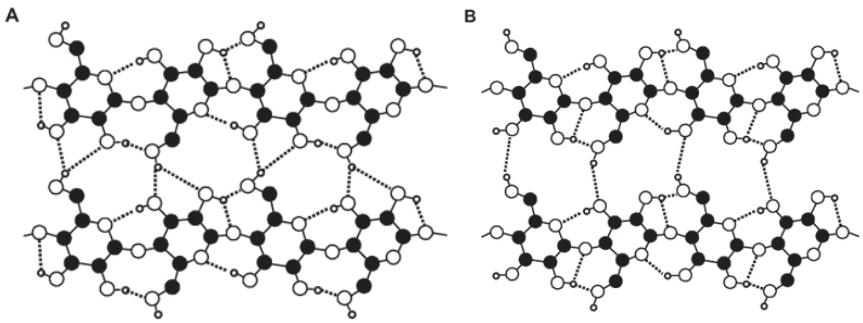

Figure 2.1.4 Cellulose Iα (A) and cellulose Iβ (B). Hydrogen bonds between the chains illustrated by dash lines. Carbon (•), oxygen (O) and hydrogen (o) atoms (Nishiyama et al., 2003).

These are not the only crystalline structures of cellulose, additional structures can be produced by alkali treatment and is a large field of research with regards to modified celluloses (Park et al., 2010). The ratio of amorphous regions of cellulose may be increased by modification through addition of functional groups such as carboxymethyl, hydroxyethyl, methyl etc. hereby gaining solubility and in some cases charge (Smidsrød and Moe, 1995). Modified celluloses are, due to their physiochemical features, widely used as additives in the food industry as well as in cosmetics, pharmaceuticals, detergents, etc. (Togrul and Arslan, 2003). These polysaccharides possess an irregular structure since they are chemically produced and originally do not occur in nature, hence the degree of substitution may vary (Smidsrød and Moe, 1995). Carboxymethyl cellulose (CMC), illustrated in Figure 2.1.5, possess polyelectrolyte properties providing charge, thus making it one of the most important water-soluble cellulose derivatives (Togrul and Arslan, 2003).

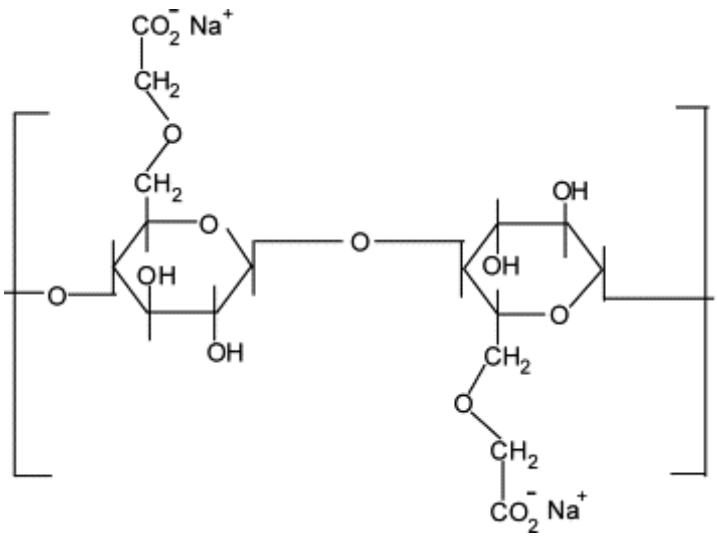

Figure 2.1.5 Chemical structure of carboxymethyl cellulose (CMC) (Biswal and Singh, 2004).

Lignin is the universal term of a large group of complex, heterogeneous, aromatic polymers, that provide rigidity to plant cell walls, protecting them from microbial degradation and physical damage (Vanholme et al., 2010). It may be described as a highly branched network of phenylpropane units which mainly function as lignin-polysaccharide complexes (Bach

Knudsen, 2001). Since lignin is not a polysaccharide it is considered an exception to the rule when defining dietary fibre. Due to the polysaccharide-complex formation, lignin precipitates during fibre analysis and is hence included in the definition (Smidsrød and Moe, 1995, Bach Knudsen, 2001).

Another fibre fraction found in both soluble and insoluble dietary fibre, may be described as hemicellulose. Existing as either a linear molecule with few side chains or as a highly branched molecule, abundant with side chains (Whistler and BeMiller, 1997). Polysaccharides included in this classification are among others xyloglucans, xylans, mannans, glucomannans, arabinoxylans and β-glucans (Scheller and Ulvskov, 2010). Nutritional sources rich in hemicelluloses include bran from corn, wheat, oats, barley and rice, as well as most fruit and vegetable skins. Hemicellulose, cellulose and lignin make up the amorphous matrix in trees and higher plants, where it provides essential mechanical properties with lignin functioning as the connective tissue between cellulose and hemicellulose (Smidsrød and Moe, 1995). However, hemicellulose has no strict chemical definition and is difficult to use in the systematic evaluation of fibre fractions. It is considered as a collective term for polysaccharides that initially are defined according to their role in plant cell walls. Therefore, the term soluble or insoluble dietary fibre will be used henceforth in this thesis.

With consideration to highly soluble NSPs, pectin, arabinoxylans and β-glucans are some of the most well documented components. Pectin exists as a linear molecule consisting of galacturonic acid, linked by α(1→4) bonds, which are substituted by α (1→2) rhamnopyranose units involving neutral sugars as side chains (Lattimer and Haub, 2010). Citrus fruits are rich in pectin and when consumed, forms a viscous gel that may provide multiple health benefits. Arabinoxylans on the other hand, are branched polysaccharides which consist of a linear β - (1 → 4) linked xylan backbone to which side residues of α-arabinofuranose units are attached via α - (1 → 3) and/or α - (1 → 2) linkages (Izydorczyk and Biliaderis, 1995). The degree of solubility depends on the quantity of arabinose side chains. Lower ratios of side chains causes the arabinoxylan molecules to bind less water and thus become more insoluble (Sternemalm et al., 2008). Arabinoxylans are the major non-cellulosic polysaccharides of cereal grains and comprise an important part of the non-starch components (Fincher and Stone, 1986). However, arabinoxylns are lower in solubility than β–

glucans, which are linked by β- (1→3, 1→4)- D-glucopyranosyl units and are often described as mixed-link β-glucans (Whistler and BeMiller, 1997). Cellulose is also composed of β - (1→4) linkages, and is a highly stiff, insoluble molecule. As with other β-linked polysaccharides, β-glucans are indigestible to humans, but due to the (1→3) linkages in β-glucans, the molecular linearity is disturbed and the molecule becomes flexible and soluble (Kumar et al., 2012).

Although current scientific communities acknowledge NSPs as dietary fibre, the definition has not always been so clear and still, one single definition remains to be determined. With this in mind, the history of the acknowledgement of dietary fibre as a part of dietary carbohydrate, in addition to the chemical definition of dietary fibre will be reviewed.
As of today, the definition of dietary fibre stated by the American Association of Cereal Chemists goes as follows: "*Dietary fibre is the edible parts of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibre exhibits one or more of either laxation, (faecal bulking and softening, increased frequency, and/or regulatory), blood cholesterol attenuation, and/or glucose attenuation"* (Kamp, 2004).

The term dietary fibre, first introduced by Hipsley in 1953, was initially used to describe plant cell walls (Bach Knudsen, 2001, Hipsley, 1953). In the 1960s attention was drawn to South Africa, where observations on diet and colonic health showed that incidents of constipation, irritable colon, haemorrhoids etc. was rarely if ever present in the population (Trowell, 1961). These observations made scientist curious whether there was a correlation between diet and western lifestyle diseases. Around 1970 the dietary fibre hypothesis was established and is based on the theory that indigestible, fibrous residues of plant foods may play an important role in human nutrition (Almy, 1981). The hypothesis proposes that increased dietary fibre consumption, may have a protective effect against lifestyle diseases such as diabetes, cancer, heart disease, and obesity (Slavin, 2005). Alongside with the dietary fibre hypothesis, it was acknowledged that inclusion of dietary fibre when calculating the calorific value of food was imperative, but the chemical definition was found to be challenging.

The first attempt to define dietary fibre from foods was based on differences in solubility and divided fibre into three categories; Crude fibre, which is the residue of plants after extraction with acid and alkali, and includes variable quantities of insoluble NSPs (Trowell, 1972a). Neutral detergent fibre (NDF), which comprises insoluble NSPs and lignin while acid detergent fibre (ADF) refers to the insoluble part of plants that majorly is comprised of cellulose and lignin (Kumar et al., 2012).

These chemical procedures did not give a very good indication of the fibre content in food, e.g. wholegrain wheat flour was ascribed a dietary fibre content of 11% and an estimated crude fibre content of 2% (Trowell, 1976). The term a*vailable carbohydrate* was defined in 1970, describing sugars and free polysaccharides that is readily accessible for digestion (Southgate and Durnin, 1970). *Unavailable carbohydrate* was used to describe polysaccharides not hydrolysable by humans, and included pectic substances, hemicelluloses, cellulose and inulin (Southgate, 1973). Bailey proposed to divide NSPs into three groups; cellulose, non-cellulosic polymers and pectic polysaccharides (Bailey, 1973). Non-cellulosic polymers included mixed-linked β-glucans, heteroxylans, mannans and xyloglucan, while pectic polysaccharides included polygalacturonic acids substituted with arabinan, galactan and arabinogalactan (Bailey, 1973). In 1972 the term dietary fibre was adopted, and included the residues derived from plant cell walls that are resistant to hydrolysis by human alimentary enzymes (Trowell, 1972a, Trowell, 1972b). The term; *dietary fibre complex*, was proposed in 1976 and included cellulose, hemicellulose and lignin in addition to all chemical compounds naturally associated with and concentrated around these structural polymers (Trowell, 1976). The refined definition was widely accepted, but was still solely based on the physiological properties of the fibre sources and the need for a physiochemical definition was requested. During the following years, scientists attempted to develop quantitative methods for defining dietary fibre (Van Soest and McQueen, 1973, Furda et al., 1979, Asp et al., 1983, Theander and Åman, 1979, Schweizer and Würsch, 1979).

The primary tools were commercially available enzymes, the degree of success was thus variable. During the late 1970s, with the purpose of improved nutritional labelling Prosky began seeking a scientific definition to quantify dietary fibre in foods (Prosky and Harland, 1979). A general consensus was achieved by 1981, through gathering the opinion of hundreds of scientists worldwide. It was concluded that the methodological research of Asp, Furda and

Schweizer was regarded to be the best approach (Prosky, 1981). Prosky and co-workers then led a multinational cooperative study, with 43 laboratories in 29 countries participating, aiming to develop a method for analysing total dietary fibre in foods. Modifying the initial enzymatic-gravimetric method made the study successful (Lee and Prosky, 1995). The method was adopted by the Association of Official Analytical Chemists (AOAC) as the first Official Method of Analysis of total dietary fibre (AOAC Official Method 985.29) (DeVries et al., 1999). The American Association of Cereal Chemists (AACC) also acknowledged the method and it became the de facto operating definition of dietary fibre.

As the need for distinction between soluble and insoluble dietary fibre emerged, AOAC 985.29 was modified so that the fraction of soluble and insoluble dietary fibre could be quantified (DeVries et al., 1999). The method of separation is somewhat arbitrary, based on the solubility of the dietary fibre fraction in a pH controlled enzyme solution, which attempts to mimic the human alimentary enzymes. However, it is far more dilute in laboratories than in vivo (DeVries et al., 1999). The method only implies solubility, whereas the degree of insolubility was still questioned. Once again the method was modified and in 1991 the Official Method 991.42, Insoluble Dietary Fibre in Food and Food Products, was adopted by AOAC and AACC (DeVries et al., 1999).

During the following years methods were developed and validated for relevance on defining dietary fibre. An international survey held in 1992, aimed to reaffirm the consensus on the physiological definition of dietary fibre. The participants agreed that the definition by Trowell from 1976 should be acknowledged, in addition to the proposition to include non-digestible oligosaccharides (Lee and Prosky, 1995). A second international survey took place in 1993, again to reaffirm the physiological definition of dietary fibre and inclusive components. The inclusion of non-digestible oligosaccharides and resistant starch was favoured by the participants (Cho et al., 1999). At the international workshop on Definition of Complex Carbohydrates and Dietary Fibre held in 1995 by AOAC, there was general agreement on the physiological definition of fibre in addition to the inclusion of non-digestible oligosaccharides in the definition (DeVries et al., 1999). An AACC expert scientific review committee was appointed in 1998 to revise and, if necessary update the definition of dietary fibre (DeVries et al., 1999). The updated version still underlined the restriction to digestion in the human small intestine as the main feature and acknowledged partial or total fermentation as part of dietary

fibre metabolism. Moreover, inclusion of oligosaccharides and the physiological effects of dietary fibre was emphasized (AACC, 2001). In 2008 the European Commission (EC) and the CODEX Alimentarius Commission (UN) agreed on a general definition on dietary fibre, and in 2009 Codex Alimetarius adopted the following definition (Alimentarius, 2009, Menezes et al., 2013);

"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 physical, enzymatic 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.*

It is still debated whether oligosaccharides (3–9 degrees of polymerisation) are to be included in the definition of dietary fibre. It is acknowledged in many countries however, until one uniform agreement is made, there will be two definitions (Howlett et al., 2010). There are currently six different definitions of dietary fibre, with the sole difference being the inclusion of oligosaccharides or not (EC, 2008, AACC, 2001, FSANZ, 2011, Canada, 2012, IOM, 2005, Howlett et al., 2010). In Norway we have to comply the definition stated by the European Union. It comprises the same paragraphs as those stated by Codex Alimentarius in 2009, with the exception that carbohydrate polymers with three or more monomeric units are to be included as dietary fibre (EC, 2008).

2.2 Viscosity of dietary fibre.

As the chemical features and definitions of dietary fibre are now established, the viscosity may be discussed. In addition to solubility, viscosity is of major influence to dietary fibres physiochemical properties. Viscosity (η), as illustrated by equation 2.2, may be described as a fluids resistance to flow, that is, the resistance to applied force (Whistler and BeMiller, 1997). A liquid flowing slowly indicates high viscosity e.g. honey has higher viscosity than water. Shear stress describes the applied force and represents pouring, mixing, chewing, swallowing etc. How fast the liquid flows is expressed by shear rate, and shear stress divided by shear rate equals the apparent viscosity (Whistler and BeMiller, 1997).

EQUATION 2.2 Viscosity (Bourne, 2002).

$$\eta = \frac{Shear\ stress}{Shear\ rate}$$

Physiochemical interactions between polysaccharides and solvents, cause enclosure/binding of liquid to the polysaccharide structure, and hence result in thickening of the mixture (Guillon and Champ, 2000). The viscosity of dietary fibres is thus closely related to water solubility, which in turn is largely influenced by the degree of lignification. Generally, the more lignified dietary fibre is, the more insoluble and non-viscous it becomes (Vanholme et al., 2010). Wheat bran, which is highly lignified, is considered to be one of the most insoluble, non-viscous fibre fractions consumed by humans.

NSPs defined as viscous dietary fibre include guar gum, pectin, arabinoxylans and β-glucans (Dikeman and Fahey Jr, 2006). β-glucans are highly water soluble, resulting in increased viscosity which may have a positive effect on digestion, colonic function and prolonged intestinal transit time (Smidsrød and Moe, 1995). Water-soluble NSPs are generally considered to be highly viscous and may delay gastric emptying and transit time. They may also act as anti-nutritive components due to enclosure of water and nutrients, hence reducing the digestion and absorption through the intestinal wall (Kumar et al., 2012).

The physiological effects typical for water-soluble, viscous dietary fibres, is their modification of the glycaemic response in both normal and diabetic human subjects in addition to reducing serum cholesterol (Whistler and BeMiller, 1997). This is considered highly advantageous with regards to prevention and management of diabetes and coronary heart disease. Increased intake of dietary fibre rich nutrients provides both viscous and non-viscous dietary fibre. The quantity differs within the different fibres and the desired effect may be affected based on this knowledge. The following section describes the physiochemical effects of consuming dietary fibre of different solubility and viscosity, and how these may affect human health.

3. The physiochemical effects of dietary fibre intake.

Now that the basic concepts of dietary fibres chemical and physiochemical features are established, the impact of increased dietary fibre intake will be discussed. The following text covers dietary fibres effect on the intestinal transit time, influence on satiety and the anti-nutritional effect exhibited by dietary fibre. Finally, anaerobic fermentation of dietary fibre and the resulting short chain fatty acids will be discussed, in addition to the positive effects dietary fibre exhibits with respect to lifestyle diseases.

3.1 Bulking effect, intestinal transit time and satiety.

Fibre is of great interest because of its positive impact to human health, especially since coronary heart disease is the leading cause of death world wide, and life style diseases such as obesity and type two diabetes is increasing at exponential rate (WHO, 2012). Dietary fibre bulking agents are important nutritional components that provide normal function to the gastrointestinal tract. Cellulose, lignin and pectin are components included in this category (Whistler and BeMiller, 1997). Cellulose and highly lignified fibre, are effective laxatives and consequently increase faecal bulk, shorten the intestinal transit time and alleviate constipation, making them interesting in body weight regulation (Kumar et al., 2012). Wheat bran is considered to be one of the richest sources of dietary fibre in human nutrition. With a total fibre content of 36,5 – 52,4 g/100g, of which 35,0 – 48,4g/100g is insoluble dietary fibre and 1,5 – 4,0 is soluble fibre (Stevenson et al., 2012). Wheat bran has been acknowledged for its laxative properties since the time of Hippocrates (460 – 377 BC.) who, based on his writings, were aware of its effectiveness in prevention of constipation (Johnson and Southgate, 1994). Wheat bran has proven to be so effective in faecal bulking that it is used as the reference group against other nutrients bulking effect (Stevenson et al., 2012). The European Food Safety Authority (EFSA) has recently approved health claims for wheat brans´ positive impact on increased faecal bulk and reduced intestinal transit time (EFSA, 2010).

The retention time in the digestive system is additionally influenced by dietary fibres water-holding capacity. Both soluble and insoluble NSPs possess high water-holding capacities, of

which the latter tend to hold less water and primarily promote faecal bulking and shorten intestinal transit time (Knudsen and Hansen, 1991). Moreover the particle size influences the substrate–enzyme contact and impact fibres binding capacity. Small particles have a greater contact surface, and may thus impact intestinal absorption by affecting water holding capacities of fibre (Potty, 1996). The effect of different particle sizes was studied in healthy, young women given coarse or fine whole meal rye bread. The faecal wet weight was higher for the coarse bread diet, however the particle size had no effect on digestibility of macronutrients, NSP or the calorific value of dietary fibre (Wisker et al., 1996). A proposed explanation for the greater effect of the coarse particles is ryes content of fibre. Whole meal rye contains both soluble and insoluble dietary fibre, of which insoluble constitutes the largest proportion, hence promoting faecal excretion. Dietary fibre from whole grain products such as barley, wheat, rye and brown rice have exhibited greater faecal energy losses than when compared to the equal amount of fibre provided from fruits and vegetables (Livesey, 1990, Livesey, 1991). Other studies supports these findings and it is thought that cereal fibre has a greater faecal bulking effect due to the three-dimensional structure of the cell wall (Wisker and Feldheim, 1990, Wisker et al., 1988). Dietary sources that comprise insoluble dietary fibre include wheat bran, whole grains and coarse vegetables e.g. broccoli, cabbage and onion, with inulin, cellulose and lignin as the main components (Kritchevsky and Bonfield, 1995).

Soluble NSPs, especially mixed-linked β-glucans, have gained prominence for decreasing blood (serum) cholesterol (Whistler and BeMiller, 1997). Additionally, β-glucans have proven effective in lowering the postprandial blood glucose and insulin response in both normal and diabetic human subjects (Chawla and Patil, 2010, Whistler and BeMiller, 1997). Major nutritional sources rich in β-glucans include oats, barley and rye (Chawla and Patil, 2010). Additional nutritional sources rich in soluble dietary fibre include amongst others legumes, vegetables and citrus fruits. Besides improving cardiovascular parameters, soluble NSP have been hypothesised to help manage diarrhoea. This was studied in hospitalized children suffering of persistent diarrhoea. One week of oral administration of 250 g/L of cooked green banana or 4g/kg of pectin in a rice based diet proved to reduce diarrhoea (Rabbani et al., 2001). Soluble NSPs may thus be useful in the prevention and management of diarrhoea when administered at small doses, however more research is needed in this field for sufficient evidence.

Concerning the different physiological effects, the total energy intake as well as the dietary fibre source is of great impact. Figure 3.1 summarizes the effect of fibre of different solubility on the human colon.

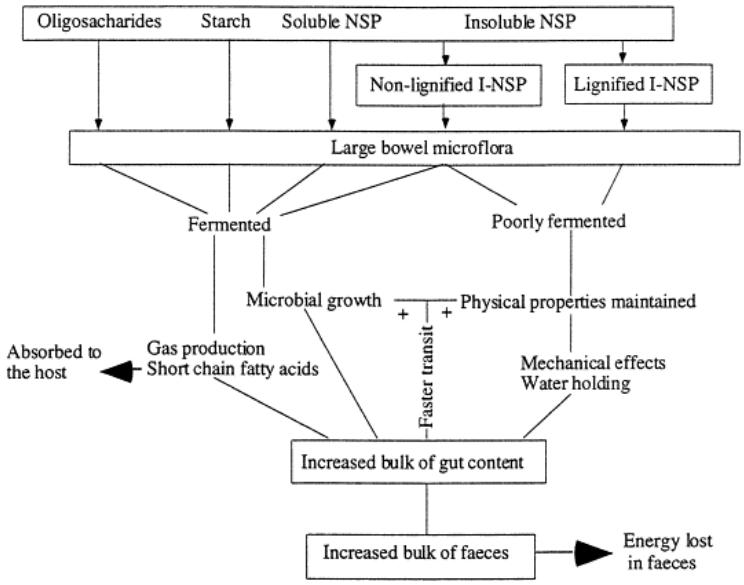

Figure 3.1 Effect of dietary fibre and undigested carbohydrates on increased bulk and transit time. The mechanisms by which dietary fibre may increase colonic and faecal weight and bulk is illustrated (Bach Knudsen, 2001).

Soluble dietary fibres are generally linked to delayed gastric emptying and in some cases associated with prolonged satiety. Modified nutrient absorption and prolonged intestinal transit time caused by soluble dietary fibre may affect the release of satiety peptides, which in turn may affect gastric emptying and signalling to the central nervous system. When exposed to nutrients, the intestinal mucosa induces release of appetite regulating peptides (hormones), namely cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) (Kristensen and Jensen, 2011). CCK, GLP-1 and PYY mainly induce satiety, GLP-1 additionally stimulate insulin secretion, while ghrelin is the only hormone, which has been found to induce hunger (Woods, 2004). Adipose tissues all over the body secrete leptin in direct proportion to the amount of body fat, inducing hunger if body fat levels fall low (Woods, 2004). Both leptin and insulin are classified as adiposity signals, thus decreasing simultaneously as body weight (Niswender and Schwartz, 2003). Consequently, insulin

secretion and leptin production are significantly influenced by the macronutrient composition of the diet, which in turn is of major impact to hunger and appetite.

These hormones are often referred to as gut-brain peptides as they are continuously secreted depending on what we eat and what state our body is in. With regard to weight control, many studies have aimed to prove the positive effect associated with dietary fibre and satiety (Burley et al., 1993, Astrup et al., 1990). One hypothesis is that soluble dietary fibre fermented in the human colon induce satiety through the release of GLP-1 (Delzenne and Cani, 2005) and PYY (Wanders et al., 2011). This was demonstrated in seven healthy, overweight and obese human subjects consuming a calorie restricted diet, providing 4 g of a highly viscous, fermentable dietary fibre/day for 16 weeks. Increased fasting plasma concentrations of PYY and GLP-1 were reported, in addition to prolonged satiety and weight loss (Greenway et al., 2007). Vitaglione et al., compared the effect of β-glucan-enriched bread to control bread in a randomized, short-term study. Healthy volunteers were allocated to an isocaloric breakfast including either 3% β-glucan-enriched bread or a control bread. The results indicated plasma ghrelin to be 23% lower and PYY 16% higher following consumption of β-glucan-enriched bread. Prolonged satiety was reported in addition to blunted glucose response (Vitaglione et al., 2009).

Enclosure of liquid due to β-glucans viscosity may result in prolonged satiety and hence increase the volume contents of the digestive system. As aforementioned, oats and barley are rich in β-glucans, and multiple studies have outlined the satiating effect compared to other whole grain products (Granfeldt et al., 1994, Schroeder et al., 2009, Lyly et al., 2009). It is assumed that viscosity is one of the main factors responsible for the positive impact on satiety and meal frequency (Zijlstra et al., 2007, Marciani et al., 2001). Marciani et al. (2001) compared the effect of viscosity on gastric emptying and satiety to that of the presence of nutrients. Twelve healthy subjects ingested high or low-viscous locust bean gum beverages either containing nutrients or a non-nutrient control. Satiety increased more as a function of increased viscosity than did addition of nutrients to the beverage. The same results were observed when adding 5g of pectin to orange juice (Tiwary et al., 1997).

The majority of scientific evidence suggests that highly viscous dietary fibre prolongs satiety. Pectin, guar gum and other highly viscous fibres may thus be useful with regard to weight

management. However, non-viscous, insoluble fibres have also proven to be effective. Inclusion of 33 g insoluble cereal fibre reduced the appetite, lowered food intake, and the glycaemic response in healthy men (Samra and Anderson, 2007). In general, dietary fibre provides texture to food, hence satiety may be induced through cephalic- and gastric-phase responses related to increased chewing resistance and retention time in the stomach (Burton-Freeman, 2000). Digestion of coarse products naturally takes longer, hence providing an extended sensation of fullness. Studies comparing coarse and fine bread products generally indicate that the coarser ones induce satiety to a larger extent than products with finely ground flour. This was demonstrated in a randomised study where healthy people were allocated to consume wholegrain and fine bread. They were to consume bread until comfortably full, approximately 83 percent of the subjects consumed more fine than wholemeal bread (Grimes and Gordon, 1978).

The confounding factors have to be acknowledged in addition to the experimental evidence that dietary fibre may cause positive effects with regard to satiety and weight regulation. Diets rich in whole-grain foods generally reflect an overall healthier lifestyle, and people who include dietary fibre in their diet will naturally lower their intake of simple carbohydrates. Even though dietary fibres contribute to the total calorific content, they are more resistant to digestion and absorption than other nutrients. Studies confirm a strong correlation between dietary fibre intake and weight loss (Tucker and Thomas, 2009, Slavin, 2005). Continuous research has been conducted to prove the positive effect dietary fibre may exhibit on weight control (Stevenson et al., 2012, Wanders et al., 2011, Brownlee, 2011). Nevertheless, even though many provide reliable results, no health claims have been accepted by EFSA (EFSA, 2010).

3.2 Dietary fibres anti-nutritional effect and influence on mineral bioavailability.

In general it has been suggested that the presence of any dietary fibre in the upper gastro intestinal tract will result in decreased absorption of nutrients, and thus reduce the nutritional value of diets (Brownlee, 2011). This is considered as a positive effect in humans with regard to life style diseases, particularly with respect to obesity. As will be described in the following text, the anti-nutritional properties of dietary fibre are to a greater extent positive, than negative with regards to human health.

Insoluble dietary fibre may exhibit anti-nutritional effects through increased faecal bulk and excretion. This was found in the study of Cummings et al. (1976), who assigned healthy human subjects to increase the intake of wheat fibre from 17 to 45 g/day for three weeks. As anticipated due to wheat fibres high content of cellulose, increased faecal weight, fat, nitrogen, and calcium excretion was observed. Increased consumption of insoluble dietary fibre may in this manner promote weight loss, which is particularly favourably with respect to obesity. Soluble dietary fibre on the other hand, may exhibit anti-nutritive effects through increased viscosity (Svihus et al., 2005), which may impair digestion of nutrients by reduced contact with the digestive enzymes (Dewettinck et al., 2008). Water soluble, viscous dietary fibres commonly reduce absorption of nutrients to a larger extent than low viscous fibres. Blunted postprandial glucose and insulin levels along with increased faecal cholesterol excretion were observed in rats fed water-soluble alginates. The gelling of alginate takes place in the stomach. Consequently the impaired glucose response and increased faecal cholesterol excretion may be due to inhibited glucose and cholesterol absorption from the small intestine (Kimura et al., 1996). The anti-nutritional effect of alginates, and other highly soluble fibres, may thus be of positive impact to people who suffer from diabetes and elevated cholesterol levels.

Loss of body fat was observed in rats fed guar gum and Solka-Floc® cellulose over a period of 28 days, guar gum was estimated to contribute 10,1 kJ/g and Solka-Floc® cellulose 1,5 kJ/g. The lost body fat was incorporated into the energy calculations, which resulted in negative calorific values of -7.1 kJ/g and -4.8 kJ/g (Davies et al., 1987). Body fat deposition may thus decrease due to dietary fibres contribution to a negative calorific value, which is highly favourable in relation to weight loss.

Inclusion of soluble NSP reduce the rate of gastric emptying, which may delay the intestinal absorption of glucose, lipids, protein and minerals (Bach Knudsen, 2001). Different levels of CMC did not appear to considerably affect apparent nitrogen digestibility (Larsen et al., 1994), while a significant decrease was observed in growing pigs when pectin was given at 75g/kg feed (Mosenthin et al., 1994). Soluble NSP have therefore received a great deal of attention with regards to animal feed. β-glucans and arabinoxylans are highly viscous and acknowledged for their anti-nutritional effects, hence degrading enzymes are added to animal feed to prevent poor digestion (Bedford, 1995). The anti-nutritional effect of soluble, viscous dietary fibre is acknowledged in animal nutrition. Humans on the other hand, are the only living creatures who drinks and eats without being thirsty or hungry. Hence, the anti-nutritional effect of soluble fibre may be of positive impact with respect to life style diseases.

Apart from increased viscosity, modification of gut functions may be an anti-nutritive effect elicited by soluble NSP. Gut modification may hinder the endogenous secretion of water, proteins, electrolytes and lipids (Montagne et al., 2003). Several authors have reported NSPs considerable effect on gut anatomy and development, and demonstrated prolonged consumption of soluble NSP to be associated with decreased nutrient digestibility (McDonald, 2001, Iji et al., 2001, Leenhouwers et al., 2006). A proposed explanation for the decreased digestibility as a function of increased viscosity is related to decreased villus length. The human small intestine contains intestinal villi and crypts, as illustrated by Figure 3.2

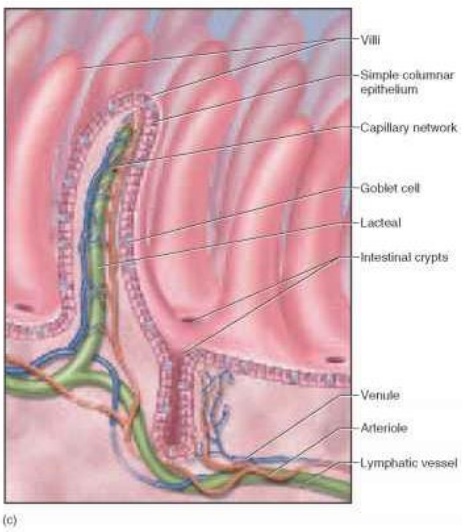

Figure 3.2 Illustrates the human small intestinal wall with villi and crypts (Saladin, 2012).

Villi and the intestinal crypts increase the surface area of the small intestine by approximately 200 times, which result in a total surface area of about 300m². Villi are responsible for absorption of nutrients and water, are only present in the small intestine and thus absent in the colon. The crypts are present throughout the intestine and are sites of cell proliferation and mucus secretion.
A proposed anti-nutritional effect of soluble NSPs is decreased villus length and increased crypt depth, which consequently reduce the contact surface and absorption capacity of the intestine (Sinha et al., 2011, Hopwood et al., 2002). Animal feeding studies with pigs have indicated that a diet high in soluble fibre will diminish villus length and increase crypt depth and in this manner impair nutrient absorption (Jin et al., 1994, Hedemann et al., 2006). The experimental diets of the aforementioned studies contained very high amounts of dietary fibre (73 – 145 g/kg/dry matter), which would affect the digestibility and intestinal function of any animal species. Further research is thus needed in this field for sufficient evidence for this hypothesis to be regarded as a substantial anti-nutritional effect of soluble dietary fibre with regards to human health. If anything, the effects of increased dietary fibre consumption by humans in especially industrialized countries, is regarded as positive.

Dietary fibre has also been found to interact with minerals and in this sense decrease mineral absorption (Sinha et al., 2011). However, in addition of binding to iron, calcium and zinc, dietary fibre possess significant binding capabilities for toxic heavy metals such as lead and cadmium, which may be present in the diet (Kroyer et al., 1995, Coudray et al., 2003). The physiochemical composition of dietary fibre, ion-exchange properties and susceptibility to the intestinal microflora impacts the bioavailability of minerals (Harmuth-Hoene and Schelenz, 1980). Bioavailability is a term used to describe the quantity of minerals absorbed and utilized by the body, compared to the total amount consumed (Fairweather-Tait, 1996). Dietary fibres effect on faecal bulk, shortened intestinal transit time and the formation of mineral-fibre complexes are some of the modes of action that is thought to impair mineral absorption (Laszlo, 1989).

The effect of a high fibre diet concerning mineral excretion and absorption was studied in human subjects given a moderate (24 g total of which 8 g soluble fibre) and a high fibre (50 g total of which 25 g soluble fibre) diet. The results indicated little difference between the two test groups. The diet providing a moderate intake of dietary fibre did not appear to pose a risk

of mineral deficiency, when consuming the high-fibre diet however, it was concluded that it is advisable to ensure an adequate mineral intake (Shah et al., 2009). A study conducted with healthy human subjects, indicated that a diet consisting of a moderate to extremely high quantities of dietary fibre poses no risk of nutrient deficiencies when concerning western populations (Rattan et al., 1981). Concerning developing countries, zinc and iron deficiency are considered as major health problems and increased dietary fibre intake may pose a risk factor for earlier onset of deficiencies (Black, 2003, Shaw and Friedman, 2011).

Both intrinsic and extrinsic factors will affect the bioavailability of minerals. Intrinsic factors include age, sex, pregnancy, health and mineral status, while extrinsic factors comprise environmental factors, nutritional status and total diet composition (Kritchevsky and Bonfield, 1995). Many sources of dietary fibre have been studied for their effect on mineral utilization, mostly in animal models. At the current time the general notion is that a moderate dietary fibre intake, does not pose a problem or interfere with mineral nutrition (Wang et al., 1994, Shah et al., 2009). "A diet high in dietary fibre may reduce mineral and nutrient density but not mineral bioavailability" (Gordon et al., 1995). When concerning susceptible groups such as children, adolescents, pregnant or elderly people, a conclusion that dietary fibre does not affect mineral absorption and metabolism cannot be stated, as these groups of the population require different dietary concerns (Gordon et al., 1995). The dietary guidelines and recommendations for daily intake of macro- and micronutrients have been made in consideration to healthy individuals, while specified guidelines for children, pregnant and lactating women and elderly people are made in addition (Helsedirektoratet, 2005). Even though the different dietary fibre sources may alter mineral density, the advice concerning increasing ones dietary fibre intake should be taken into consideration. The benefits of increased dietary fibre consumption outweigh the possibly decreased mineral and nutrient density. Despite this knowledge, the effects of the individual fibre sources are significantly different. For this reason, the general notion that increased fibre consumption may influence mineral and nutrient density is retained.

Increased consumption of NSPs generally increases the faecal loss of both organic (fat, protein, carbohydrate) and inorganic (vitamins and minerals) compounds. NSP lowers in this manner the nutritional value of the diet and is attributed as the anti-nutritional effect of dietary fibre (Johnson and Southgate, 1994). The effects dietary fibre exhibit on digestion, relate to

the chemical composition and physiochemical properties. Solubility, viscosity and water holding capacity of dietary fibre are of major influence, and it is evident that the effect must be expressed for each fibre source in order to fully evaluate fibres effect on digestive processes. The reduced digestibility may additionally be caused by intrinsic factors present in dietary fibre. Particularly legumes may contain high levels of these and will be discussed in larger detail in section six. Nevertheless, with regards to mineral utilization and the anti-nutritional effects, increased dietary fibre intake is not considered a risk for normal, healthy people. The benefits of increasing the intake of nutrients rich in dietary fibre outweigh the possible anti-nutritive effects, and it is considered positive rather than negative with regard to human health.

3.3 Microflora and metabolic end results of anaerobic fermentation.

The indigenous microflora (normal microflora) within all healthy humans consists of approximately 10^{14} microbial cells inhabiting all parts of the body. Sites such as the respiratory tract, the genitals and the intestine are more densely populated than e.g. the skin (Tannock, 1994). Due to the acidic environment in the stomach, the microbial population of the human intestine increase towards the colon. The human colon is approximately 1,5 meters long, 6,5 cm in diameters and consist of four regions: the cecum, colon, rectum and anal canal. The main function of the colon is to form and store faeces in addition to reabsorption of water and electrolytes. It also comprises the colonic microflora, which includes approximately 800 bacterial species, with the largest population occupying the cecum and ascending colon. The colonic microflora is predominantly anaerobic and has been found to include gram-negative rods of the genus *Bacteroides,* which may represent up to 30% of the total microbial flora (Gibson, 1999). Other identified bacteria include *Bifidobacteria, Clostridia, Eubacteria* and *Lactobacilli,* gram-positive cocci, coliforms, methanogens and dissimilatory sulphate-reducing bacteria. Some of these bacterial strains possess the ability to synthesise vitamin B and K. Certain strains of *Lactobacillus* and *Bifidobacterium* are able to produce folate (vitamin B9), which is essential for normal cell growth and replication, thereby being important during pregnancy for sufficient development of the foetus (Rossi et al., 2011). Vitamin K exists in two forms, vitamin K1 (phylloquinone) and vitamin K2 (menaquinone), and are essential cofactors for proper blood clotting. K1 may be obtained from a diet rich in green leafy vegetables, while K2 may be synthesised by intestinal bacteria. Lactic acid bacteria, particularly *Lactococcus lactis ssp.* and *Leuconostoc lactis ssp.* have proven to produce significant amounts (Conly and Stein, 1992, Morishita et al., 1999). The bacterial population of the colon and the appendix, which is rich in lymphocytes, thus constitutes an important part of our immune system but may additionally act as potential pathogens. E.g. *Bifidobacteria, E.coli* and *Lactobacilli* strains are commensal in the colon by inhibiting the growth of pathogens and improving the immune response, but are pathogenic when transferred to other sites in the body (Rycroft et al., 2001).

Dietary fibre that escapes digestion in the small intestine may be fermented by the colonic microflora and may only in this manner contribute to the energy obtained by humans. Anaerobic fermentation in the colon results in short-chain fatty acid (SCFA) (C_2-C_6)

production, increased bacterial- and faecal biomass, hydrogen, carbon dioxide and methane production in addition to altered colonic pH (Roberfroid, 1993, D'Argenio and Mazzacca, 2000). Proteins may also be fermented, resulting in the same end products in addition to branched chain fatty acids (Cummings and Macfarlane, 1997). Undigested components reaching the colon along with sloughed epithelial cells and mucus thus contributes to the total energy yield (Kumar et al., 2012). NSP-rich diets have proven to considerably stimulate the growth of *Bifidobacterium* and several strains of *Lactobacillus enterococcus ssp.*, as well as resulting in high concentrations of SCFAs (Shen et al., 2012, Gibson et al., 1995). With regards to stimulation of particularly *Bifidobacteria* and *Lactobacillus,* soluble fibres are believed to exhibit the greatest effect (Olano- Martin et al., 2002). The solubility of dietary fibre is of major impact to the end products of fermentation by means of altered microflora and consequently pH, and total SCFAs produced. Upon reaching the colon, soluble dietary fibre may be readily fermented, which is considered highly advantageous as regards colonic health and function (Allison Db, 2009, Lattimer and Haub, 2010). Low doses of fermentable dietary fibre has also been proposed to help recovery from diarrhoea in children, due to the enclosure and binding to water (Buddington and Weiher, 1999).

As apposed to soluble fibres, insoluble dietary fibres are less available for fermentation, but may be influenced by interfering factors such as particle size (Guillon and Champ, 2000). The level of lignification is also of major impact to fermentability of dietary fibre and hence SCFA production. Generally, non-lignified sources such as pectin, result in greater fermentation rates than lignified sources such as wheat bran (Stephen, 1994, Knudsen and Hansen, 1991). Insoluble dietary fibre are poorly digested and passes the colon without severe fermentation, resulting in low concentrations of SCFAs (Tucker and Thomas, 2009). As noted, insoluble fibres generally promote faecal bulk and excretion, which in turn increase SCFA excretion. Increasing the intake of wheat fibre by a threefold resulted in significantly increased faecal SCFA excretion, but did not alter the colonic concentration (Cummings et al., 1976). These findings indicate that in spite of increased faecal losses, anaerobic fermentation causes increased production of SCFAs to an extent in which maintains the concentration.

Acetic (C_2), propionic (C_3) and butyric (C_4) acid in addition to lactic acid are amongst the SCFAs that are produced, metabolized and made available as energy(Kumar et al., 2012). These are saturated aliphatic monocarboxylic acids, which also are referred to as volatile fatty

acids (Livesey and Elia, 1995). The different dietary fibres impact the production rate of the individual fatty acids. Fermentation of pectin results in approximately 80% acetate while guar gum generally result in larger quantities of butyrate (Kritchevsky and Bonfield, 1995). SCFAs exhibit several health promoting effects and have been found especially efficient in promoting colonic function by enhancing the growth of beneficial bacterial strains. Moreover SCFAs have a trophic effect on the epithelial cells of the colon, meaning that they impact their proliferation and differentiation. This is thought to be one of the mechanisms by which SCFAs have a protective effect against colon cancer (D'Argenio and Mazzacca, 2000).

Most of the microbial fermentation takes place within the proximal colon, which includes the cecum, the ascending colon, the hepatic flexure, the transverse colon and the splenic flexure (Edwards and Parrett, 1999).

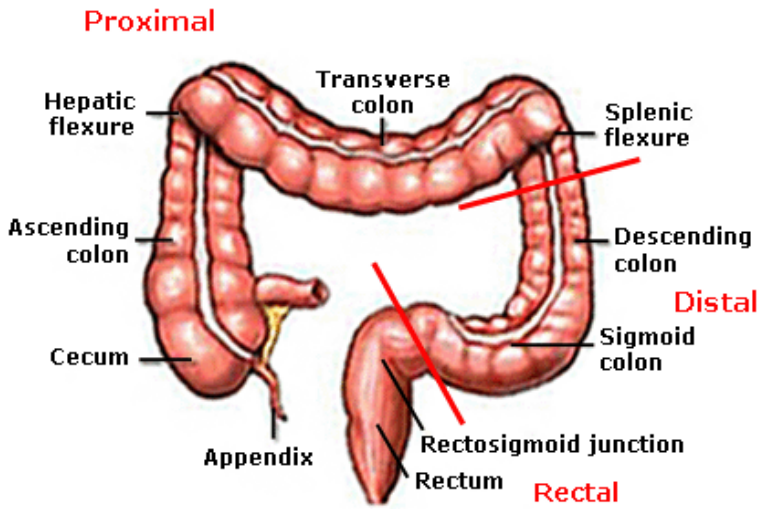

Figure 3.3.1 Illustrates the proximal, distal and rectal colon with the majority of anaerobic fermentation undergoing in the proximal colon where pH ≈ 5,5 – 6,7 (Ontario, 2010).

SCFAs are rapidly absorbed, predominantly in the proximal colon simultaneously with water and electrolytes (Mortensen and Clausen, 1996, Herrmann et al., 2011). Due to the fact that SCFAs principally are weak acids, they are ionized as a result of the slightly alkaline environment in the colon (pH 5,5 – 6,7) (Cummings et al., 1987, Bergman, 1990). This leads to a decreased colonic pH, which is associated with protection against colorectal cancer by inhibiting formation of carcinogens. The lower pH also induce decreased production of

secondary bile acids, which have potential tumour promoting properties (Nagengast et al., 1995, Wong et al., 2006). Increased SCFA production may by this mechanism exhibit cancer protective features (Kumar et al., 2012, Thornton, 1981). Moreover, SCFAs are important for maintaining homeostasis as well as being the colonocytes' (epithelial cells of the colon) main source of energy (Ritzhaupt et al., 1998, Clausen and Mortensen, 1994).

The decreased colonic pH causes a fraction of the SCFAs to be protonated, these are subject to non-ionic passive diffusion and are readily transported across the epithelium (Herrmann et al., 2011). However, the majority of SCFAs holds anionic features due to ionization and demands carrier-mediated transport to be taken up and transported across the colonic epithelium (Herrmann et al., 2011, Ritzhaupt et al., 1998). Monocarboxylate transporter (MCT)-1 is responsible for the transport of organic acids (Herrmann et al., 2011). Recent hypotheses suggests that impaired MCT-1 populations may increase the risk of colon cancer, due to decreased availability of SCFAs (Ritzhaupt et al., 1998). Carrier-mediated transport across the epithelial membrane is regulated by H^+- and Na^+ transport. SCFAs are thus involved in electrolyte and acid-base balance, which in turn impacts their absorption and utilization as an energy source (Miyauchi et al., 2004, Herrmann et al., 2011, Panel on the Definition of Dietary Fiber, 2001). Once taken up into the cells the SCFAs undergo mitochondrial β-oxidation before they enter the citric acid cycle to yield CO_2, water and energy (Demigné et al., 1999). The protective role of SCFAs was demonstrated by inducing experimental ulcerative colitis through inhibiting β-oxidation of SCFA in rats (Roediger and Nance, 1986). The absorption of SCFAs is therefore related to colonic health and is greatly influenced by microbial activity, diet and life style.

About 95% of SCFAs produced from anaerobic fermentation are absorbed by the colonic mucosa. The colonocytes employ SCFAs in the following order, with butyrate being their main source of energy, while propionate and acetate are utilized by muscle, brain and liver; butyric acid > propionic acid > acetic acid (Clausen and Mortensen, 1994, Kamp, 2004). SCFAs stimulate the colons main function and consequently promote anti-diarrhoeal properties, individuals that undergo partial or total colectomy therefore repetitively experiences diarrhoea (Scheppach, 1994). Colonic fermentation of dietary fibre results in acetate being produced in the largest extent, comprising approximately 67 % of the overall SCFA production (Cummings and Macfarlane, 1997). The fermentation ratio, independent of

the dietary fibre source, is always greatest for acetate, followed by propionate and butyrate (Kritchevsky and Bonfield, 1995). A general molar ratio of 60:20:18 for acetate, propionate and butyrate has been found to be an acceptable estimate (Cummings and Macfarlane, 1997). Acetate is generally produced through oxidative decarboxylation of pyruvate, while butyrate is produced by reduction of acetoacetate generated from acetate. Two main routes produce propionate; the "acrylate pathway" from lactate and acrylate or the "dicarboxylic acid pathway" involving fixation of CO_2 to form succinate which is subsequently decarboxylated (Cummings, 1981). The majority of butyrate is oxidized by the colonocytes, while minor quantities are converted to ketone bodies or CO_2 and the remaining parts are metabolized by the liver (Bergman, 1990, Clausen and Mortensen, 1994). The conversion to ketone bodies occurs only at low blood glucose concentrations, as one of the body's mechanisms of providing the brain and erythrocytes with glucose, which is their main source of energy (Demigné et al., 1999). Butyrate has several times been associated with the decreased risk of lower bowl cancer, by the protective effect against tumour formation, cell-cycle arrest and apoptosis of transformed colonocytes. Apoptosis is induced by the inhibition of the enzyme histone deacetylase, which compacts the structure of chromatin and hence play a central role in cellular function, and may in this manner affect tumour cell formation (McIntyre et al., 1993, Wong et al., 2006, Goodsell, 2003). The majority of propionate is metabolized by the liver and has proven to exhibit cholesterol-lowering effects. The mechanisms by which this occurs is uncertain, but several studies indicate that propionate is involved in the inhibition of cholesterol and fatty acid synthesis (Hosseini et al., 2011). Acetate is minimally metabolized by the colonocytes, and is transported to the liver where it is included in long chain fatty acids synthesis and ketone body production. It is further transported to the portal system where it acts as an energy source for the periphery (Clausen and Mortensen, 1994). Acetate also increases colonic blood flow and ilael motility, thus promoting normal digestion and function (Scheppach, 1994, Kritchevsky and Bonfield, 1995).

As above-mentioned the physiochemical features of dietary fibre are closely related to the rate of fermentation. Barry et al. (1995) and Salvador et al. (1993) studied the fermentation rate of different fibre sources by in vitro incubation with human faecal inoculum. The findings of both studies were analogous. Higher fermentability was correlated with higher degradability, which resulted in elevated SCFA ratios (Figure 3.3.3). The fibre sources of more soluble features had high degradability in comparison to the more insoluble ones (Figure 3.3.2). Barry

et al.,(1995) applied Solka-Floc® as cellulose source, hence a very low fermentability (7,2%) was observed, maize bran was also nearly unaffected (6,2%). 59,5% of sugar beet fibre was degraded, while pectin (97,4%) and soybean fibre (91,9%) was degraded to the largest extent. Simmilar results were obtained by Salvador et al., who supported the conclusion that solubility was highly associated with fermentability (Salvador et al., 1993). It was found in both stidies that as the soluble fibre fraction increased, degradability, gas production and SCFA production increased.

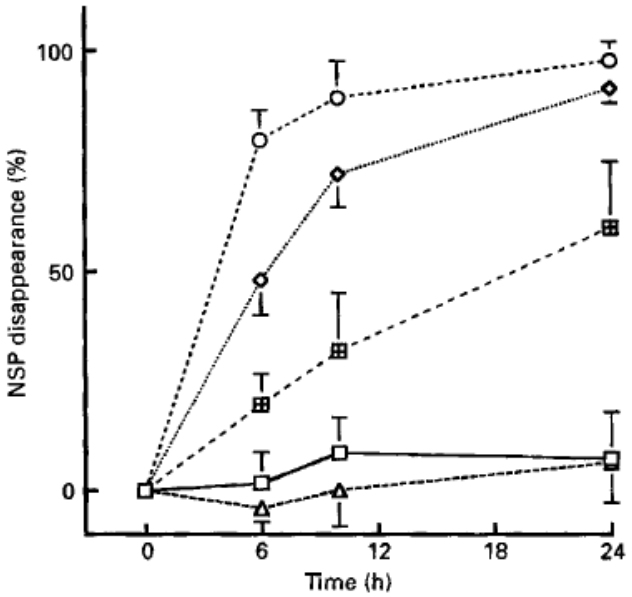

Fig. 2. Disappearance of NSP during the *in vitro* fermentation of cellulose (—□—), soyabean fibre (···◇···), pectin (– –○– –), maize bran (– –△– –) and sugarbeet fibre (– –⊞– –) using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are shown by vertical bars.

Figure 3.3.2 Degradability of five different fibre sources after 24 hours of in vitro incubation (Barry et al., 1995).

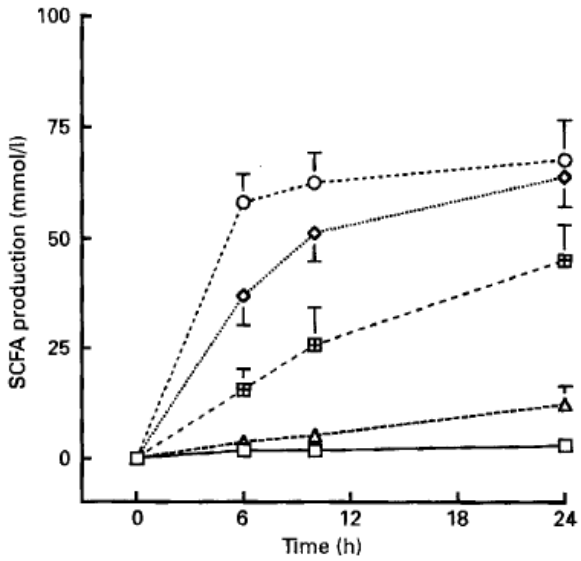

Fig. 3. Production of short-chain fatty acids (SCFA; mmol/l) during the *in vitro* fermentation of cellulose (—□—), soyabean fibre (···◇···), pectin (—○—), maize bran (—△—) and sugarbeet fibre (—⊞—) using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are shown by vertical bars.

Figure 3.3.3 SCFA (mmol/l) produced during 24 hours of in vitro incubation with human faeces (Barry et al., 1995).

Since the proximal colon is principally inaccessible it is difficult to conduct in vivo studies. Animal studies are more expensive but offer results that are comparable to those expected in human models. If human studies are made, indirect measurements of faecal or plasma SCFAs and breath hydrogen are most common (Edwards and Parrett, 1999). Nevertheless, in vitro measurment of fibre degradability may provide a good indication of the apparent fermentability. Guillon et al. (1995) incubated pea hull fibre and apple fibre with human faecal inoculum. After 24 hours 75% of the pea hull fibre and 42% of the apple fibre could be recovered. The findings were in good correlation with what was expected, since pea hulls mainly consist of insoluble fibre, while apple fibre is rich in pectin. Reliable evidence from similar studies have indicated that substrate recovery after 24 and 48 hours relate well to SCFA production and may hence be used as an indicator of fermentability (Titgemeyer et al., 1991). Although limitations such as rapid absorption in the colon needs to be taken into consideration, in vitro studies are regarded as acceptable estimates for determining the quantity and production ratio of SCFAs (Canibe and Bach Knudsen, 2002).

Anaerobic fermentation of dietary fibre is thus considered to be highly positive with respect to colonic health and function. The consequential decreased pH as a result of increased SCFA production is associated with multiple health benefits. With respect to the positive results of anaerobic fermentation, it is the total effects exhibited by dietary fibre, which contributes to the positive end results (Beyer-Sehlmeyer et al., 2003). Increased colonic fermentation is induced by increased dietary fibre intake, which in turn positively influences other bodily functions. Blood glucose, cholesterol and nutrient digestibility will also be influenced and the benefits of increased dietary fibre intake may be highly favourable in relation to prevention and management of lifestyle diseases. This is reviewed in larger detail in the following section, and highlights which types of dietary fibre that is considered to exhibit the greatest effects with respect to obesity, type two diabetes mellitus and coronary heart disease.

3.4 Positive impact of increased dietary fibre consumption in relation to management and prevention of life style diseases.

As it has been reviewed, dietary fibre may exhibit an abundance of health benefits. Increased intake has been found to alter gut transit time, stimulate the colonic microflora, increase stool weight, influence appetite, absorb toxins and modify the absorption of fats, sugars, minerals and bile acids (Chaplin, 2003). The recommended daily intake of dietary fibre is 25g/day for women and 35g/day for men, respectively (Helsedirektoratet, 2011a). The Norwegian population consumes approximately 20g/day. By increasing the amount to the recommended, the population could lower their energy intake in addition to the risk of life style diseases such as coronary heart disease, obesity and type 2 diabetes (Helsedirektoratet, 2011b). To accomplish this, a diet high in whole grain products, fruits, berries, vegetables and legumes is recommended (Helsedirektoratet, 2011b). This theory is supported by the World Health Organization report from 2003 on diet, nutrition and prevention of chronic disease (WHO, 2003). Including and increasing the amount in ones diet may offer severe, positive alteration to peoples colonic health and gastrointestinal function. The prevalence of life style diseases seen in industrialized countries is overwhelming and inclusion of dietary fibre is thought to be one of the modifiable dietary factors, which may exhibit protective effects. The following text gives an overview of the life style diseases of greatest influence to the world population: Obesity, type two diabetes and coronary heart disease, and dietary fibres' positive effect in prevention and management of these.

During the last quarter of the 20th century dietary fibre has emerged as a leading dietary factor in the prevention and treatment of life style diseases. High fibre intake is associated with reduced serum cholesterol, blood pressure and risk of coronary heart disease and certain types of cancer, in addition to improved weight management, glycaemic control, and gastrointestinal function (Anderson et al., 1994). Metabolic syndrome or syndrome X, are two of the terms used to describe risk factors associated with an increased risk of developing coronary heart disease (CHD) and type two diabetes (Novo et al., 2008). The risk factors, listed in Table 3.4 may occur individually but are often associated. A person must be diagnosed with at least three risk factors to be diagnosed with metabolic syndrome (Helsedirektoratet, 2009).

Table 3.4 Risk factors associated with development of metabolic syndrome (Helsedirektoratet, 2009).

Risk factor	**Definition**
Large waist line	>102 cm for men and >88 cm for women.
High triglyceride levels	>1,7mmol/l
HDL-cholesterol	<1,03 for men and <1,29 for women
Blood pressure	>130/>85 mm Hg
Fasting plasma glucose	>5,6 mmol/l

HDL: High-density lipoprotein cholesterol.

Obesity, CHD and type two diabetes have rapidly become the major lifestyle diseases in both industrialized and developing countries. Asia, North Africa, Latin America and the Middle East have experienced exponential economic growth during the past decades and hence improved living standards within the populations. The shift from a primarily agricultural lifestyle to one that is dominated by modern technology and industry, has also led to the adoption of industrial countries bad habits in diet and lifestyle. Resulting in what is described as the nutrition transition. This describes the shift from a balanced, varied diet combined with a high level of physical activity, to a nutrient dense diet combined with little or no physical activity (Popkin, 2012). The fact that low- and middle-income countries have reached or exceeded the obesity levels of that in the USA and Western Europe in less than 25 years, has led to an escalation in nutrient-related non-communicable diseases (NR-NCD) (Popkin, 2012). Type 2 diabetes, obesity and CHD are amongst the NR-NCD that are of highest prevalence and are expected to increase in the future.

Obesity, is one of the main risk factors for development of CHD, type 2 diabetes and certain types of cancer and is defined by having a body mass index (BMI) of ≥30 (Flegal et al., 2013). Approximately 20% of the adult Norwegian population suffers from obesity while

nearly 50% of the Swedish adult population are classified as overweight (BMI ≥25) (Helsedirektoratet, 2009, Helsedirektoratet, 2011b). As stated, this trend takes place mainly because of the energy intake/physical activity imbalance. Roughly 66% of the US adult population are overweight or obese (Lattimer and Haub, 2010). If diet and lifestyle trends does not change, there is an estimated 33% and 130% rise in obese and severe obese (BMI ≥ 35) prevalence in the American population during the future decades (Finkelstein et al., 2012). Dietary fibre has received a great deal of attention when concerning weight and weight loss, several studies indicate an inverse relationship between dietary fibre intake and weight gain (Koh-Banerjee et al., 2004, Liu et al., 2003). Generally, people who consume increased levels of dietary fibre tend to have decreased dietary fat intake, which consequently decrease the metabolisable energy (Lattimer and Haub, 2010). Adding dietary fibre to diets naturally reduce the quantity of energy yielding nutrients, which consequently decrease the available energy. Decreased digestibility as caused by dietary fibre may additionally be attributed to increased faecal bulk and viscosity (Lattimer and Haub, 2010). By increasing the intake of fruits, vegetables, whole grains and legumes, more dietary fibre will be consumed, which in turn may help reduce the epidemic of obesity seen in developed countries.

In relation to type 2 diabetes mellitus (T2DM), which develops as a result of decreased insulin sensitivity and hyperglycaemia, the nutritional carbohydrate source is of particular interest (Diabetikerforbundet, 2008). Insulin is secreted by the β-cells in the pancreas when blood glucose is elevated. Upon development of T2DM the pancreas either fails to produce enough insulin or the insulin sensitivity is decreased, therefore T2DM is additionally referred to as insulin-independent diabetes. Insulin-dependent diabetes on the other hand, occurs as a result of the pancreas being unable to produce insulin. The prevalence of T2DM was practically non-existing before flour milling was mechanized (Trowell et al., 1976). The industrialization of food production made refined commodities more available and affordable to the public, resulting in decreased consumption of whole foods and beginning of the era of chronic life style diseases. Smoking, lack of physical activity and obesity are also considered significant risk factors for development of T2DM (Diabetikerforbundet, 2008). Approximately 347 million people worldwide are currently suffering from diabetes of which 90% is T2DM (Danaei et al., 2011). Diet is thought to be one of the modifiable risk factors in development of T2DM, and whether the carbohydrates are of simple or complex structure may highly impact the glycaemic response (Priebe et al., 2008). The glycaemic response, the postprandial

blood glucose response to food in comparison to a reference group such as glucose or white bread, is classified as the glycaemic index (GI) or glycaemic load (GL) (Lattimer and Haub, 2010). A high GI implies that the food causes a larger rise in blood glucose response, than low GI foods. Studies indicate that the inclusion of GI in the planning of ones diet may help manage T2DM, and even delay and/or prevent development (Silva et al., 2009). However, recent findings imply the effect of GI to be of more modest effect on development of T2DM than previously suggested (Sluijs et al., 2013). Increasing the dietary fibre intake has thus proved to be more efficient in prevention/management of T2DM. High-fibre diets have demonstrated improved insulin sensitivity, which may occur due to the viscosity of soluble fibres (Anderson et al., 1994). An inverse relationship has also been observed between fasting insulin and insoluble fibre. This was demonstrated when whole grain products were added to mixed diets of young men and women, and subsequently divided according to solubility. Compared to that of soluble fibre, the insoluble fibre proved to reduce the insulin response to a larger extent (McKeown et al., 2002). Insoluble fibre may also help stabilise and lower serum glucose concentrations. This was demonstrated when diets rich in insoluble fibre were given to cats and dogs suffering from insulin dependent diabetes mellitus (Nelson et al., 2000, Kimmel et al., 2000). However, viscous dietary fibre is thought to exhibit the greatest effect with respect to blunted blood glucose. Dikeman and Fahey Jr. (2006) found consumption of viscous NSPs to reduced postprandial blood glucose, regardless of whether the dose was high or low. Development of T2DM has also been inversely related to whole grain consumption (de Munter et al., 2007, Montonen et al., 2003).

Whether people are suffering from insulin dependent or non-insulin dependent diabetes it is recommended to increase the dietary fibre intake, as advised by The British- and Canadian Diabetes Association together with the European Association for the Study of Diabetes (Anderson et al., 2004). In spite of the recent studies that indicate a limiting link between development of T2DM and the GI of food, it is recommend by the Norwegian diabetes association to comprise GI in the diet (Diabetikerforbundet, 2008). Low GI foods are generally good fibre sources, and by comprising the GI of food, dietary fibre is naturally incorporated into ones diet (Anderson et al., 2004). It was recently concluded that an increased intake of soluble dietary fibre, above the recommended daily intake; improved the glycaemic control, decreased hyperinsulinemia, and lowered plasma lipid concentrations in patients with type 2 diabetes (Chandalia et al., 2000). The majority of evidence supports the

notion that a diet high in dietary fibre, consisting of low GI foods in addition to thirty minutes of moderate-intensity physical activity on most days, will aid weight management and lower the risk of developing type 2 diabetes and CHD (Brand-Miller et al., 2009, Kendall et al., 2010).

As noted, coronary heart disease (CHD) is currently ranked as the leading cause of death worldwide, and it is estimated that 7,3 million deaths in 2008 occurred due to CHD (WHO, 2012). CHD is caused by atherosclerosis, which is constriction of the arteries as a result of the build-up of fats and plaque, this reduces the blood flow to the heart and may result in angina, heart attack or in worst case heart failure (Mackay et al., 2004). Obesity, T2DM and hypercholesterolemia are some of the risk factors associated with development of CHD. Hypercholesterolemia is the term used to describe elevated blood cholesterol levels, in terms of an imbalance between HDL- (high density lipoprotein), triglycerides and LDL- (low density lipoprotein) cholesterol (UMMC, 2011). While the "good" HDL-cholesterol has a protective effect against CHD, LDL-cholesterol is classified as "bad" and has the opposite effect (NHI, 2013). Studies support the hypothesis that an active lifestyle and a diet low in saturated fat and rich in vegetables, fruits and wholegrain products will prevent/postpone development of CHD (Lattimer and Haub, 2010). Soluble dietary fibre has proven to exhibit hypocholesterolaemic effects in both humans and animals (Kritchevsky and Bonfield, 1995). Kritchevsky and Bonfield, 1995, conducted a study where hypercholesterolaemic and healthy male and female subjects consumed oat bran and bean products. They reached the conclusion: "*Foods rich in soluble dietary fibre have significant hypocholesterolaemic effects*". These are products acceptable to most people, and consumption of reasonable quantities may reduce serum cholesterol by 10 – 15% and by as much as 20 – 30%, if added to fat-restricted diets (Kritchevsky and Bonfield, 1995). The LDL-cholesterol lowering effect by β-glucans, pectins and guar gum is well documented. The aforementioned soluble fibres have efficiently lowered LDL-cholesterol without much altering of the HDL- or triglyceride levels (Theuwissen and Mensink, 2008). Pectin, when given at 10 g/day, proved to lower the risk of developing CHD by decreasing plasma cholesterol by 5 – 10% (Truswell and Beynen, 1992). If one wants to consume 10g of pectin through a varied diet, this is equivalent to approximately 1 kg fruit or vegetables (Truswell and Beynen, 1992). Concerning people who are accustomed to western diets, this may pose as a problem. Especially with consideration to elderly people, which constitutes the largest proportion of the population requiring cholesterol-lowering

medications. It may thus be easier for people to add pectin as a supplement. Moreover, soluble dietary fibre may reduce emulsification and (re)absorption of bile acids and additionally result in increased bile acid excretion due to increased viscosity. Increased bile acid excretion promotes bile acid synthesis from cholesterol, which will increase LDL-cholesterol uptake by the liver (Cohn et al., 2010). Insoluble fibre may also be effective due to increased faecal bulk and excretion. Consumption of wheat fibre over a period of three weeks increased faecal bile acid excretion from 199 +/- 46 mg/day to 279 +/- 46 mg/day (Cummings et al., 1976). This knowledge makes it possible for people suffering from hypercholesterolemia to stabilize and manage their elevated serum cholesterol levels without medications. Dietary fibre provides far less side effects than cholesterol-lowering medications. However, for this knowledge to be used actively to manage elevated cholesterol levels, health professionals as well as consumers will need much more education and understanding.

Inclusion of dietary fibre may thus lower the risk of developing life style diseases and acknowledged nutrients include vegetables, whole cereals, legumes and nuts (Salas-Salvadó et al., 2006). Whole grains and legumes generally provide more starch than dietary fibre, and may not be the best choice regarding that one of the predominant lifestyle diseases in industrialised countries is obesity. Vegetables on the other hand, may contain up to 50% dietary fibre, and may thus constitute a more efficient source of NSP without adding additional calories to the diet. A diet based on whole grains, an abundance of fruits and vegetables as the main carbohydrate source, and adequate intake of omega-3 fatty acids, is thought to offer protective effects against CHD, obesity and development of T2DM (Hu and Willett, 2002, Morin et al., 2004, Montonen et al., 2005). Consumption of dietary fibre may improve several blood parameters, however the individual fibre source will highly impact the result. The risk of developing T2DM has been inversely linked to whole grain consumption, which contains both fibre fractions (McKeown et al., 2002, Montonen et al., 2003). As aforementioned, soluble dietary fibre exhibits hypocholesterolaemic effects, hence it is wise to include β-glucans, pectins and guar gum in ones diet with respect to CHD (Theuwissen and Mensink, 2008, Cohn et al., 2010). Improved weight management and weight loss has been observed after increased dietary fibre consumption (Slavin, 2005, Tucker and Thomas, 2009). Weight gain has also been inversely linked to consumption of whole grains, cereal fibre and nuts (Koh-Banerjee et al., 2004, Bes-Rastrollo et al., 2009). Even though nuts are extremely

energy dense and provide additional calories to the diet, they offer great health benefits when consumed in moderate doses and are thus considered healthier alternatives than processed products. All things considered, inclusion of nutrients rich in dietary fibre has proved to be protective against development of life style diseases. Such nutrients will in most cases, induce lower postprandial blood glucose and insulin response and protect against development of CHD and obesity. Additionally they offer a more favourable nutrient composition. Whole grain flour offers a healthier carbohydrate composition than refined flour and is also an important source of antioxidants, phytochemicals, vitamins and minerals, particularly B vitamins (Dewettinck et al., 2008). The interaction of these beneficial nutrients in addition to the digestive properties they provide, is thought to be the mechanisms that are favourable in the treatment and prevention of obesity, diabetes, coronary heart disease and certain types of cancer (Lattimer and Haub, 2010).

As it has been demonstrated by the reviewed studies, decreasing the energy value of diets is also one of the effects exhibited by dietary fibre and is considered highly beneficial with respect to the above-mentioned lifestyle diseases. Consequently, the importance of energy calculation and the experimental work of Atwater and other food scientists, in addition to the energy conversion factors are reviewed in the following section.

4. The development of energy systems and energy conversion factors.

The energy content of nutrients differs by means of chemical composition, digestibility and absorption by the human body. Thanks to scientists desire for knowledge, the energy provided by fat, protein and carbohydrates have been estimated over the past century. The following text describes the history of the development of food energy systems and the progress up to the present values used for commercial nutrient labelling. Furthermore the accuracy of the calorific value of ethanol and protein will be discussed.

4.1 Determining the nutritional value of food.

Energy is stored in food as chemical energy and is used by the human body for maintenance (heart beat, active transport, synthesis of enzymes, hormones and much more) and work (physical activity). The availability of energy varies within foods, is highly associated with the chemical composition and may be measured by determining the heat production (oxidising the food by burning) (McDonald et al., 2002). The energy is most commonly measured in calories (kcal) or kilojoule (kJ) per gram of oxidised food, of which 1 kcal is equal to 4,1868 kJ. Principles in thermodynamics indicate that the same results are obtained when nutrients are completely combusted in a bomb calorimeter in the presence of oxygen, as when fully oxidized to H_2O and CO_2 by living organisms (Elia and Cummings, 2007). A large range of metabolic steps occurs during digestion of nutrients. Not all nutrients are as readily available as others, and the end products of oxidation in the human body differ greatly from that of a bomb calorimeter. Therefore, the variations between gross, digestible, metabolisable and net metabolisable energy may be significant (Elia and Cummings, 2007). The following text describes energy utilization in humans and is illustrated by Figure 4.1.

The heat generated from complete oxidation of a unit of food is known as its gross energy (GE) or heat of combustion (McDonald et al., 2002). GE is measured by a bomb calorimeter and illustrates the energy consisting within the food before digested (McDonald et al., 2002). Digestible energy (DE) is the energy that remains in food after the heating value of faeces is subtracted. By additionally subtracting the energy lost through energy-containing compounds in urine and combustible gasses (methane etc.) one is left with metabolisable energy (ME).

Metabolisable energy (ME) represents the energy available for use by the body and is the starting point for Atwater´s calculations (Svihus, 2007). The ME is affected by many factors, the main ones being those influencing digestibility, hence both the quantity and quality of dietary fibre in nutrients are of major impact. The net metabolisable energy (NME) may be calculated by subtracting the energy lost to heat as a result of digestive and turnover processes. NME thus equals ME minus heat lost to the environment and represents the energy the body may further convert to energy and use for maintenance (McDonald et al., 2002). The energy available for use by the human body is thus different from the energy derived from a bomb calorimeter. It has taken scientists over a century to develop the systems for calculating and understanding that net metabolisable energy is the energy readily available as energy source.

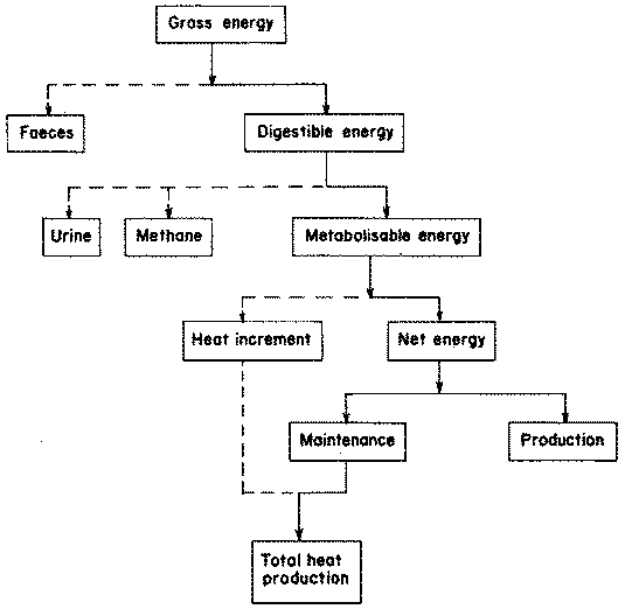

Figure 4.1 Schematic representation of energy utilization by the human body. Energy losses are in dash lines (McDonald et al., 2002).

The German physiologists Voit (1831 – 1932) and Rubner (1854 – 1932) were both pioneers in measuring the human body's energy requirements by use of calorimeters. Voit built the first whole body indirect calorimeter for measuring the energy expenditure in humans. Rubner combined direct and indirect calorimetry and became the first scientist to study energy requirements in small animals and children (Nichols, 1994). He also determined the energy values for protein, fats and carbohydrates through bomb calorimetry, which was determined

to be 4,1 kcal/g, 9,3 kcal/g and 4,1 kcal/g (Maynard, 1944). These findings were widely accepted throughout Europe, however Wilbur Olin Atwater (1844 – 1907), who was one of Rubners students, wanted to seek more knowledge. He made the first system for determining the energy of nutrients in the United States of America and built his studies on Voit´s and Rubner´s findings. The energy system was made by Atwater and his colleagues and was classified as The Atwater general factor system. In 1896 Atwater completed the first human calorimeter in the United States, subsequently the energy needs of the population and the calorific value of food were surveyed (Nichols, 1994).

The main difference between their methods was that Rubner determined the GE of protein, fat and carbohydrates while Atwater corrected for faecal and urinary losses (Maynard, 1944). Atwater made the conclusion that the energy yield from food when oxidised by the human body was equal to that derived from bomb calorimeters when corrected for the losses in faeces and urine, i.e. the ME (Atwater, 1910). Through use of respiratory calorimeters and current knowledge of the nutrient content of food led to his determination of the energy conversion factors. Additionally he summarized dietary studies of groups of people from different areas in the United States (Atwater and Benedict, 1903, Atwater, 1910). The aim of his studies was to develop food composition tables, dietary guidelines through nutrient requirements, and to improve public health by estimating the energy requirements of the average population. Through nutritional information he could help improve the health and wellbeing of the poorer parts of the population, and also improve consumer economy. Emphasising that cheese also was a good source of protein enabled the poorer parts of the population to afford to meet the need for dietary protein (Nichols, 1994).

The conversion factors were determined by multiplying the heat of combustion with the percentage of availability, listed in Table 4.1. The percentage of availability was estimated by comparing literature and results from experiments conducted by Atwater and colleagues (Merrill and Watt, 1973). Subtracting the faecal and urinary losses from the gross energy determined the availability of fat and carbohydrate. Protein may further be oxidized to urea in addition to creatinine, uric acid and other nitrogenous end products by the human body. Consequently, protein was corrected for unoxidized material in faeces and urine. Equation 4.1 illustrates Atwater’s definition of availability with respect to protein.

EQUATION 4.1 Atwater´s estimation of available protein (Merrill and Watt, 1973).

$$\frac{Nitrogen\ in\ food - Nitrogen\ in\ faeces}{Nitrogen\ in\ food} \times 100 = coefficient\ of\ availability.$$

A correction factor was estimated to be 1,25 (5kJ) kcal/g digested protein, by measuring the heat of combustion in addition to the nitrogen content of urine from forty-six human subjects (Merrill and Watt, 1973).

Table 4.1 Atwater´s factors for the heat of combustion and "available energy" values of nutrients.

			"Available energy"
Nutrient	Heat of combustion (kcal/g)	Availability (%)	(kcal/g) total nutrients
Protein: Animal Vegetable	5,65 5,65	97 85	4,25* 3,55*
Fat: Animal Vegetable	9,40 9,30	95 90	8,95 8,35
Carbohydrate: Animal Vegetable	3,90 4,15	98 97	3,80 4,00

* Corrected for unoxidized material in urine. Subtracted for 1,25 after calculating the available energy. Adapted from Merrill and Watt (1973).

The heat of combustion for fat, protein and carbohydrates were derived from mixed diets and the conversion factors for protein, fat, carbohydrate and alcohol, which was also acknowledged as an energy source, was determined to be 16,7 kJ/g, 37,4 kJ/g, 16,7 kJ/g and 28,9 kJ/g, respectively(Atwater, 1910). From their study, Atwater and Bryant concluded that from average American diets 61% of protein, 92% of fat and 5% of carbohydrate came from animal origin and the residual from vegetable sources. On this basis they made the conclusion to round off the conversion factors to 17 kJ/g, 38 kJ/g and 17 kJ/g when determining the energy value of protein, fat and carbohydrate, and 29 kJ/g for alcohol (Atwater and Bryant, 1900, Merrill and Watt, 1973).

The fact that the Atwater conversion factors are based on American mixed diets is one of the limitations when calculating the energy values of diets. As with the current American diet, the majority of protein came from animal origin and the carbohydrate was largely based on highly milled, refined flour (Maynard, 1944). Another drawback is Atwater´s referral to "total carbohydrate" also termed "carbohydrate by difference", which is the difference between 100 g food and the sum of fat, protein, water and ash. He also assumed that starch was the major component of carbohydrate-rich nutrients and therefore assigned the calorific value of 17 kJ/g to all vegetables, cereals and other plant based commodities (Atwater, 1910, Merrill and Watt, 1973). This has led to serious over- and underestimation of the energy value of carbohydrate rich nutrients. However, it is important to stress that Atwater never intended for the general factors to be assigned to individual foods, but to varied diets (Maynard, 1944).

The general factor system has been widely used, in large extent because of its simplicity. As earlier noted it only calculates the metabolisable energy (ME) of carbohydrates, fats, proteins and alcohol, this made scientist question the systems accuracy and resulted in revaluation of the Atwater factors.
Southgate and Durin acknowledged that not all fractions of carbohydrates consist of starch, and may not be readily available for digestion. This resulted in what is described as the extensive general factor system (FAO, 2003). A factor for available carbohydrate was added in 1970 by Southgate and Durin and was expressed as monosaccharide, with an energy value of 16kJ/g (Southgate and Durnin, 1970). This change acknowledged that different values are attained from carbohydrates, depending on the availability (FAO, 2003). A general energy value of 8,0kJ/g (2,0kcal) dietary fibre was first suggested in 1998 by the Food and

Agriculture organisation (FAO) and the World health organisation (WHO). Since the energy made available from dietary fibre highly depends on the degradability and thus fermentability, the use of 8,0 kJ/g as a general factor has been much debated. This will be discussed in larger detail in section five.

The extensive general factor system distinguished total from available carbohydrate, and made it possible to determine the energy from monosaccharides. Consequently, factors other than fat, protein and carbohydrates were acknowledged as sources of energy (FAO, 2003). However, it was the Atwater specific factor system that took into account that nutrients have different chemical compositions and hence provide different amounts of energy (FAO, 2003). It was introduced in 1955 by Merrill and Watt, and in contrast to the general Atwater system, it acknowledged that the bioavailability as well the digestibility of proteins, fats and carbohydrates varies, depending on the food source (Merrill and Watt, 1973). The Atwater specific factor system is based on approximately 50 years of research and uses different factors for fat, protein and carbohydrates for individual nutrients. This results in marginally different values when comparing the ME of fibre rich diets to experimentally determined ones (FAO, 2003, Wisker et al., 1988). Factors for protein range from 10,2 kJ/g for some vegetables, to 18,2 kJ/g for eggs. Fat varies from 35 kJ/g to 37,7 kJ/g and total carbohydrate from 5,56 kJ/g to 17,4 kJ/g (Merrill and Watt, 1973). Merrill and Watt found the energy value of foods in American mixed diets to be 5% higher, when using the general factors rather than the specific factors. The general factors tend to overestimate the energy content hence the specific factors provide more accurate values. Charrondiere et al. (2004) supported this assertion after studying the food supply data from nine countries with different diets. The differences between general and specific Atwater factors were found to account for 50–320 kJ/capita/day. This is a negligible amount, however the largest differences were observed in countries, of which diets mainly were plant-based, indicating that larger miscalculations would occur in carbohydrate rich diets. Differences in energy calculations were also observed within the same nutrients. This was largely due to differentiation of carbohydrate and dietary fibre, which differ between countries and thus result in diverse energy values for the same commodities and carbohydrate rich diet (Charrondiere et al., 2004).

In addition to the differences seen within energy calculations, it was found in the late 20th century that the analytical methods used to determine the nutritious value of food had not

been revised since the time of Atwater. The demand for proper food labelling of new products increased simultaneously as large differences were observed in the listed values and those found by new studies. An example is cholesterol in eggs, which in 1988 indicated to be 15 – 20% lower than the ones listed in Handbook nr.8 (Watt and Merrill, 1963). In order to minimize such errors within nutritional labelling and consumer information, the methods for analysing nutrient composition were revised. The analytical methods were successfully revised, however the systems for determining the energy value still differ a great deal. Different energy systems are used around the world with regard to food labelling and energy calculations. In addition to the three Atwater systems the net metabolisable energy (NME) system was made to make the calculations more accurate. Unlike the Atwater factors, which determine the ME of food, the NME-system includes the energy lost through heat production, so that the energy made available through ATP (adenosine triphosphate) production could be calculated (FAO, 2003, Elia and Cummings, 2007). The NME values have been estimated to be: Fat (37 kJ/g), protein (13 kJ/g), available carbohydrate (16 kJ/g), fully fermentable carbohydrate (8 kJ/g) and alcohol (26 kJ/g) (Livesey, 2001). Combustible-, digestible-, metabolisable- and net metabolisable energy are some of the energy systems used for food labelling. This causes confusion among the consumers and whether the NME system should be used instead of the ME, has regularly been up for debate (Elia and Cummings, 2007). It was concluded by the FAO in 2003, that the ME factors rather than the NME factors are to be used (FAO, 2003). The factors to be applied are approximately the same as Atwater first determined; 17kJ/g for proteins, 37kJ/g for fat and 29kJ/g ethanol. The conversion factor for available carbohydrates (total carbohydrate minus dietary fibre) should be 17kJ/g and 16 kJ/g when expressed as monosaccharide equivalents. Dietary fibre should be determined according to the AOAC Official method 985.29 and applied the conversion factor of 8kJ/g (FAO, 2003, Omsorgsdepartementet, 2009, EC, 2008). Though it has taken scientists over a century to develop approved, standardized analytical methods for determining the nutrient value of food, it is owing to the extensive work of Rubner, Voit and Atwater that we today are able to calculate the energy of diets. Although energy utilization by the human body is acknowledged and the metabolisable pathways are surveyed, the accuracy of the energy conversion factors is still debated. Accordingly, the accuracy of the energy conversion factors of ethanol and protein will be reviewed in the following subsection, before the energy obtained from dietary fibre is reviewed in larger detail in section five.

4.1.2 Energy conversion and accuracy of the energy values of ethanol and protein.

The ME value of 29kJ/g ethanol was first determined by Atwater and Rosa in 1899. Several studies have questioned the accuracy of this value and have suggested that the actual energy contents is much lower than the ME value assigned to ethanol (Atwater and Rosa, 1899, Rumpler et al., 1996). 80% of the metabolisable energy from ethanol was found to be utilized when compared to carbohydrates in healthy, non-alcoholic human subjects by using indirect calorimetric measurements (Suter et al., 1994). Weight loss was observed in human subjects, when up to 25% of the energy from carbohydrates was substituted with ethanol (Pirola and Lieber, 1972, Rumpler et al., 1996). When the equivalent amount of energy was exchanged with chocolate, the subjects gained weight.

The diminished energy utilization of ethanol, may be justified by the hepatic microsomal ethanol oxidizing system (MEOS), which is an additional pathway to alcohol dehydrogenase and NADH oxidation (Lieber et al., 1970). MEOS activity increases simultaneously as tissue concentrations of alcohol and yields 10 ATP per mole oxidized ethanol, while alcohol dehydrogenase yields 16 ATP (Lieber, 1991, Rumpler et al., 1996). This is thought to be a contributing factor to why alcoholics often are malnourished. Studies conducted by Lieber on alcoholic subjects, proved that MEOS activity increased simultaneously as chronic ethanol consumption, which influenced fat metabolism and promoted oxidative damage to liver cells (Lieber, 2003, Lieber, 1999). Lieber concluded, with respect to body weight and alcoholics, that calories may not fully count because the body is not able to completely utilize the ethanol (Lieber, 2003). In normal humans, moderate alcohol consumption will constitute an efficient energy source that is rapidly degraded by alcohol dehydrogenase (Lieber, 1991). The net energy provided by alcohol varies between individuals and depends on lifestyle, weight and level of physical activity, of which all impact the calorific end result. However, it is proposed that alcoholics derive less energy from alcohol than non-alcoholic persons, in large due to the increased MEOS activity (Lieber, 2003, Suter, 2005). As noted the calorific value of ethanol derived from bomb calorimetry has been determined to be 29 kJ/g. Since energy deficiency is only seen in subjects undergoing chronic abuse of large amounts of alcohol the advice that ethanol provides 29kJ/g is concerned realistic with respect to healthy, social drinking people.

With regards to the metabolisable energy of proteins, Atwater determined the heat of combustion for many nutrients, and on this basis calculated the average heat of combustion for protein in mixed diets. When determining the calorific value of meat, fat-free muscle tissue was applied and found to contribute 23kJ/g. The same factor was applied to milk protein, while eggs were estimated to contribute 24kJ/g based on data of the protein proportion of egg whites and yolk, assuming that limited non-protein nitrogen (NPN) was present (Atwater, 1910). Upon revising the Atwater general factors, Merrill and Watt found that when applied to protein, some miscalculation occurred (Merrill and Watt, 1973). As described earlier, Merrill and Watt generated the Atwater Specific factors for a range of nutrients. They found, as Atwater, apparent digestibility percentages of nutrients and multiplied them with the heat of combustion, hereby determining the physiological fuel value (Merrill and Watt, 1973). Comparing the specific factors to the general factors resulted in a 44 percent variance within the ME of proteins. Nevertheless, Merrill and Watt (1973) concluded that the Atwater general factor of 17kJ/g for protein provided a reasonable estimate, when calculating the calorific value of average diets. The Specific factors were more suited for use when determining the calorific value of certain foods or diets with a distinct purpose (infant formulas, weight loss diets etc.). Moreover the correction factor of 5kJ/g of available protein was questioned, due to large variations in the calorie-nitrogen ratio found in the urinary samples Atwater based his findings on (Merrill and Watt, 1973). However, they concluded that the correction factor of 5kJ/g of available protein was agreeable as a proximate value. Merrill and Watt continuously revised the specific factors and acknowledged that the values obtained when applying the general factors commonly resulted in elevated values. Alongside with Southgate and Durin´s proposal of a factor for available carbohydrate, it was acknowledged that the need for a more detailed energy system was necessary. Hence, the adoption of the NME system was proposed as a more suitable approach for calculating the calorific value of proteins. The principal reason being that ME only gave an estimate of the energy provided by nutrients (Livesey, 1988).

Proteins are particularly thermogenic, which means that more work is required for their digestion and absorption than e.g. simple carbohydrates, which may contribute to decreased energy efficiency and thus lead to weight loss. The NME of proteins have been calculated by use of human calorimeters and net ATP yield and estimated to be 13 kJ/g (Livesey, 2001, Paddon-Jones et al., 2008). The aim of nutrient labelling determined by the FAO is to inform the consumer, and assist in the selection of healthy food. This is one of the main reasons why scientists have stressed the need to modify the calorific values with regards to food labelling.

Nevertheless, proteins contribute approximately 15% of the total energy to average diets in industrialized countries (Helsedirektoratet, 2011b). On this basis FAO (2003) considered 17kJ/g protein to be regarded as an acceptable estimate for the energy contributed to average diets. This is supported by the EU Directive 90/496/EEC, which stated that protein in diets on average provides 17 kJ/g, and that this value shall be applied for nutrition labelling purposes (EC, 2008). As with the definition of dietary fibre, Norway has to comply the definitions stated by the EU Directive.

The conversion factors of ethanol and protein have been extensively studied, and are still being revised. Since ethanol is an efficient energy source, which is rapidly absorbed, the ME value of 29 kJ/g is regarded as an acceptable estimate for the energy obtained by normal, social drinking people. The debate on whether the ME (17 kJ/g), or NME (13 kJ/g) value of protein should be used for nutritional labelling is persisting within scientific communities. Even though proteins require more work to be digested and absorbed than ethanol, the ME value of 17 kJ/g protein is considered an acceptable value to be applied to the mixed diets of healthy people.

Many studies have been conducted to determine the accuracy of the energy conversion factors and the specified factors are assumed to provide acceptable approximations of the true energy value. Nevertheless, when calculating the energy of NSP-rich diets the proposed factor of 8kJ/g dietary fibre has been found to overestimate the calorific value. As reviewed in the following section, 8kJ/g dietary fibre is assumed to result in large miscalculations.

5. Energy obtained from dietary fibre through anaerobic fermentation.

Determining the nutritive value of food is important for calculating the calorific value of diets, which may be decreased by adding dietary fibre. Consequently, the proposed factor by the European Commission of 8kJ/g dietary fibre is considered to result in large miscalculations. The energy contributed by dietary fibres is very diverse, cellulose is estimated to contribute very little energy to the diet, while pectin and other water soluble fibres may be readily fermented and contribute to the total energy yield. Due to the anti-nutritional effect of dietary fibre, the energy from other nutrients may not fully count. The degree of fermentability therefore needs to be taken into consideration, since it is of major influence to dietary fibres reduction or contribution to the calorific yield. Even if fibre is fully fermented, the total energy may not be available for metabolism. With this in mind, the calorific value of dietary fibre, and the energy obtained through anaerobic fermentation will be discussed in larger detail in the following text.

The apparent digestibility of nutrients may be reduced by dietary fibre, and many studies have emphasized that the conversion factor of 8kJ/g (2 kcal/g) overestimates the energy value of NSP-rich diets (Goranzon et al., 1983, Göranzon and Forsum, 1987, Merrill and Watt, 1973). Göranzon and Forsum (1987) conducted two balance experiments with 20 healthy human subjects given two high fibre diets, providing between 33 and 74 g dietary fibre per day. The ME of both diets was calculated by use of the Atwater general and specific factors. It was concluded that the specific factors represented the most accurate values when calculating the ME of NSP-rich diets. The Atwater general factors have also been found to overestimate the ME of mixed diets by up to 11 % (Zou et al., 2007). Use of the specific values when determining the ME of NSP-rich diets has thus proven to be more accurate and pose as a more precise estimate (Brown et al., 1998, Zou et al., 2007). This assertion is supported by the findings of Wisker and Feldheim (1990), who studied digestibility when human subjects were given high- or low fibre diets based on fruits and vegetables. Digestibility proved to be lower when the high fibre diet was consumed. The specific factors and the factor expressed as monosaccharide by Southgate and Durin, were found to be most consistent with the predictable energy of the diets. The ME of dietary fibre from fruits and vegetables was estimated to contribute approximately 3kJ/g (Wisker and Feldheim, 1990). An analogous conclusion was reached when healthy, non-constipated, young women were given cereal

based mixed diets either high or low in dietary fibre, consisting of pasta, bread and bread rolls with varying contents of wholegrain flour. The low and high fibre diet contained 9,2g/d and 37,8g/d of dietary fibre from cereals. The high fibre diet was calculated to provide an additional 502kJ when compared to the low fibre diet, respectively. The total faecal and urinary energy losses were estimated to be 653kJ when consuming the high fibre diet, which exceed the additional 502kJ (Wisker et al., 1988). The proposed explanation is that the whole grain products of the high fibre diet may have promoted faecal bulk and excretion to a larger extent than the low fibre diet. Dietary fibre was estimated to provide 8kJ/g and 5kJ/g in the low and high fibre diet. Accordingly, the conclusion emphasized that digestibility was lower and more energy was lost than gained from the diet high in dietary fibre from cereals.

Increased fibre consumption is generally inversely related to fat and protein digestibility in mixed diets, in large due to increased faecal excretion and decreased nutrient absorption (Baer et al., 1997, Wisker et al., 1988). Increased quantity of dietary fibre naturally reduces the starch fraction of the diet. Additionally, interactions among fibres, fat and protein may lead to decreased apparent digestibility. Several clinical trials have investigated the digestibility of macronutrients when consuming a low fibre, high fat diet as opposed to a low fat, high fibre diet. They all concluded that digestibility was considerably lower when subjects consumed the high fibre, low fat diet (Baer et al., 1997, Miles, 1992). In addition to dietary fibres anti-nutritional effect on digestibility, the lower energy yield when consuming high fibre diets may be due to colonic degradation of starch. Starch hydrolysed in the small intestine yields approximately 4 kcal/g, however when reaching the colon only yields 3 kcal/g or less (Wisker et al., 1988).

Dietary fibre may thus decrease the nutritional value of food, but may also be fermented by the colonic microflora, which possesses enzymes that are able to degrade NSP. Anaerobic fermentation of dietary fibre results in SCFAs as the main end product and is the only way for humans to obtain energy from NSPs. For this reason, we get more energy from dietary fibre than what we are able to digest on our own (Cummings, 1981). Animal scientists have for a long time acknowledged short chain fatty acid metabolism as an additional energy source in ruminants. While concerning humans, interest in this field has increased over the past decades, simultaneously as knowledge of the positive effects anaerobic fermentation exhibits on colonic health. The energy yield provided to the body by SCFAs is somewhat 70-80% in

ruminants and 5-10% in non-ruminants, which explains the different levels of interest (D'Argenio and Mazzacca, 2000, Mortensen and Clausen, 1996). As aforementioned, the SCFA yield is closely related to fermentability, and it is estimated that up to three-quarters of the calorific value of NSP may become available to the body through SCFAs (Johnson and Southgate, 1994). On average this equals fermentation of approximately 70% of NSP and is in agreement with intestinal balance studies and net production rates (Elia and Cummings, 2007, Livesey and Elia, 1995).

At a baseline level, SCFAs may be present at concentrations of approximately 100mmol/l in the human colon (Clausen et al., 1991). Nevertheless, relatively few studies have been conducted with regards to dietary fibres calorific value in terms of SCFA production and absorption. Elia and Cummings (2007) proposed an illustration of the calorific yield from anaerobic fermentation of NSP and other unavailable carbohydrates in the human colon (Figure 5). The conversion factor of carbohydrates determined by Atwater, was applied as the energy value of NSP and unavailable carbohydrate from a cereal based, mixed diet. It was assumed that approximately ≈ 60% of NSP was fermented into SCFAs, of which most was absorbed and metabolised by tissues, or lost as faecal biomass. Of the initially available energy in dietary fibre, both fermentable and non-fermentable, 51% was lost to faeces, 3,5% to gaseous products and the remaining 45,5% accounted for metabolisable energy (Elia and Cummings, 2007). Additionally, the NME was determined by subtracting 5% lost as heat of fermentation and 5% as gaseous energy from the proposed ME. However, these values differ between individuals. Whole body calorimeter studies indicated that 3,8 ± 2,8% of fermentable energy was lost as gaseous energy (Poppitt et al., 1996).

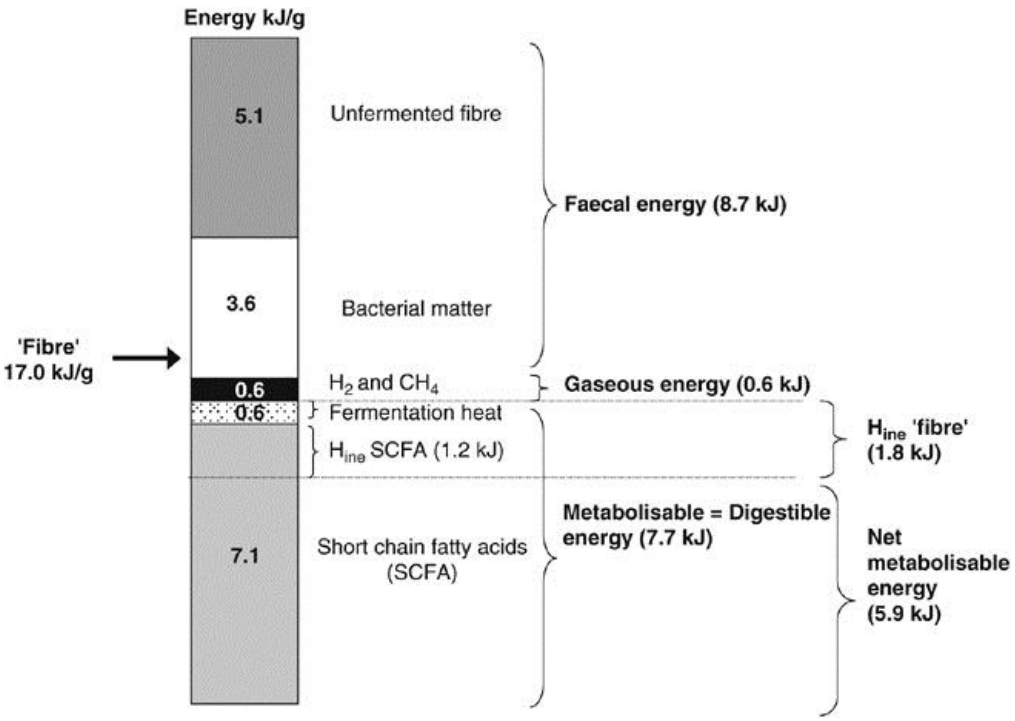

Figure 5 The calorific yield of cereal NSP when consumed in a cereal based mixed diet (Elia and Cummings, 2007).

Figure five provides an agreeable illustration of the energy contributed from cereal carbohydrate. However, the carbohydrate reaching the colon is assigned the energy conversion factor of 17kJ/g. This results in 7,7kJ/g of metabolisable energy, and may be attributed as an agreeable estimate for carbohydrates in NSP-rich diets. However, it is considered a very high calorific estimate for the energy obtained from SCFAs resulting from anaerobic fermentation of dietary fibre. Compared to energy- balance studies examining the effects of cereals in humans, the faecal loss is considered to be very high (Wisker et al., 1988, Livesey, 1990). The high faecal loss and the high calorific yield of SCFAs indicate Figure 5 to be unfit as an estimate for the energy yield from anaerobic fermentation in humans. Furthermore, the quantity of dietary fibre resulting in SCFAs is very diverse and is in addition to the anti-nutritive effect, a contributing factor to why 8kJ/g dietary fibre is assumed to overestimate the calorific value of NSP-rich diets. Livesey and Elia (1995) suggested 100g of fully fermentable carbohydrate to result in 61g SCFAs when mixed diets were given to humans. However, later findings indicated fermentation of starch to yield up to 60g SCFA/100g, while arabinogalactans and pectin yielded approximately 35 – 54g SCFA/100g (Cummings and Macfarlane, 1997). The rate of fermentation thus differs widely, varying

from practically zero, which is the case for Solka-Floc® cellulose, to 95% fermented with regards to pectin (Livesey and Elia, 1995). However, the rate of fermentation is not equivalent to the ratio of SCFAs produced. Guar gum, which is completely fermented, contributes approximately 60% of its energy to SCFAs, while pectin yields somewhat less. Furthermore large differences in the fermentation rate has been observed within humans consuming the same diet (Livesey and Elia, 1995, Southgate and Durnin, 1970). The source of dietary fibre is thus of major impact to SCFA production, quantity excreted as faecal biomass and gaseous flatus.

With consideration to the calorific value of SCFA, Merill and Watt (1955) were the first to propose organic acids in certain commodities as potential energy sources. Citrus fruits, cranberries, gooseberries, sauerkraut and pickled apples were some of the commodities used for measuring the organic acid content. The calorific value of acetic acid was determined by use of bomb calorimetry and found to be 14,6kJ/g, respectively. This is in accordance with later findings of the gross energy values determined by bomb calorimetry, which additionally estimated the values for propionate and butyrate. Acetic, propionic and butyric acid were estimated to contribute 14,6. 20,8 and 24,9kJ/g dry matter (McDonald et al., 2002). With respect to ATP, which yields 31kJ/mol of oxidised materiale, acetate, propionate and butyrate have been estimated to result in 8, 13.5 and 20 moles of ATP (McDonald et al., 2002). As noted the approximated base line concentration of SCFAs present in the human colon is equal to 100mmol/l. Considering that 100mmol equals 0,1mol puts a perspective view on the energy humans are able to obtain from SCFAs metabolism.

The variations in SCFAs produced are significantly dependent on fermentibility. Non-fermentable, unavailable NSP is assumed to contribute 0 kJ/g, while fully fermentable NSP is assumed to contribute 8 kJ/g (Livesey and Elia, 1995). Wheat bran has been found to contribute 4,2kJ/g, while β-glucan contributed 9,1kJ/g (Livesey, 1992). The calorific value of dietary fibre is thus highly dependent on the degree of fermentability when exceeding 20 g/day in mixed diets (Livesey, 1990). A proposed ME value based on these differences is suggested to be a better approximation of the energy derived from NSP rich diets. Dietary fibre contains both fermentable and non-fermentable fibre fractions, hence an intermediate value of 6,2 kJ/g is suggested to be a more accurate estimate (Livesey, 2001).

The energy obtained from dietary fibre through anaerobic fermentation is ultimately determined by the degradability of dietary fibre and susceptibility to the colonic microflora. SCFAs are rapidly absorbed and utilized by the human body, colonic health and function is thus closely related to the calorific end results of anaerobic fermentation. These in addition to the negative influence dietary fibre exhibits on energy utilization are the determining factors of the energy yield of dietary fibre. The findings of the reviewed studies indicate that dietary fibre decrease the energy value of diets by contributing to increased energy losses in relation to the intake. The conversion factor of 8kJ/g dietary fibre is thus assumed to overestimate the energy value of NSP-rich diets.

6. Pea fibre.

In addition to the literature analysis, experiments have been conducted with respect to pea hull fibre. Pea fibre may be very beneficial with respect to postprandial blood glucose and insulin secretion, which makes it an interesting source of fibre for scientific research regarding type two diabetes mellitus. This section of the thesis first reviews the anti-nutritional factors, which may be present in legumes and pulses, followed by pea fibres physiochemical properties, chemical features and effects of consumption. Additionally, fermentability of pea fibres and the resulting SCFAs will be reviewed.

6.1 Anti-nutritional components present in legumes and pulses.

Pulses and legumes have long been important constituents of human nutrition, primarily due to their easy cultivation, content of starch, protein and other nutrients. Legumes and pulses are rich sources of dietary fibre and are thus acknowledged for comprising anti-nutritional factors (Vadivel and Janardhanan, 2000, Vadivel and Janardhanan, 2001). Components included in this category are phytic- and oxalic acid, protease inhibitors, lectins, saponins and phenolic compounds, which may interfere with nutrient utilization (Martine, 2002).

Legumes may be rich in phytic- and oxalic acid, which may chelate metal ions such as zinc, calcium and magnesium and so influence the mineral bioavailability (Graf, 1983, Soetan and Oyewole, 2009). Additionally, as a result of evolution, plant protease inhibitors have evolved as a defence mechanism against insect attacks, damage to the plant etc. (Birk, 2003). Included in this category are, among others; trypsin-, chymotrypsin and α-amylase inhibitors, which have been found to possess both carcinogenic and anti-carcinogenic properties (Kennedy, 1993). High concentrations are found in animal and plant tissues, the most effective protease inhibitors possess chymotrypsin inhibitor activity and may be found in soybean, chickpea and potato (Martine, 2002). Amylase-inhibitors forms a complex with amylase, of which complex formation depend on multiple factors such as pH, temperature and inhibitor concentration (Thompson, 1993). Amylase-inhibitors may in this manner reduce the glycaemic response and may thus have a positive impact on insulin production and secretion (Taufel et al., 1991). Amylase-inhibitors are sold under the commercial name “Starch blockers”, but have failed to

prove effective in weight loss trials (Bo-Linn et al., 1982, Carlson et al., 1983). This is a field of great interest with concern to diabetic subjects as well as weight loss/regulation. However, the effect of amylase-inhibitors depends on the chemical purity. Improved glucose response was observed in healthy human subjects given 2,9g of purified inhibitor with a 650 calorie meal (Boivin et al., 1987). Decreased plasma glucose and insulin were also observed in healthy, normal and diabetic human subjects given purified amylase-inhibitor with 50 g of starch (Layer et al., 1986).

Lectins are found in the seeds of plants and are, like protease inhibitors, involved in plants defence mechanism towards insect attacks and other forms of degradation. Lectins, or phytohaemagglutinins, are sugar-binding proteins that specifically bind polysaccharides, and subsequently are able to bind and agglutinate red blood cells (Martine, 2002). Legumes are the main source of lectins in human nutrition and have exhibited toxicity through growth-inhibition in animal studies in addition to diarrhoea, nausea, bloating and vomiting in humans (Liener, 1989). Most lectins are toxic, though the degree may vary between species and proper heat treatment eliminates or reduce their toxicity due to denaturation of the lectine structure (Martine, 2002).

Saponins are commonly found in legumes, especially soy, and due to their amphipathic features, they have been used as detergents for centuries. They are glycosides composed of a lipid-soluble aglycone linked to a triterpene or steroid structure which is attached to water-soluble sugar residues (Martine, 2002). Saponins are acutely toxic if injected intravenously, due to interaction with cholesterol in the erythrocyte membrane. However, naturally occurring saponins in plants are non-toxic to humans (Birk et al., 1980, Oakenfull and Sidhu, 1990). Saponins are thus able to bind cholesterol and/or bind to bile acids and may exhibit hypocholesterolemic effects, which has been illustrated in numerous animal studies (Afrose et al., 2010, Afrose et al., 2009, Zhao et al., 2008).

When these compounds are reviewed, emphasis is regularly focused on the toxic and anti-nutritional effects. However, processes such as heat treatment, fermentation, in addition to sprouting and germination commonly leads to detoxification (Soetan, 2008). Legumes and pulses are commonly soaked and cooked before being consumed, which consequently reduce and/or inactivate the anti-nutritional components. Heat processing additionally increase starch

digestibility and makes the soluble dietary fibre fraction more readily available for digestion. This is due to redistribution of insoluble and soluble NSP and loss of non-fibre material such as free sugars (Periago et al., 1996). Peas are categorized as legumes, and are thought to offer a healthier nutrient composition than e.g. white bread, pasta or potatoes. Moreover, peas contribute dietary fibre to the diet, which has proven to exhibit positive effects on multiple health parameters. The positive impact of peas and pea residuals will now further be reviewed with regards to human health.

6.2 Pea fibres positive impact on human health, chemical composition and resulting SCFAs from anaerobic fermentation.

During the past decades attention has been turned to health benefits associated with pea consumption. Peas nutrient density enables them to meet the dietary needs of the estimated 800–900 million undernourished individuals worldwide, and is also a valid source of essential vitamins and minerals, such as selenium and folate (Dahl et al., 2012). Additionally the intermediate level of amylose causes digestibility of pea starch to be significantly lower in comparison to starch from refined cereals and potatoes. This was demonstrated when healthy, human subjects were given soups containing pea starch. An overall reduction of 54% in serum insulin concentration was observed when compared to corn starch (Seewi et al., 1999). The same results were obtained from a randomized controlled trial conducted with human subjects in a crossover study. Banana bread, biscotti and spaghetti containing either whole yellow pea flour or whole-wheat flour were studied for the effect on postprandial blood glucose. Each serve provided 50 g of available carbohydrate. Banana bread and biscotti, contained 100% whole pea flour and resulted in decreased postprandial blood glucose, while pasta exhibited no effect. The whole pea flour pasta contained only 30% pea flour, to make the product more palatable. The residual flour fraction consisted of white wheat durum, which may have caused insufficient blunting of the glycaemic response (Marinangeli et al., 2009). Peas are thus regarded as low glycaemic commodities (Hoover et al., 2010). Starch from peas (*Pisum sativum L.*) is also naturally gluten-free, and is hence an excellent choice for people who suffer from celiac disease (Hall, 2009). Several experiments based on animal nutrition have also demonstrated that peas may replace soy as a valid source of both starch and protein (Cozzi et al., 2010, Nalle et al., 2011). Additionally pea fibre possesses high water binding capacity and has been found to bind and hold more fluid than cereal fibre (Hall, 2009).

However, water holding capacity is generally greater for whole pea flour than for pea outer fibre (Daveby and Aman, 1993).

Peas serve as a rich source of NSP, which is located in the seed coat (outer fibre), commonly referred to as the hull, and the cotyledon (inner fibre) (Dahl et al., 2012). The hull is composed primarily of insoluble NSP, while the cotyledon contains NSPs of varying solubility (Guillon and Champ, 2002, Tosh and Yada, 2010). Pea cotyledon mainly consist of pectic polysaccharides, with arabinose and galacturonic acid as the primary monomers followed by xyloglucans and cellulose (Canibe and Bach Knudsen, 2002). Pea hulls have been estimated to contribute 857g/kg dietary fibre (NSP and lignin) of which cellulose make up the major proportion (Canibe and Bach Knudsen, 2002). These findings are in accordance with the ones of Lebet et al. (1998) who found pea hulls to contain 875±12 g/kg of dietary fibre, of which 62±8 g/kg was soluble and 813±4g/kg was insoluble. The insoluble fraction is composed of high levels of crystalline cellulose (83 – 84%), while the soluble fraction mainly consists of pectic polysaccharides and xylans (Canibe et al., 1997, Daveby and Aman, 1993). Pea hulls have a higher quantity of NSP than wheat bran, are virtually tasteless, and have useful physicochemical properties when included in ones diet (Weightman et al., 1994).

Pea hulls have proven to decrease dry matter digestibility (Cadden et al., 1983) and may in this manner acts as effective bulking agents. The intestinal transit time of elderly long-term care residents was significantly reduced by addition of 4 g/day to the diet (Dahl et al., 2003). Pea hull fibre may thus aid in normal bowel function and movements and is additionally assumed to blunt the insulin response in both normal and diabetic human subjects. Insulin resistance was compared in hypercholesterolaemic, overweight human subjects given muffins containing whole pea flour, pea hull fibre or white wheat flour equivalent to half a cup of pulses (approximately 50 g/d) (Marinangeli and Jones, 2011). Whole pea flour and the pea hull fibre reduced fasting insulin levels by 13,5% and 9,8%. Compared to the control group, the insulin resistance was reduced by 25% (Marinangeli and Jones, 2011). Pea fibre may thus positively impact the insulin response, which may also affect the glucose response in a positive manner. This was observed in a crossover experiment conducted in Norway including ten Pakistani immigrant women. Addition of pea hull fibre resulting in 17g dietary fibre/100g bread, reduced postprandial blood glucose. The women ingested regular coarse bread or pea hull fibre enriched-bread, with both providing 25 g available carbohydrates

(Lunde et al., 2011). The results indicated that pea hull fibre enriched bread significantly reduced postprandial blood glucose (Lunde et al., 2011).

The favourable fibre content is hence advantageous in prevention and treatment of T2DM and may additionally reduce cholesterol by decreasing reabsorption of bile acids (Trinidad et al., 2010, Cohn et al., 2010). Examination of pea fibres effect on cardiovascular health in young, healthy human subjects were found to reduce fasting and postprandial triglyceride concentrations. However, fasting cholesterol concentrations remained unchanged (Sandstrom et al., 1994). No significant results were observed in serum total -, HDL- or LDL-cholesterol from a crossover study with diabetic (T2DM) and non-diabetic human subjects, consuming peas (50g of available carbohydrate) for two weeks (Trinidad et al., 2010). The negligible effect may have been caused by the short study period or the amount of peas consumed. Significant improvement of cardiovascular parameters has been demonstrated in other studies of diets containing mixed legumes (lentils, chickpeas, peas and beans) (Abete et al., 2009, Hermsdorff et al., 2011).

Due to differences in solubility, pea hull fibre has been found to be fermented to a lower extent than pea cotyledon fibre (Lebet et al., 1998). Cellulose that has a higher quantity of amorphous regions is more susceptible to the colonic microflora. However, research indicates that the quantity of crystalline cellulose is not the factor of greatest impact to enzyme degradability and thus fermentability (Park et al., 2010). Naturally, the chemical composition of pea hull fibre is a contributing factor to low fermentation rates. But confounding factors such as water holding capacity, particle size, retention time in the digestive tract and microfloral composition are all of great influence to the fermentative the end results. Reducing the particle size slightly improved the water holding capacity of pea hulls, but limited effect was observed in fermentability (Guillon and Champ, 2000, Auffret et al., 1994). Fermentation rates were studied in pigs fed four diets containing dehulled barley, barley hull, pea cotyledon or pea hull. A drastic drop was seen in hydration levels from ileum/caecum to faeces within the pigs having consumed the diet containing pea hull fibre. The soluble fractions of pea hull fibre are degraded in the ileum/caecum, resulting in the most resistant fraction reaching the colon (Canibe and Bach Knudsen, 2002). Consequently, the degree of fermentation was higher for the diet containing pea cotyledon than the diet based on pea hull fibre.

As noted, dietary fibre is predominantly fermented in the proximal colon. However, slow depolymerisation of xylose and glucose from pea hulls results in low fermentation rates of these at the distal part of the colon (Canibe et al., 1997). In spite of the prolonged fermentation of these monosaccharides in the colon, SCFA concentrations have remained modest. Multiple studies have found moderate fermentation rates when including pea hulls in test diets, of which an acceptable estimate of acetic, propionic and butyric acid is approximated to equal the general molar ratio of 60:20:18 (Lebet et al., 1998, Guillon et al., 1995). Since most of the fermentable fraction has been degraded prior to reaching the colon pea hulls are fermented to a low extent. Addition of pea hulls to diets may thus lower the calorific value, since the energy made available through anaerobic fermentation is limited. Pea hulls may additionally contribute to decreased insulin secretion and postprandial blood glucose response, and is considered a valid fibre source that may exhibit positive health effects when replacing a fraction of starch in the diet of humans. This was found in the thesis of Ragnhild Tokvam Aas and the study of Marianne Haug Lunde. On this basis, the objective of this thesis was determined and will be described in larger detail in the following section.

7. Experimental data.

The positive effect of incorporating pea hull fibre in diets has made it an interesting field of research. When applied to bread, pea hull fibre has been estimated to contribute 0,95 kcal/g, which resulted in a decreased calorific value of 4,6 kcal/g added pea hull fibre (Aas, 2011). Blunting of postprandial blood glucose (Lunde et al., 2011) and insulin (Marinangeli and Jones, 2011), makes it a fibre source of great interest with regards to diabetes. Moreover, with consideration to pea hull fibres positive characteristics regarding taste and gluten-free status, it is considered a palatable and acceptable fibre application to commonly eaten foods.

This thesis has its origin in these findings and is based on continued research in these fields. Ragnhild Tokvam Aas and Marianne Haug Lunde both conducted studies that applied pea hull fibre, collected from yellow peas (*Pisum sativum* L.), which was pulverized and added to bread. The purpose of the experimental part of this thesis is to study the fermentability of pea hull fibre when added to bread and consumed by healthy human subjects. This was conducted by analysing the faecal samples collected during Ragnhild´s research. The fermentation rate was measured by using high-performance liquid chromatography. Additionally, the faecal and bread samples collected from Ragnilds work were analysed for quantity of neutral detergent fibre (NDF) to determine the NDF-digestibility of pea hull fibre.

Furthermore, pea hull fibres impact on healthy human subjects was studied by analysing the blood parameters collected after consumption of control bread and pea hull fibre enriched bread. The blood parameters, the results of Marianne Haug Lundes study, were statistically analysed to determine if significant difference was observed after consumption of pea hull fibre enriched bread, as compared to the control bread.
Ragnhilds thesis and Mariannes study will now briefly be reviewed, along with a short overview of the principles of high-performance liquid chromatography and NDF-analysis.

7.1 Ragnhilds thesis.

In 2011 Ragnhild Tokvam Aas conducted her thesis in public health at the Norwegian University of Life Sciences (UMB). With regard to the conversion factor stated by the European Union of 8kJ/g (2 kcal/g) dietary fibre, the aim was to determine the energy value of pea hull fibre. Concerning the global prevalence of increasing obesity, her hypothesis proposed that by replacing a fraction of carbohydrates in bread with pea hull fibre, the calorific value would decrease (Aas, 2011).

The experimental design included four identical diets consisting of breads and toppings, with the only differential factor being the fibre content. The four breads, denominated bread 1, 2, 3 and 4, was analysed to contain 9.8, 17.1, 24.4 and 29.8 g dietary fibre/100 g dry matter. Bread 1 mainly consisted of whole wheat and contained 9,8 g dietary fibre/100 g dry matter, it did not contain pea hull fibre and was used as the control bread. Bread 2, 3 and 4 were diluted with increasing quantities of pea hull fibre as a percentage of dry matter. Bread 2, 3 and 4 were analysed to contain 7,5. 15 and 22,5 g pea hull fibre/100g dry matter (Table 7.1).

TABLE 7.1 Dietary fibre and pea hull fibre contents of the test breads (g/100 g dry matter).

Bread	**Dietary fibre (g/100 g)**	**Pea hull fibre (g/100 g)**
1 – control.	9,8	0
2 – contain pea hull fibre.	17,1	7,5
3 – contain pea hull fibre.	24,4	15
4 – contain pea hull fibre.	29,8	22,5

Additionally, 0,5% of an indigestible marker, titanium dioxide (TiO_2), was added to each bread so that starch digestibility could be determined. Ten healthy men were recruited and given one test-bread each week over a period of four weeks. During each test week they were to only consume the selected bread for two days before faecal samples and blood glucose measurements were collected (Aas, 2011). A gelatine capsule containing E 142 (Brilliant green) was taken after lunch on the second day of each test period, to be used as a marker for collecting the correct faeces. After the faecal samples were collected they were packaged in double plastic bags and frozen before being freeze-dried and homogenized by use of a coffee grinder for three minutes.

Inclusion of pea hull fibre reduced the energy content of bread by 4,6 kcal per gram added pea hull fibre. This is largely due to the fact that pea hull fibre has a low energy value and is less readily fermented. The reduced energy content may also be a result of decreased blood glucose response and starch digestibility. The study supported the proposition that dietary fibre has a lower energy value than the factor of 8kJ/g determined by the EU directive (Aas, 2011).

7.1.2 High-performance liquid chromatography (HPLC).

High-performance liquid chromatography (HPLC) may determine and quantify organic acids and carbohydrates in organic solutions. It was thus the chosen analytical method to determine the quantity of short chain fatty acids (SCFA) produced after consumption of pea hull fibre enriched bread. The use of high-performance liquid chromatography has increased rapidly since the early 1970s, and has provided improved convenience and availability, in addition to improved resolving power detection and quantification (Lima and Abdalla, 2002).

The sample to be analysed is injected into the HPLC- apparatus, which contains a mobile phase that flows continuously through the apparatus by means of a pump producing high pressure. Upon injection of the sample, the mobile phase transports the sample through the column, which contains the stationary phase and is located at the heart of the apparatus. The column, which holds a positive charge, is the site where separation of the components of interest takes place. Separation occurs due to interactions of the stationary phase, the mobile phase and the sample. Organic acids predominantly have a negative charge, and for this reason may bind to the column-material (stationary phase) to varying degrees, and are then detected by an ultraviolet (UV)- and/or infrared (RI)-light-detector. The detector compare the samples to standards of organic acids, resulting in a chromatogram with different peaks, each indicating a certain organic acid (Østlie and Vegardud, 2012).

7.1.3 Neutral detergent fibre digestibility.

Digestibility of food may be measured by adding indigestible markers to the diet. This was done in Ragnhilds thesis to determine starch digestibility. Addition of 0,5% titanium dioxide to bread made it possible to determine the quantity of bread the faecal samples represented.

On this basis, it was decided that the neutral detergent fibre (NDF) digestibility should be calculated in this study. The quantity of titanium dioxide was known from Ragnhilds thesis, and it was necessary to determine the quantity of NDF in bread and faecal samples to calculate digestibility. NDF analysis was performed to determine the NDF content in the test breads and the faecal samples.

The NDF-analytical method, the filter bag technique, is based on the method of Ankom Technologies Corporation and has been widely accepted (ANKOM, 2011). The samples are placed in filter bags and rinsed with acetone prior to and after being heat treated with neutral detergent solution by using an Ankom^{200} Fibre Analyser. The neutral detergent solution dissolves the plant cell contents, while the cell wall remains intact. The non-dissolved fraction (NDF) consists of hemicellulose, proteins bound to the cell wall, cellulose, lignin and silicates. The soluble fraction "neutral detergent solubles" (NDS), which precipitates consist of lipids, sugar, organic acids, water soluble compounds, pectin, starch, nitrogen (not originating from proteins) and water soluble proteins.

Starch has limited solubility in neutral detergent solutions, and if not decomposed results in elevated NDF values upon analysis. Addition of the enzyme α-amylase, which hydrolyses starch into monosaccharides, results in decomposition and precipitation of the starch fraction. Applying heat stable amylases additionally inactivates interfering factors such as other enzymes, which may dissolve fibrous material. Following processing in the Ankom^{200} Fibre Analyser, the filter bags are dried prior to calculation of the NDF quantity of the samples. The result of this procedure is often referred to as aNDF, where *a* stands for amylase. Further in this thesis, for simplicity's sake, NDF will be used to describe aNDF.

7.2 Bread test conducted by Marianne S. H. Lunde at the faculty of medicine, the University of Oslo.

A research group at the University of Oslo found intake of pea hull fibre enriched bread containing 17g dietary fibre/100g, to considerably blunt postprandial blood glucose (PPG) as compared to regular bread (Lunde et al., 2011). These findings raised the question whether regular use of pea hull fibre enriched bread over a period of time, might also beneficially influence fasting values of blood glucose, and other diabetes/CHD risk factors. The interest of the experiment was based on the knowledge that 90 000– 120 000 Norwegians were diagnosed with diabetes mellitus in 2004 (Stene et al., 2004). Additionally an increased percentage of type 2 diabetes was observed, of which younger people are affected every year (Nolan et al., 2011).

They recruited eighty healthy, ethnic Norwegian volunteers of both sexes above 25 years of age. The subjects were to replace their habitual bread with the test breads throughout the study. The study comprised of two experimental periods of 19 – 22 days, that each was separated by a washout period of two weeks to ensure that one test period had no residual effect on the next. The breads used in the study had the same basic ingredients, but differed in that the fibre bread was added pea hull fibre as the major fibre component, in addition to minor quantities of alginate, pectin and dextrose as listed in Table 7.2.
The control bread consisted mainly of wheat and contained 4,5 g dietary fibre/100 g dry matter. The fibre bread was added pea hull fibre in such quantities that g dietary fibre/100g dry matter increased from 4,5g/100g to 13,5g/100g. The breads were assigned by drawing of lots and the subjects consumed the control and the fibre bread in a randomized 2 x 2 crossover design.

TABLE 7.2 Ingredients list of the control and fibre bread.

Bread	**Ingredients listed in decreasing order.**	**Dietary fibre/100g dry matter.**
Control bread	Water, whole wheat flour, wheat flour, canola oil, dried sourdough of wheat, yeast, salt, wheat gluten, emulsifier (E472e), wholemeal rye, flour treatment agent (E300), enzymes.	4,5 g/100g
Fibre bread	Water, wheat flour, pea fiber, whole wheat flour, wheat gluten, canola oil, dried sourdough wheat, yeast, salt, alginate, pectin, dextrose, emulsifier (E472e), wholemeal rye, flour treatment agent (E300), enzymes.	13,5 g/100g

During the test periods the subjects were to exchange their bread of habitual choice with the bread they were assigned. They were requested to consume bread for at least two meals every day. It was essential that they did not alter their diet or level of physical activity during the experimental period (this was actually an exclusion criteria). They were also asked to document the amount of bread they consumed each day. Midway in the study period they were given a questionnaire to make sure that they had not changed their lifestyle and eating habits. During consultations the subjects were asked to meet fasting, having only ingested water from 21.00pm the night before. The blood parameters were measured by fasting blood samples and included: blood glucose, cholesterol, triglycerides, uronic acid, HDL- and LDL-cholesterol, insulin, C-peptide, mCRP, weight, height, diastolic and systolic blood pressure and pulse. Fasting blood glucose was additionally measured by submission of blood samples to Fürst. All subjects were to attend four consultations of which consultation one was a blank test, consultation two was conducted after the first test period. Consultation three was

conducted after the washout period, before the final test period, and finally consultation four was conducted after the last test period. Good correlation was observed between bread habit and the consumed amount during the test periods, however no significant differences were observed in blood parameters.

The results have been evaluated in relation to pea hull fibres effect on blood parameters, and whether consumption of pea hull fibre enriched bread will be of benefit as compared to control bread. Out of the initially eighty enrolled subjects, seventy completed the study protocol. The quantity of consumed bread slices ranged from 26 – 174 for fibre bread, and from 26 – 168 for the control bread. All of the reported quantities of bread slices are included in the calculations.

7.2.1 Research questions.

1. How will increasing the quantity of pea hull fibre in bread from 0 to 22,5g/100g affect colonic fermentation in humans? Will there be a significant difference in the SCFA ratio by means of increasing quantities?
2. To what extent will increasing quantities of pea hull fibre affect NDF-digestibility? Is NDF-digestibility associated with fibre fermentability?
3. How will increasing the quantity of dietary fibre from 4,5g/100g to 13,5g/100g by adding pea hull fibre to bread influence blood parameters such as glucose, insulin, triglycerides and cholesterol?

7.2.2 Hypotheses.

1. Fermentation of pea hull fibre will be moderate, and increased levels of pea hull fibre in bread will have insignificant impact on the fermentation rate.
2. NDF-digestibility will decrease simultaneously as increasing levels of pea hull fibre. NDF-digestibility is associated with fermentability.
3. Reduced postprandial blood glucose, insulin, triglycerides and cholesterol levels are expected after consumption of the fibre bread in comparison to the control bread.

8. Materials and methods.

8.1 Sample preparation technique for HPLC – analysis.

The freeze-dried faecal samples collected from the ten healthy men after consumption of the four test diets was analysed for short chain fatty acids. This amounted to a total of forty freeze-dried faecal samples that were subsequently prepared and analysed. The sample preparation technique used in this work is based on the method for determining organic acids in dairy products (Marsili et al., 1981). The standard method for preparation of samples for HPLC-analysis of dairy products is performed as follows:
Mixing 1 gram of sample with 2,5 ml of ionised water (dH_2O), 0,2 ml of 0,5 M sulphuric acid (H_2SO_4) (Merck, Darmstadt Germany) and 8,0 ml acetonitrile (CH_3CN) (Merck).

Modification of the method to determine the optimal quantity of sample and solvent for accurate readings was conducted by diluting samples with different quantities of solvent. One sample was prepared by using 1 gram of faeces mixed with 2ml of ionised water. However, 1 gram was too much and the sample had to be further diluted for the UV-detector to be able to analyse it. It was determined that 0,5 grams of faecal sample mixed with 2,5 ml of ionised water corresponded to a dilution that the UV-detector could read.

The samples were mixed in Eppendorf tubes (VWR, Pennsylvania, USA) before being centrifuged (Centrifuge 5804 R, Hamburg, Germany) at 2500 rpm for ten minutes. Some samples were additionally run at 5000 rpm and 8000 rpm to see if this was more beneficial. It was concluded that 2500 rpm was sufficient. Following centrifugation, the supernatant was extracted and sulphuric acid and acetonitrile were added. 100µl of sulphuric acid was added to the first sample, followed by addition of 2ml of acetonitrile at a time. It was determined that addition of 200µl of sulphuric acid and 8ml of acetonitrile diluted the sample sufficiently. The samples were then mixed (Multi RS-60, Biosan) for 30 minutes before being centrifuged (Kubota 2010, Tokyo Japan) at 3500 rpm *(1470 g)* for 15 minutes. Finally the samples were transferred to vials (Chromacol LTD, Herts UK) by use of a syringe filter (PTFE 13mm Syringe filter, pore size 0,2µm Becton Dickinson) and syringe (Becton Dickinson, Madrid, Spain), before being sealed with caps (VWR). The syringe was rinsed with acetone (Sigma-Aldrich, St. Louis, USA) between transfers of each sample.

Laboratory personnel subsequently analysed the samples by use of the HPLC-apparatus consisting of an auto-injector 200 series (Perkin Elmer: Shelton, Connecticut, USA), pump series 200 (Perkin Elmer), UV detector 200 series (Perkin Elmer), RI detector series 200 (Perkin Elmer) and LC oven 101 (Perkin Elmer). Software System, Total Chrom version 4.1 (Perkin Elmer) and 900 serial interface (Perkin Elmer).

The organic acids were separated on an Aminex HPX-87H column (Bio Rad: Hercules, CA, USA). The mobile phase consisted of 5 mM sulphuric acid, flowing at 0,4 ml / minute at 32°C.

The rate of acetate and propionate may be determined by HPLC analysis, while GC is required to analyse the quantity of butyrate. Hence, the ratio of acetate and propionate were the only determined SCFAs in this thesis. SCFAs are generally identified by means of UV – detection (Perkin Elmer), at 210 nanometres. When detecting acetic acid, an unknown component eluted simultaneously, and detection had to be conducted by RI (infrared) – detection (Perkin Elmer). Propionic acid detection was achievable by UV – detection.

8.2 Conversion factor for the HPLC – results.

The standard method for preparing HPLC-samples is based on the use of 1 g sample, when preparing the samples for this thesis, 0,5 g was used. A conversion factor was calculated and multiplied with the determined parts per million (ppm) values to estimate the correct quantities of SCFA from 1g of human faeces.

Standard solution for dairy products:

1 gram sample + 2,5 ml H_2O + 0,2 ml H_2SO_4 + 8,0 ml acetonitrile

2,5 ml H_2O + 0,2 ml H_2SO_4 + 8,0 ml acetonitrile = 10,7 ml

$\frac{1g}{10{,}7ml}$ = 0,0934 g/ml

Faecal samples: 0,5 gram sample + 2,5 ml H_2O + 0,2 ml H_2SO_4 + 8,0 ml acetonitrile

$\frac{0{,}5g}{10{,}7ml}$ = 0,0467 g/ml

Conversion factor: $\frac{0{,}0934\ g/ml}{0{,}0467\ g/ml}$ = **2**

8.3 Neutral detergent fibre analysis.

The total of forty freeze-dried faecal samples and the four breads from the test diets was analysed for neutral detergent fibre (NDF). This equalled forty-four samples, which were subsequently analysed for NDF-quantity by laboratory personnel. The analytical method was based on the method of Ankom Technologies Corporation (ANKOM, 2011). The filter bag technique is based on 0,5g of sample being weighed and heat sealed in filter bags, which retain 95% of particles larger than 30µm. The filter bags (F57, Ankom, Fairport, N.Y) were weighed prior to addition of samples (W_0) and after 0,5 g of sample was applied (W_1). The filter bags were placed in a glass jar, subsequently covered with acetone (Sigma) and sealed, before turned twelve times followed by ten minutes of infusion. Rinsing with acetone was performed twice.

Air-drying of the bags was subsequently carried out in a fume cabinet until the bags were dry, and the odour of acetone had vanished completely. When the filter bags were completely dry, they were placed in the bag compartment in the Ankom^{200} Fibre Analyser (Ankom 220, Fairport, N.Y.), and immersed in neutral detergent solution. Thereafter 20 g of sodium sulphite (VWR), 4 mL heat stable α-amylase (Ankom) and 1900 to 2000 mL of NDF-solution (Ankom) was added before the Ankom^{200} Fibre Analyser was run for 75 minutes. The bag compartment was then emptied of solution, and rinsing for five minutes with 4 mL of heat stable α-amylase and 1900 to 2000 mL of NDF-solution commenced. This was performed twice, followed by one final round of rinsing without α-amylase. Subsequently, the bags were placed in a beaker, filled with acetone and allowed to infuse for 3 – 5 minutes before drying in the fume cabinet. The bags had to be completely dry and odour free before they were dried overnight at 103 ± 2 °C, and then cooled in a desiccator and weighed (W_2). Finally, calculation of the quantity of NDF was performed, using the formula:

$$\frac{(\mathrm{W2} - (W0 \times F))}{W1} \times 1000 = quantity\ NDF\ in\ sample\ (\frac{g}{kg})$$

W_0 = weight of bag

W_1 = weight of sample weighed

W_2 = weight of sample + bag after drying

F = correction factor bag = 0,9987

Neutral detergent fibre (NDF) digestibility was calculated according to the following equation:

EQUATION 8.3 NDF – digestibility.

$$1 - \left(\frac{titanium\ concentration\ in\ bread}{titanium\ concentration\ in\ faeces}\right) \times \left(\frac{NDF\ concentration\ in\ faeces}{NDF\ concentration\ in\ bread}\right)$$

8.4 Blood parameters from Marianne S. H. Lundes study.

The blood parameters from the human subjects were grouped according to fibre- and control bread. The quantity of bread slices consumed during the test periods were then analysed for each subject. When the data had been sorted, the quantity of consumed bread slices were plotted against the blood parameters in a scatter plot. The parameters that were analysed and processed in this thesis includes insulin, blood glucose, cholesterol (total, HDL and LDL) and triglycerides.

8.5 Statistical analyses.

Linear regression analysis was conducted for SCFA production, NDF-quantity in bread and faeces, and blood parameters by use of Microsoft Excel 2011, which additionally generated standard deviation analysis for the SCFA results.

The significance of NDF-digestibility in relation to pea hull fibre in bread was determined by use of one-way analysis of variance, conducted by use of the GLM procedure within a 95% confidence interval.

Conducting a T-test within a 95% confidence interval by use of Microsoft Excel 2011 generated the p-values of the blood parameters. The p-values generated from the analysis of variance and the T-test may be interpreted in the following manner:

$P < 0,01$ indicates a tendency towards significant effects.

$P < 0,05$ indicates significant effects.

9. Results.

9.1 Short chain fatty acids produced from anaerobic fermentation of pea hull fibre.

SCFA production was highest for subjects having consumed the control bread, which contained 9,8 g dietary fibre/100 g. Bread 2, 3 and 4, which contained 7,5. 15 and 22,5 g pea hull fibre/100g and resulted in marginally different values. As illustrated by Figure 9.1.1 and 9.1.2, standard deviation indicated no significant differences between the SCFA ratios in relation to increasing quantities of pea hull fibre.

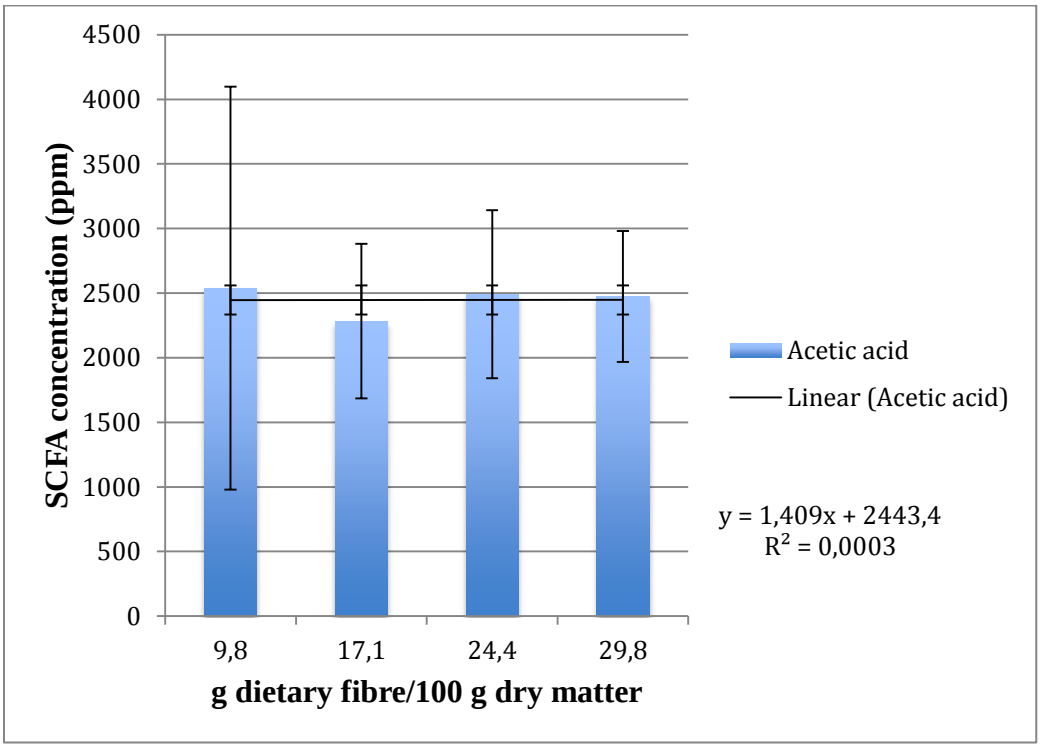

Figure 9.1.1 Standard deviation and linear regression analysis for the average concentration of acetic acid produced after consumption of bread 1 (containing 9,8 g dietary fibre/100 g), 2, 3 and 4 (containing 17,1. 24,4 and 29,8 g dietary fibre/100 g). Generated by use of Microsoft Excel 2011.

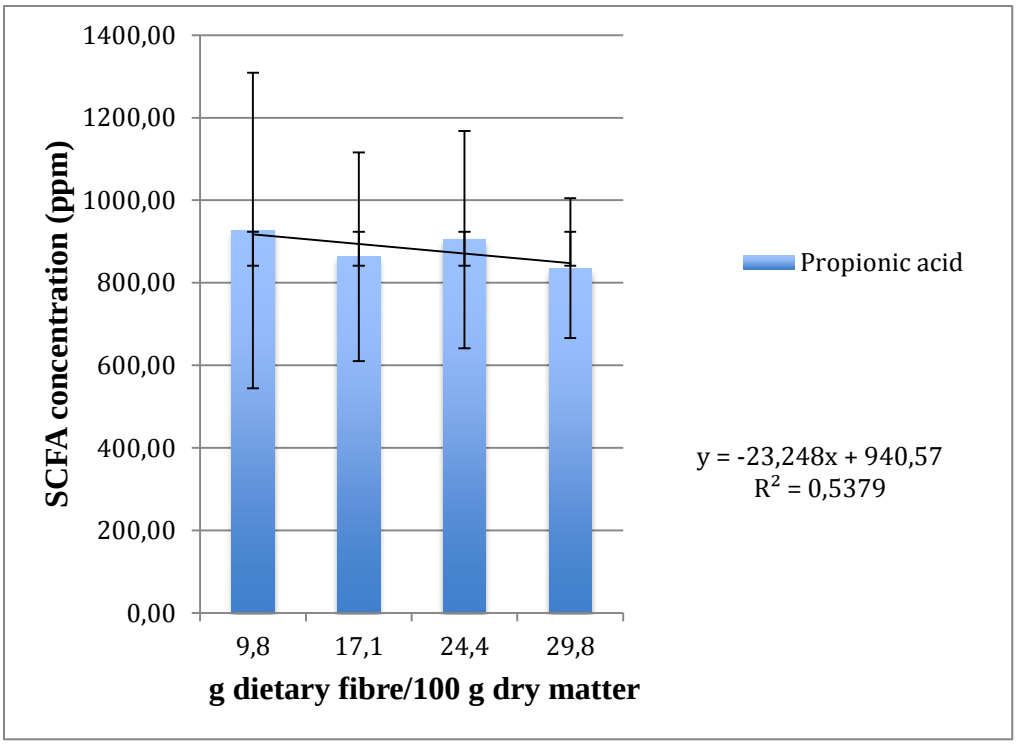

Figure 9.1.2 Standard deviation and linear regression analysis of the average concentration of propionic acid produced after consumption of bread 1 (containing 9,8 g dietary fibre/100 g), 2, 3 and 4 (containing 17,1. 24,4 and 29,8 g dietary fibre/100 g). Generated by use of Microsoft Excel 2011.

9.2 NDF – Digestibility of pea hull fibre.

NDF excreted as faecal biomass was in good correlation with which bread the subjects had consumed (Figure 9.2). One-way analysis of variance demonstrated NDF-digestibility to be significantly lower for bread 3 and 4 in comparison to the control bread. Bread 2 was not different from either of the other breads (Table 9.2).

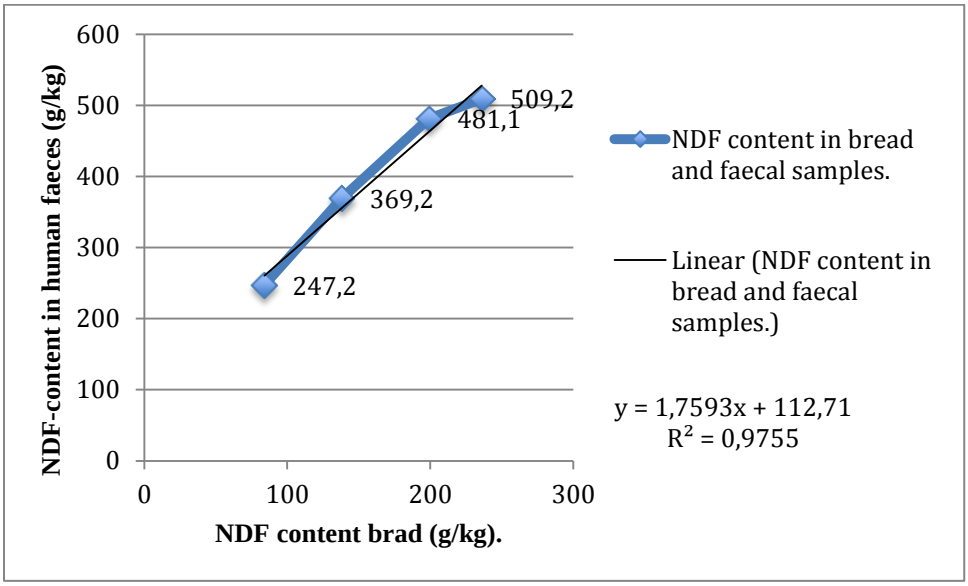

Figure 9.2 Linear regression analysis of the average NDF content (g/kg) present in test bread and excreted as human faeces, generated by use of Microsoft Excel 2011.

Table 9.2 NDF-digestibility of pea hull fibre consumed as bread 1, 2, 3 and 4. Generated by one-way analysis of variance, by use of the GLM procedure with a 95% confidence interval in relation to pea hull fibre content in breads.

REGWQ Grouping		Bread	NDF-digestibility
	A	1	0,36
B	A	2	0,28
B		3	0,22
B		4	0,24

REGWQ: Ryan-Einot-Gabriel-Welsch Q multiple comparison test, dissimilar letters signify significant differences, similar letter indicate insignificantly differences; NDF: Neutral detergent fibre.

9.3 Effects on blood parameters by consumption of pea hull fibre enriched bread.

Linear regression analysis and T-test indicated insignificant differences in the blood parameters by increasing the quantity of dietary fibre in bread from 4,5g/100g to 13,5g/100g by adding pea hull fibre.

Table 9.3.1 Linear regression analysis of blood parameters after consumption of control- and fibre bread, generated by use of Microsoft Excel 2011.

Blood parameters	Bread	R^2 value	Slope
Insulin	Control bread	0,015	0,111
	Fibre bread	0,011	0,075
Blood glucose	Control bread	0,064	0,0045
	Fibre bread	0,095	0,0053
Blood glucose (Fürst)	Control bread	0,062	0,0038
	Fibre bread	0,074	0,0049
Triglycerides	Control bread	0,00039	- 0,0004
	Fibre bread	0,00428	0,0012
Total cholesterol	Control bread	0,013	0,0046
	Fibre bread	0,028	0,0059
HDL-cholesterol	Control bread	0,00025	- 0,0002
	Fibre bread	0,00456	- 0,0009
LDL-cholesterol	Control bread	0,018	0,0048
	Fibre bread	0,033	0,0059

R^2: the coefficient of determination; Slope: gradient of a straight line; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 9.3.2 Mean values of blood parameters after consumption of control- and fibre bread and the resultant p-values generated by use of T-test in Microsoft Excel 2011.

Parameter	Control bread	Fibre bread	P-value
Insulin (mmol/l)	50,467	46,083	0,394
Blood glucose (mmol/l)	4,972	4,987	0,865
Blood glucose (Fürst) (mmol/l)	5,266	5,313	0,564
Triglycerides (mmol/l)	1,242	1,249	0,933
Total cholesterol (mmol/l)	5,629	5,636	0,970
HDL-cholesterol (mmol/l)	1,468	1,470	0,965
LDL-cholesterol (mmol/l)	3,575	3,556	0,917

P-value: calculated probability; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

10. Discussion.

The purpose of this thesis was to conduct an extensive literature review of dietary fibre and the effect non-starch polysaccharides exhibits on human health. Increased consumption of soluble dietary fibre may exhibit hypocholesterolaemic effects, blunt postprandial blood glucose and the insulin response, while insoluble fibre primarily contributes to increased faecal bulk and decreased intestinal transit time. Dietary fibre has been found highly effective in decreasing the nutrient availability and thus the calorific value of food. Determining the energy value of food is thus important in nutritional research. For this reason the development of energy systems and the conversion factors for determining the energy value of nutrients were reviewed. Dietary fibre was reviewed in relation to the accuracy of the conversion factor of 8kJ/g dietary fibre. The findings of the reviewed studies indicate that 8kJ/g dietary fibre tends to overestimate the energy value of NSP-rich diets.

In addition to the literature analysis, data from studies regarding the effect of adding pea hull fibre to bread were processed and analysed. The purpose was to determine the fermentability of pea hull fibre and the extent to which NDF-digestibility of pea hull fibre was affected when consumed by healthy, human subjects. Additionally, the effect of adding pea hull fibre to bread in relation to blood parameters was studied. The results indicate a low fermentation rate, which was not significantly affected by increased quantities of pea hull fibre in the diet. NDF-digestibility decreased as a function of increasing pea hull fibre content, and was found to be significantly different for bread three and four in relation to the control bread. No significant differences were observed within blood parameters.

The possible significance of the experimental results in relation to other scientific findings is discussed in the following text, along with possible sources of error. Since pea hull fibre is the source of research, the discussions are assessed based on this, and does not encompass other fibre components of peas.

10.1 Pea hull fibres impact on colonic fermentation.

How will increasing the quantity of pea hull fibre in bread from 0 to 22,5g/100g affect colonic fermentation in humans? Will there be a significant difference in the SCFA ratio by means of increasing quantities?

10.1.1 SCFA production resulting from anaerobic fermentation of pea hull fibre.

The present study indicates no significant differences in the fermentation rate in relation to increased pea hull fibre content in bread when consumed by normal, healthy human subjects. The ratio of acetic acid was more stable (Figure 9.1.1) than propionic acid (Figure 9.1.2), which decreased to a larger extent as a function of added pea hull fibre. The proportion of pea hull fibre that was fermented indicated an initially low fermentability, which slightly decreased as the fibre amount increased. However, no significant correlation was observed (Figure 9.1.1 and 9.1.2). It may therefore be suggested that moderate fermentation of pea hull fibre takes place, and is negligibly affected by quantity in the diet. Similar results were obtained in a recent study, which fermented pea hulls in vitro with dog faecal inoculum (Calabro et al., 2013). As aforementioned, the findings of Lebet et al. (1998) and Canibe and Bach Knudsen (2002) emphasized similar results, demonstrating fibres of more soluble features to be fermented to a greater extent. The insoluble features of pea hull fibre make it particularly inaccessible to the colonic microflora, and it was expected that SCFA production would be modest. The observations of Canibe et al. (1997), suggests that even though distal fermentation of glucose and xylose from pea hull fibre occurs during colonic fermentation, SCFA concentrations remain modest.

As expected, acetic acid was the major SCFA produced, followed by propionic acid. The R^2 – value for propionic acid production was equal to 0,53787, which means that 53,7% of the variance in SCFA-production is related to the amount of pea hull fibre in the different breads (Figure 9.1.2). However, standard deviation of the mean values demonstrated that there were no significant differences between the produced quantities of propionic acid as a result of increasing quantities of pea hull fibre (Figure 9.1.2). Large internal variations in the SCFA production rate, exceeding 1000ppm, were observed in both acids. Standard deviation of the mean values proved these variations to be insignificant for acetic and propionic acid (Figure

9.1.1 and 9.1.2). Several in vitro fermentation studies, have reported large variations in SCFA production, depending on the faecal flora and substrate fermentability (McBurney and Thompson, 1989, Livesey and Elia, 1995). It appears that pea hull fibre passed the colon without severe degradation, wich was verified by the NDF-recovery in faecal samples compared to the quantity in breads (Figure 9.2). The hypoyhesis that fermentation of pea hulls would be moderate, and SCFA production would be insignificantly affected by increasing the quantity of pea hull fibre is thus retained. Possible sources of error may have occurred as a result of the study design, as noted the faecal samples were packaged in double plastic bags and frozen prior to freeze-drying and homogenization. A proportion of the short chain fatty acids may have evaporated prior to or during freeze-drying, which may have influenced the results. Moreover, the samples were stored frozen for more than one year prior to this study, which may have caused further degradation of the SCFAs and consequently influenced the results.

10.2 The significance of pea hull fibre in relation to NDF – digestibility.

To what extent will increasing quantities of pea hull fibre affect NDF-digestibility? Is NDF-digestibility associated with fibre fermentability?

10.2.1 NDF – digestibility of pea hull fibre.

The poor susceptibility of pea hull fibre to colonic degradation is reflected in NDF-digestibility. The quantity of NDF in the four test breads and faecal samples from the human subjects provides a good indication of digestibility of pea hull fibre in the human colon (Figure 9.2). The p-value generated by one-way analysis of variance for NDF-digestibility was determined to be 0,0054, which is lower than 0,05 and thus regarded as significant. NDF-digestibility was significantly different for bread three and four, when compared to the control (Table 9.2). The quantity of pea hull fibre in bread three and four equalled 15 and 22,5g/100g dry matter. It is therefore reasonable to assume that digestibility of pea hull fibre is lower than that of wheat fibre in the control bread, and when applied to bread equal to or above these quantities will decrease NDF-digestibility. Similar results were observed in an animal feeding study, of which pigs were fed different sources of fibre. Digestibility of pea hull fibres was found to decrease as the level of incorporation increased (Dierick et al., 1989). This is

supported by the findings of Stanogias and Pearce (1985), who fed pigs' pea hulls at four levels of NDF. Pea hulls were added to feed in such a manner that NDF-content increased from 75 – 300 g/kg dry matter. The higher NDF-content resulted in significant ($P < 0,001$) lower apparent digestibility of NDF and indicated a consistent level of decline when added pea hull fibre (Stanogias and Pearce, 1985). In addition to decreased NDF-digestibility, it is reasonable to assume that pea hulls may decrease the digestibility and absorption of other nutrients. This is supported by Aas (2011) who found a significant decrease in starch digestibility as a function of increased pea hull fibre in bread. Several authors have made similar observations, nutrient digestibility proved to decrease nearly linearly with increasing NDF-content from pea hull fibre in the diet of pigs (Wenk and Zürcher, 1990, Cadden et al., 1983).

Based on these findings it is considered reasonable to assume that addition of pea hull fibre may decrease the calorific value of diets in addition to energy absorption of nutrients. The hypothesis that NDF-digestibility will decrease simultaneously as increasing levels of pea hull fibre is thus retained.

10.2.2 SCFA production in relation to NDF – digestibility.

NDF-digestibility decreased as a function of increasing quantities of pea hull fibre in bread. Significant differences in NDF-digestibility were observed for bread three and four as compared to the control bread (Table 9.2). Despite the slight decrease in SCFA production, there were no significant differences between the control- and the fibre breads (Figure 9.1.1 and 9.1.2). Based on earlier studies, these results were as expected and confirms that pea hull fibre passes the colon without severe fermentation (Calabro et al., 2013, Guillon et al., 1995). As indicated by Barry et al. (1995), solubility is closely related to fermentability. Significant association between NDF-digestibility and total SCFA production was observed in vegetable and cereal fibres, of which greater NDF-digestibility generated increased SCFA concentrations (McBurney and Thompson, 1990). NDF-digestibility may thus provide an indication of fermentability. However, since NDF-digestibility decreased and SCFA production was insignificantly affected by increasing quantities of pea hull fibre, the hypothesis that NDF-digestibility is associated with fibre fermentability must be discarded in this thesis.

10.3 The effect pea hull fibre enriched bread elicits on blood parameters.

How will increasing the quantity of dietary fibre from 4,5g/100g to 13,5g/100g by adding pea hull fibre to bread influence blood parameters such as glucose, insulin, triglycerides and cholesterol?

10.3.1 Influence on blood parameters.

Addition of pea hull fibre to bread resulting in increasing the quantity of dietary fibre from 4,5 g/100g to 13,5 g/100g did not result in significant differences in this study. None of the regression analysis of the blood parameters resulted in R^2 – values exceeding 0,09, most were considerably lower (Table 9.3.1). This means that at best 9% of the variance in blood parameters is related to the amount of pea hull fibre in the fibre bread. Furthermore, the p-values generated from the T-test were very high, ranging from 0,39 for insulin to 0,97 for total cholesterol (Table 9.3.2). The T-test was conducted within a 95% confidence interval, which indicates statistical significance if the P-values are below 0,05. The p-values generated in this study thus indicated no differences between the groups.

Regression analysis of the postprandial blood glucose (PPG) measured directly and submitted to Fürst, indicated no significant differences by consumption of pea hull fibre enriched bread (Table 9.3.1). The p-values ($p = 0,86$ and $p = 0,56$) additionally indicated insignificant differences in PPG between the control- or fibre bread (Table 9.3.2).
Although inclusion of pea hull fibre failed to result in significant differences in this study, other studies have successfully demonstrated pea hull fibres blunting effect on PPG. As aforementioned Lunde et al. (2011) found blunting of PPG when adding pea hull fibre to bread equal to 17g dietary fibre/100g. Similar results were observed when comparing wheat bran to pea fibre, of which the latter induced a reduction of 65% in PPG in normal human subject when added as 15 g/100g (Hamberg et al., 1989). Marinangeli et al. (2011) induced decreased circulating glucose levels in hamsters ($p = 0.03$), by replacing 10 % of corn starch with pea hull fibre. Based on these findings, it may be assumed that by adding pea hull fibre to diets, PPG may decrease.

There were no significant differences in insulin secretion after consumption of the control- or fibre bread. Both resulted in R^2 – values equal to 0,01 (Table 9.3.1) and a p-value of 0,39 (Table 9.3.2). This study thus failed to demonstarte declined insulin levels as an effect of pea hull fibre, however this has been demonstarted by other studies. Hamberg (1989) observed a reduced insulin response, although there were no significant differences from the control. Marinangeli and Jones (2011) found the insulin resistance to decrease significantly ($P<0.05$), compared to the control diet in hypercholesterolaemic and overweight human subjects. Marinangeli et al. (2011), demonstrated reduced insulin levels ($p = 0.032$), as compared to the control diet. A recent study found pea hull fibre to significantly decrease fasting and glucose-stimulated insulin secretion ($P<0,05$), when included as 8 % in rats diets over a period of four weeks (Whitlock et al., 2012). These findings imply that pea hull fibre may blunt the postprandial insulin response, however further research is needed for sufficient evidence on pea hull fibres impact on the insulin response in normal, human subjects.

None of the blood lipid parameters were significantly affected by consumption of fibre bread (Table 9.3.1 and 9.3.2). The p-values of the blood lipids were all above 0,9, which indicates no significant differences between the control and the fibre group. Similar results have been observed in hamsters, pea hulls produced negligible effects on circulating cholesterol and triglyceride levels as compared to the control (Marinangeli et al., 2011). However, prior to the study, the hamsters were fed a hypercholesterolemic diet, which may have influenced the results. Several authors such as, Hermsdorff et al. (2011) and Sandstrom et al. (1994) have demonstrated significant improvement in cardiovascular parameters. Sandstrom et al. (1994) found pea fibre to significantly decrease fasting and postprandial triglyceride concentrations ($P<0,05$) in young, healthy human subjects. However, the studies indicating improved blood lipid profiles as induced by consumption of pea fibre, include consumption of whole peas, and have proven to be very effective with regard to LDL-cholesterol and triglycerides (Martins et al., 2004). The hypocholesterolaemic effect has so far only been elicited by whole pea fibre containing the cotyledon, involving the soluble dietary fibre fraction of peas. The modest effect elicited by pea hull fibre on cholesterol and triglyceride levels, is most likely linked to the insoluble features of pea hulls. As noted, soluble dietary fibre has proved to significantly lower serum blood lipid concentrations, whole pea fibre may thus pose as a more efficient component for this particular purpose.

However, type 2 errors may be an erroneous acceptance of the null hypothesis, namely that there is no correlation between pea hull fibre intake and blood parameters. A type 2 error is defined by rejection of an alternative explanation, which actually is correct (over cautiousness). As previously referred, several studies have demonstrated improved concentrations of insulin and PPG when including pea hull fibre in diets. The confined effect in the present work may have been induced by several factors. The study design may have caused limitations for pea hull fibre to induce significant results. The subjects were requested to consume the given test breads for at least two meals every day. This reflected the natural bread habits of the participants, and consequently resulted in great varieties in quantity of bread slices consumed by subjects within both test periods. The variance in intake may have contributed to insufficient blunting of the blood parameters. The experimental design was thus unfit to determine whether addition of pea hull fibre to bread equal to 13,5g dietary fibre/100 g dry matter, would exhibit any effects on blood parameters.

11. Conclusion.

The results of the studies in this literature review indicates that increased dietary fibre intake will positively influence multiple health parameters in humans. Dietary fibre is also acknowledged for decreasing the nutritive value of food and may thus be effective in prevention of development of lifestyle diseases. The reviewed studies supports the assertion that the energy value of 8kJ/g dietary fibre stated by the EU directive, is assumed to result in large miscalculations when determining the energy value of NSP-rich diets.

The present study has also demonstrated that fermentability of pea hull fibre is limited, and results in moderate SCFAs concentrations. Moreover, significant differences in NDF-digestibility were observed when pea hull fibre was added to bread at quantities equal to or above 15g pea hull fibre/100g dry matter. The decreased NDF-digestibility as a result of increasing quantities of pea hull fibre indicates an initially low fermentability, which is lower than that of fibre from wheat.

Addition of pea hull fibre in such quantities that g dietary fibre/100g dry matter increased from 4,5g to 13,5g, resulted in insignificant alterations in insulin, PPG, triglycerides and cholesterol (total, HDL- and LDL-). As noted, significant effects have been demonstrated by other studies. The study design was thus unfit to determine whether addition of pea hull fibre to bread, equal to 13,5g dietary fibre/100 g dry matter, would alter blood parameters when consumed as bread by healthy, human subjects. There is a possibility that eventual effects elicited by pea hulls diminish in other interaction effects. Nevertheless, consumption of products containing pea hull fibre may help increase the daily intake of dietary fibre to the recommended and additionally offer health benefits. However, further research is needed to determine the effect pea hull fibre exhibits in healthy, humans.

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13. Attachments.

Attachment 1. Detected ppm values of acetate and propionate after consumption of the four pea hull fibre enriched breads.

		Propionic acid/UV-detektor		Acetic acid/RI-detektor		
Bread	Sample ID	ppm	Calculated ppm values	ppm	Calculated ppm values	g dietary fibre/100g dry matter.
1	21	730,19	**1460,38**	2916,45	**5832,90**	**9,8**
1	22	607,31	**1214,62**	1477,92	**2955,84**	**9,8**
1	23	359,28	**718,56**	710,32	**1420,64**	**9,8**
1	24	275,81	**551,62**	788,31	**1576,62**	**9,8**
1	25	595,63	**1191,26**	1408,74	**2817,48**	**9,8**
1	26	783,3	**1566,60**	2487,19	**4974,38**	**9,8**
1	27	328,65	**657,30**	783,68	**1567,36**	**9,8**
1	28	233,2	**466,40**	487,07	**974,14**	**9,8**
1	29	447,15	**894,30**	911,01	**1822,02**	**9,8**
1	30	273,5	**547,00**	723,52	**1447,04**	**9,8**
2	1	324,31	**648,62**	1171,79	**2343,58**	**17,1**
2	2	381,27	**762,54**	937,97	**1875,94**	**17,1**
2	3	498,15	**996,30**	1449,52	**2899,04**	**17,1**
2	4	360,82	**721,64**	999	**1998,00**	**17,1**
2	5	577,44	**1154,88**	1055,38	**2110,76**	**17,1**
2	6	655,28	**1310,56**	1828,71	**3657,42**	**17,1**
2	7	520,62	**1041,24**	1293,23	**2586,46**	**17,1**
2	8	430,33	**860,66**	1043,97	**2087,94**	**17,1**
2	9	201,61	**403,22**	729,45	**1458,90**	**17,1**
2	10	365,5	**731,00**	905,97	**1811,94**	**17,1**
3	31	255,9	**511,80**	955,64	**1911,28**	**24,4**
3	32	661,81	**1323,62**	1651,08	**3302,16**	**24,4**
3	33	525,17	**1050,34**	1718,15	**3436,30**	**24,4**
3	34	290,19	**580,38**	1325,51	**2651,02**	**24,4**
3	35	418,48	**836,96**	961,94	**1923,88**	**24,4**
3	36	618,68	**1237,36**	1616,85	**3233,70**	**24,4**
3	37	517,96	**1035,92**	1422	**2844,00**	**24,4**
3	38	529,08	**1058,16**	1106,49	**2212,98**	**24,4**
3	39	340,38	**680,76**	858,41	**1716,82**	**24,4**
3	40	364,22	**728,44**	842,36	**1684,72**	**24,4**
4	11	299,32	**598,64**	1242,97	**2485,94**	**29,8**
4	12	335,02	**670,04**	970,85	**1941,70**	**29,8**
4	13	555,31	**1110,62**	1609,06	**3218,12**	**29,8**
4	14	386,97	**773,94**	1402,89	**2805,78**	**29,8**
4	15	469,24	**938,48**	1376,03	**2752,06**	**29,8**
4	16	502,86	**1005,72**	1558,16	**3116,32**	**29,8**
4	17	442,66	**885,32**	1261,52	**2523,04**	**29,8**
4	18	488,26	**976,52**	1169	**2338,00**	**29,8**
4	19	405,09	**810,18**	1023,75	**2047,50**	**29,8**
4	20	292,98	**585,96**	755,65	**1511,30**	**29,8**

***Bold typeface shows the values used in the calculations.**

Attachment 2. Analysed quantities of neutral detergent fibre (NDF) g/kg in test breads and human faeces.

		NDF content	Mean value
	Sample ID	g/kg	
	Bread 1	84	
	Bread 2	138	
	Bread 3	199	
	Bread 4	236	
Bread 1	Faeces 21	260	247,2
	Faeces 22	289	
	Faeces 23	213	
	Faeces 24	245	
	Faeces 25	243	
	Faeces 26	237	
	Faeces 27	248	
	Faeces 28	232	
	Faeces 29	251	
	Faeces 30	254	
Bread 2	Faeces 1	340	369,2
	Faeces 2	361	
	Faeces 3	370	
	Faeces 4	379	
	Faeces 5	379	
	Faeces 6	383	
	Faeces 7	394	
	Faeces 8	319	
	Faeces 9	390	
	Faeces 10	377	
Bread 3	Faeces 31	473	481,1
	Faeces 32	463	
	Faeces 33	517	
	Faeces 34	520	
	Faeces 35	488	
	Faeces 36	502	
	Faeces 37	448	
	Faeces 38	466	
	Faeces 39	469	
	Faeces 40	465	
Bread 4	Faeces 11	439	509,2
	Faeces 12	512	
	Faeces 13	515	
	Faeces 14	513	
	Faeces 15	479	
	Faeces 16	527	
	Faeces 17	504	
	Faeces 18	511	
	Faeces 19	548	
	Faeces 20	544	

Attachment 3. Measured blood parameter after consumption of control bread (4,5g dietary fibre/100g dry matter).

Glu	Kol	TG	HDL	Insulin	LDL	FG	Quantity of bread slices consumed.
4,9	7	2,41	1,3	82	5	5,3	116
5,3	5,2	0,87	1,2	126	3,7	5,0	142
5,1	6,8	0,61	2,9	46	2,9	5,5	108
5,3	7,7	1,27	2,1	57	4,6	5,3	56
6,1	6,3	1,65	0,9	59	5,1	6,3	74
5,3	6	0,7	2,1	33	3,2	5,2	60
4,7	5,1	0,8	1,1	57	3,4	5,1	86
4,9	4,7	0,76	1,1	76	3,3	4,8	63,5
4,6	5,6	0,98	1,5	93	3,5	4,5	26
5,3	3,6	0,86	1,1	55	2	5,3	92
4,7	4,3	0,64	1,5	23	2,4	4,9	168
4,5	7,6	1,46	1,5	28	5,4	5,6	138
5,5	4,3	0,9	0,9	85	3	5,6	77
5,4	5,4	1,85	1	70	3,6	5,8	99
4	6	2,06	1,4	108	4	5,0	66
5	4,2	0,71	1,2	68	2,5	5,5	92
4,7	6,6	0,98	2,2	20	3,4	5,3	86
4,3	3,9	1,15	1,3	40	2	5,0	28
4,9	5,3	1,44	1,2	46	3,5	5,1	
4,5	3,8	0,9	1,5	20	1,9	4,8	57
4,6	5,9	1,38	1,4	33	3,7	5,2	120
4,6	4,3	1,24	1,7	38	2	4,8	
4,9	6,8	1,5	1,3	116	5,1	5,2	86
4,5	6,2	0,81	1,9	21	3,7	5,6	63
4,8	4,3	0,75	1,4	25	2,5	5,0	42
5,6	5,2	1,36	1	54	3,8	5,9	65
5,7	6,6	2,63	0,9	79	4,3	6,0	92
5,2	4,8	1,57	0,9	96	3,5	6,0	
4,8	4,9	0,73	1,8	21	2,6	5,1	70
5,3	7,3	1,55	1,5	76	5,4	5,8	73
5	7,4	2,33	1,4	56	5,2	5,3	42
4,9	6,9	1,35	1,1	49	5,5	5,5	87
6,7	4,9	0,8	1,4	46	3	6,7	
4,8	5,9	0,84	1,3	27	4,4	5,2	55
6,2	3,8	0,83	1,3	68	2	6,1	100
5,9	7,8	1,29	1,9	30	5,6	5,3	
5,2	6,3	2,06	1,2	46	4,1	5,7	65
4,7	7,1	1,19	1,7	33	4,9	4,9	96

5,5	5,4	2,53	1,4	86	3,1	5,6	81	
5,2	4,9	1,36	1,3	20	3	5,1	56	
4,6	4,7	0,76	1,5	18	2,5	4,9	98	
4,4	5,5	0,57	2,1	29	2,9	4,7	68	
6,4	5	0,91	1,4	59	3,1	6,0	103	
5,1	8	1,96	1,6	45	5,5	5,5	89	
5	3,8	0,89	1,6	29	1,7	5,1	58	
5,2	5,7	1,08	1,5	72	3,6	5,5	46	
4,7	6,2	1,59	1,3	48	4,2	5,2	60	
4,9	5,3	0,9	1,7	39	2,9	4,8	38	
4,6	6,1	0,57	1,6	16	3,8	4,6	65	
4,6	7,1	0,96	2,1	34	4,4	4,8	69	
5,6	5,5	1,17	1	34	3,9	5,9	108	
4,3	7,5	1,31	1,8	14	5	5,0	77	
3,7	4	0,71	1,2	40	2,4	4,5	36	
5,5	7,2	1,01	2,1	34	4,6	6,2	74	
5,1	5,6	2,07	1,2	45	3,2	5,5		
6,1	6,7	1,21	1,6	139	4,9	6,8		
4,4	5,4	0,83	2,1	25	2,7	5,2	47	
5	4,9	2,19	1,4	78	2,9	5,2		
4,7	4,9	1,76	1,1	59	2,9	5,2	57	
4,6	5,7	1,3	1,5	20	3,9	5,1		
5,7	5,6	0,61	2,1	25	3,1	6,2	114	
4,5	5,9	0,92	1,3	48	4,3	5,4		
4,6	5,4	2,23	1,2	163	3,5	5,0		
4,5	4,6	0,44	2	30	2,2	5,2		
4,7	6,2	1,21	1,4	27	4,2			
5,1	5,8	1,63	1	52	4	5,7	37	
4,6	3,9	0,65	1,6	23	2	5,2	34	
5	4,7	0,76	1,6	53	2,5	5,6	73	
4,3	5,3	0,66	1,8	21	3,1	5,0	68	
4,6	5,1	1,16	1,1	20	3,5	4,9		
4,6	5,2	0,91	1,5	41	3,2	4,3		
5,4	6,9	2,39	1,5	38	4,5	6,0	37	
5	6,5	2,72	1	50	4,9	5,6		
4	3,8	1,11	1	76	2,1	5,1		
4,7	6,4	0,88	1,8	29	4,2	5,1		
4,972	5,62933333	1,24173333	1,468	50,4666667	3,57466667	5,266	57,1133333	**Mean**
6,7	8	2,72	2,9	163	5,6	6,83333333	168	**Max**
3,7	3,6	0,44	0,9	14	1,7	4,33333333	26	**Min**

Glu: Fasting blood glucose; **Kol**; Total cholesterol; **TG**: Triglycerides; **HDL**: High-density lipoprotein cholesterol; **LDL**: Low-density lipoprotein cholesterol; **FG**: Fasting blood glucose measured by submission at Fürst.

Attachment 4. Measured blood parameter after consumption of fibre bread (13,5g dietary fibre/100g dry matter).

Glu	Kol	TG	HDL	Insulin	LDL	FG	Quantity of bread slices consumed.
5,2	4,2	1,21	1,2	19	2,4	5,2	54
5	4,6	0,72	1,5	18	2,3	5,2	99
5,4	7,3	2	1,6	50	4,8	5,8	85
4,6	4,2	0,87	1,8	59	2	5,0	45
5,7	6,2	2,89	1,1	68	4,2	6,4	101
6	6,9	1,33	1,9	40	4,3	6,5	71
5,3	5,7	1,39	1,3	175	3,9	5,7	
5	5,5	0,73	2	34	3	5,3	58
5,5	5,6	1,81	1,4	142	3,7	5,9	
4,6	5,4	3,05	1	83	3,1	5,5	
5,6	5,6	0,59	1,9	33	3,3	5,8	113
4,6	5,4	2,23	1,2	163	3,5	5,0	
4,7	6,2	1,21	1,4	27	4,2		
4,6	5,1	1,16	1,1	20	3,5	4,9	49
4,8	6,3	1,88	1,3	45	4,1	5,6	37
5,3	3,9	1,41	1,1	56	1,9	5,6	56
5	6,8	0,87	1,2	58	5,3	5,2	146
4,4	5	0,82	1,1	63	3,3	5,0	119
5,1	6,9	0,67	2,8	26	3,1	5,2	71
5,6	7,2	1,03	2,1	26	4,2	5,8	77
5,2	4,6	0,84	1,1	86	3,1	5,1	42
4,6	3,7	0,42	1,3	14	2,1	5,1	55
4,7	3,8	0,4	1,4	29	2	4,8	150
4,3	5,9	2,13	1,3	57	4,1	4,4	38
4,8	4,8	1,01	1,3	66	2,9	5,5	76
4,6	5,1	1,1	1,2	63	3,3	5,3	
5,8	4	1,31	1,4	58	2	5,2	
4,6	4,3	0,66	2	30	1,8	5,6	
4,7	6,2	0,68	2	23	3,7	5,1	64
5,6	6,9	1,91	0,9	42	5,2	6,1	84
4,9	4,7	0,74	2	22	2,2	5,7	54
6	6,6	1,48	1,5	81	4,9	6,2	82
4,6	7,7	1,92	1,6	32	5,5	5,5	
6,3	4,7	0,71	1,3	24	3,1	7,0	
5,1	5,5	0,66	1,4	27	4	5,8	61
5,2	6,3	2,06	1,2	46	4,1	5,7	
5,4	5,5	1,55	1,6	57	3,1	5,7	109
4,5	5,6	0,58	2,3	17	2,7	4,5	104
6,2	4,7	0,69	1,5	31	2,8	6,8	109
4,2	4,3	0,79	1,2	23	2,6	4,5	27
4,3	5,7	1,46	1,2	45	3,8	5,3	84
4,8	5	0,41	1,7	15	2,9	5,2	52
4,7	6,6	0,72	1,5	28	4,3	4,8	65
4,1	7,9	0,81	2,3	37	5	4,9	60
5	7,5	1,28	1,8	20	5	5,1	76
4,4	4,5	1,07	1,3	30	2,8	4,8	66
5,1	5,6	2,07	1,2	45	3,2	5,5	50
4,6	5,7	1,3	1,5	20	3,9	5,1	
4,4	6,1	1,43	1,4	48	4,2	5,2	57
4,1	4,8	0,85	2,1	22	2,1	4,7	61
4,8	6	1,84	0,9	58	4,2	5,2	46
4,1	4	0,65	1,5	20	2,1	4,3	33
5	4,3	1,2	1,2	30	2,2	5,5	84
4,4	5,4	1	1,8	21	3,1	4,4	63
4,6	5,2	0,91	1,5	41	3,2	4,3	
5,2	6,3	2,12	1	30	4,8	5,7	
4,7	6,4	0,88	1,8	29	4,2	5,1	
5,8	6,2	2,06	0,9	62	4,5	5,9	106
4,7	6	0,75	1,9	27	3,3	5,1	60
4,6	5,4	1,03	1,5	34	3	5,3	75
4,7	5,5	0,66	1,5	33	3,5	4,4	26
5,2	7,8	1,36	1,5	30	5,5	5,4	174
5,8	4,4	0,79	0,9	53	3	6,0	92
5,8	6,4	2,36	1	76	4,6	6,0	94
4,5	6,1	0,71	2	19	3,2	5,2	86
4,5	5,8	2,17	1,4	63	3,6	5,0	98

4,7	6,2	1,78	1,2	123	4,6	5,3	60	
5,3	4,9	1,14	0,9	54	3,6	5,9	50	
5,9	6,7	2	1,2	88	5,1	6,3	90	
5,7	3,8	1,25	1,3	37	1,9	5,8	95	
5,7	7,1	1,43	1,8	22	4,9	6,4	36	
4,6	7,6	0,99	1,7	25	5,5	5,1	105	
4,9875	5,63611111	1,24986111	1,47083333	46,0833333	3,55694444	5,31388889	59,4375	**Mean**
6,3	7,9	3,05	2,8	175	5,5	6,96666667	174	**Max**
4,1	3,7	0,4	0,9	14	1,8	4,3	26	**Min**

Glu: Fasting blood glucose; **Kol**; Total cholesterol; **TG**: Triglycerides; **HDL**: High-density lipoprotein cholesterol; **LDL**: Low-density lipoprotein cholesterol; **FG**: Fasting blood glucose measured by submission at Fürst.