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Intake of sourdough bread and effects on gut symptoms and gut microbiota compared to baker's yeast bread – a randomized controlled study

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Abstrakt

Bakgrunn: Surdeigsbrød er sett å ha mindre innhald av FODMAPs, og kan derfor vere ei alternativ kjelde til fiber for dei som opplev mageplagar.

Hensikt: Hensikta med denne studia er å undersøke endringar i tarmsymptom og tarmflora etter inntak av surdeigsbrød samanlikna med gjærbrød.

Metode: Studia er ei dobbeltblinda randomisert kontrollert overkrysset studie med varigheit på totalt fem veke. Tjue friske deltakarar som rapporterte skepsis til å ete brød og/eller milde til moderate mageplager ved inntak av brød, vart rekruttert. Deltakerane fekk både surdeigsbrød og gjærbrød og måtte ete minst 200 g dagleg, i ei veke kvar. Bortsett frå hevingsmiddel var oppskrifta lik for begge brøda. Begge brøda vart analysert for næringsinnhold. Tarmsymptom vart målt med ved hjelp av spørjeskjema etter kvar visitt, og avføringsprøver vart avlagt dagen i forvegen. Avføringsprøvene vart analysert av Bio-me, for 107 forskjellege artar av bakteriar.

Resultat: Surdeigsbrødet var oppdaga å innehalde mindre FODMAPs, og spesielt fruktose, samanlikna med gjærbrødet. Spesielt fruktose var lavare. Total score på tarmsymptom og diare var signifikant forskjelleg mellom gruppene (P=0.033, 0.043, henholdsvis), med reduksjon etter surdeigsbrød og auking etter gjærbrød. Vidare vart fleire bakterieartar sett å vere signifikant auka etter inntak av surdeigsbrød samanlikna med gjærbrød: *Alistipes putrenidis* (P=0.042), *Alistipes Shahii* (P=0.015), *Bifidobacterium adolscentis* (p=0.042), *Bifidobacterium longum* (p=0.012), og *Bifidobacterium longum subsp longum* (P=0.027).

Konklusjon: I denne studia fann vi at surdeigsbrød reduserte tarmsymptom og auka førekomsten av fleire gunstige tarmbakteriar samanlikna med gjærbrød. Desse resultata indikerer at dei som opplev mageplager tøler surdeigsbrød betre enn gjærbrød, og at dette kan skuldas det lavare innhaldet av FODMAPs.

Abstract

Background: Sourdough bread has been shown to contain less FODMAPs than baker´s yeast bread, and therefore might be an alternative for people with gastrointestinal symptoms.

Aim: The aim of the present master project was to investigate gut symptoms and changes in the gut microbiota upon consuming sourdough bread compared to bread baked with yeast.

Methods: This randomised double-blind controlled cross-over study lasted for five weeks. Twenty healthy participants reporting scepticism towards consuming bread and/or mild to moderate gut symptoms upon consumption of bread were recruited. Participants were supplied with either sourdough bread or baker´s yeast bread for one week each and consumed a minimum of 200 g of bread daily. Except for the leaving agent, both types of bread were baked using a similar recipe. Gut symptoms experienced by the participants were measured using questionnaires on the day of visitation, and faecal samples were collected a day in advance. The faecal samples were analysed for 107 different species of bacteria.

Results:
The sourdough bread was found to contain less FODMAPs–fructose in particular–than the baker´s yeast bread. Gastrointestinal symptoms and diarrhoea levels differed significantly across the two groups (P= 0.033, 0.043, respectively). Sourdough bread yielded reduced levels, and baker's yeast bread yielded increased levels. Several bacteria species were found to be prominently expressed in the faecal samples of the participants who consumed sourdough bread and reduced in those who consumed baker's yeast bread. These species included: *Alstipes putrenidis* (P=0.042) *Bifidobacterium adolscentis* (p=0.042), *Bifidobacterium longum* (p=0.012), *Bifidobacterium longum subsp longum* (P=0.027), and *Alistipes Shahii* (P=0.015).

Conclusions: The findings indicate that, compared with baker's yeast bread, sourdough bread consumption cause less gut symptoms and leads to the proliferation of several gut bacteria. This suggests that people experiencing gut symptoms may be more tolerant of sourdough bread. This may be due to sourdough bread's lower levels of FODMAPs.

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Abbreviations

BSC	Bristol Stool Scale
CNS	Central nervous system
CRF	Case report form
CRP	C-reactive protein
CVD	Cardiovascular disease
DF	Dietary fibre
FFQ	Food frequency questionnaire
FGID	Functional gastrointestinal disorders
FODMAPs	Fermentable Oligo-, Di-, Monosaccharides and Polyols
FOS	Fructo-oligosaccharides
g	Grams
GBA	Gut-brain-axis
GI	Gastro-intestinal
GOS	Galacto-oligosaccharides
GSRS-IBS	Gastrointestinal Symptom Rating Scale-irritable bowel syndrome
H_2	Hydrogen
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant irritable bowel syndrome
IBS-D	Diarrhoea predominant irritable bowel syndrome
IBS-M	Mixed irritable bowel syndrome (diarrhoea and constipation)
IBS-U	Unsubtyped irritable bowel syndrome
INSDF	Insoluble dietary fibre
IQR	Interquartile range
kcal	Kilocalorie
kg	Kilograms
kJ	Kilojoule
LMWDF	Low Molecular weight dietary fibre
mmol	Millimol
NCD	Non-communicable diseases
NSP	Non-starch polysaccharide

NSD	Norwegian Service of Research Data Approval
PI-IBS	Postinfectious IBS
RCT	Randomised Controlled/Clinical Trial
REK	Regional Committees for Medical and Health Research Ethics Sør-Øst
RO	Resistant Oligosaccharides
RS	Resistant Starch
SCFA	Short chain fatty acid
SD	Standard deviation
T2D	Type 2 diabetes
TG	Triglyceride
SDF	Soluble dietary fibre
V	Visit
y	Years

1.0 Introduction

1.1 Gastrointestinal disorders

The burden of health problems related to the gastrointestinal tract has become widespread, and its global incidence has risen in the past century (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014). Gastrointestinal diseases are diseases which can affect the entire gastrointestinal tract, from the oesophagus to anus. In recent years, there has been a particular increase in gastrointestinal disorders in Europe (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014), with the highest rates of incidence being seen among the very young and the elderly. Additionally, as the European population continues to age, this burden is expected to increase (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014). While there is substantial variation in the trends of diseases in different countries, issues in the lower abdomen have been most commonly observed across the globe (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014). Many factors have been observed to influence the gastrointestinal tract and its motility, including stress, medication, pregnancy, exercise and diet (Sperber et al., 2021). Thus, it is not uncommon for many people to experience problems related to the gut (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014). In most cases, symptoms are harmless and will pass with time. However, in cases of persistent or severe discomfort, gastrointestinal conditions may require medical attention as well as changes to one's diet and lifestyle habits. The severity of these symptoms can vary greatly depending on the specific type of gastrointestinal disease or on the individual. Gastrointestinal disorders often complicate everyday life and are a great burden to those affected (Saha, 2014). Even in patients who report mild symptoms, a gastrointestinal diagnosis is associated with more frequent doctor visits, increased medicinal use and a lower quality of life. Moreover, studies indicate that individuals who seek help only account for half of those with gastrointestinal disorders that should be attended to. Many of these conditions, if inadequately treated, can lead to potentially life-threatening complications (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014).

Conditions in the gastrointestinal tract are often defined as either structural or functional disorders (Sperber et al., 2021). Structural disorders are those in which structural abnormalities

result in bowel dysfunction, often leading to corrective surgery (Maunder, 1998). The most common diagnoses of structural disorders include haemorrhoids, diverticular disease, colon polyps, colon cancer and inflammatory bowel disease (IBD) (Anderson et al., 2014; Farthing et al., 2014; Roberts et al., 2014). IBD, which primarily includes Chrone's disease, ulcerative colitis and indeterminate colitis, is believed to affect more than 6.8 million people worldwide and is characterised by a non-infectious chronic inflammation of the gastrointestinal tract (Alatab et al., 2020; Podolsky, 1991).

Functional gastrointestinal disorders, however, do not involve structural or biochemical abnormalities but rather disorders of the functions of the gastrointestinal tract. Functional gastrointestinal disorders can affect any part of the gastrointestinal tract, including the oesophagus, stomach and intestine, and are more prevalent than structural disorders (Sperber et al., 2021). Functional gastrointestinal disorders are often classified in accordance with the concerned area. The intestine alone includes sub-categories which include IBS, functional bloating, functional constipations and functional diarrhoea (Amundsen, 2016; Saha, 2014). Functional disorders have a prominent place within "functional somatic syndromes" (Saha, 2014). Consequently, x-rays, blood tests, and endoscopies are often inconclusive and are not applicable diagnostic procedures. Functional gastrointestinal disorders can be related to any combination of motility disturbances, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota and altered central nervous system processing (Sperber et al., 2021). Additionally, a spectrum of psychiatric commodities is frequently present in people with functional gastrointestinal disorders, including stress, anxiety and depression (Riedl et al., 2008; Staudacher, Mikocka-Walus, & Ford, 2021). Even today, functional gastrointestinal disorders are generally less understood than structural disorders and, in most cases, highly challenging to both diagnose and manage.

1.2. Irritable Bowel Syndrome

1.2.1. Prevalence, definition and diagnosis

IBS is the most common functional gastrointestinal disorder and is defined by the World Health Organisation (WHO) as "a functional bowel disorder in which recurrent abdominal pain is associated with defecation or a change in bowel habits" (Fraberger, Call, Domig, & D'Amico, 2018; Quigley et al., 2012). Current data indicates that 7–15% of the population worldwide is affected by IBS, with an estimated prevalence of 12% in Europe and 10% in Norway (Struyf, Verspreet, & Courtin, 2018, Werlang, Palmer & Lacy, 2019, Fraberger et al., 2018). Additionally, in Western countries, IBS has been observed to develop more frequently in women than in men (Adeyemo, Spiegel, & Chang, 2010). However, despite its substantial prevalence and global impact, IBS is still poorly understood (Sperber et al., 2021). IBS is a multifactorial disorder (Enck & Mazurak, 2018) which mostly affects the large intestine but can affect the small intestine in some cases (Amundsen, 2016; Quigley et al., 2012). Typical IBS symptoms are abdominal pain, bloating and altered bowel habits combined with intermittent diarrhoea or constipation (Bellini et al., 2020; Saha, 2014). IBS patients can be further categorised into subtypes according to their most prevalent symptoms. These subtypes include predominant diarrhoea IBS (IBS-D), predominant constipation IBS (IBS-C) and a mix of diarrhoea and constipation IBS (IBS-M). Patients who do not fit into any of these subtypes are categorised as unsubtyped (IBS-U). However, it is not uncommon for patients to alternate between classifications over time. Additionally, bacterial gastroenteritis has been identified as a risk factor for IBS, and patients who develop IBS as a consequence are classified as postinfectious IBS (PI-IBS) (Raskov, Burcharth, Pommergaard, & Rosenberg, 2016).

There are currently no diagnostic biomarkers available for IBS; symptoms often vary between individuals and can have striking similarities to those of other gastrointestinal disorders (Fraberger et al., 2018). Therefore, diagnosing IBS can be challenging (Fraberger et al., 2018; Werlang, Palmer, & Lacy, 2019). Currently, IBS is diagnosed using a combination of several symptoms-based diagnostic tools, including the recoding of personal medical history, a physical examination (Werlang et al., 2019) and use of the Rome IV criteria. In general, abdominal pain must occur in association with at least one symptom, including altered stool

frequency, relief of pain following defecation and altered stool form or appearance (Hellström & Benno, 2019). The symptoms must occur at least one day per week in the last three months and must have been recurring at least six months prior to the diagnosis. Symptoms such as pain and change in bowel habit are frequently used to distinguish IBS from other functional gastrointestinal disorders (Hellström & Benno, 2019).

1.2.2 Pathogenesis of IBS

The exact pathophysiology of IBS remains uncertain, but several factors have been implicated in the pathogenesis of IBS, including dysregulation in the gut-brain axis (GBA), altered gastrointestinal motility and gut microbiota, bacterial overgrowth, food sensitivity, carbohydrate malabsorption, intestinal inflammation, immune activation, visceral hypersensitivity, post-infectious reactivity and brain-gut interactions (El-Salhy, Hatlebakk, Gilja, Kristoffersen, & Hausken, 2020; Meydan et al., 2020; Saha, 2014).

Both a dysregulated BGA and altered gut microbiota are believed to be central in the pathogenesis of IBS symptoms (Raskov, et al., 2016; Werlang et al., 2019). The GBA is a bidirectional neuro-humoral pathway that integrates brain and gastrointestinal functions (Raskov et al., 2016) and regulates functions in the gut, including secretion, motility and blood flow (Saha, 2014). Because intestinal signals are also involved in the regulation of central nervous system (CNS) responses, changes in the diversity and richness of the gut microbiota are considered fundamental factors for both the initiation and maintenance of IBS in most patients (Raskov et al., 2016). Alterations in gut microbiota composition are commonly accompanied by small intestinal bacterial overgrowth, which is believed to contribute to the development of several prominent symptoms in IBS patients, including abdominal pain, bloating and altered bowel functions (Amundsen, 2016; Basseri, Weitsman, Barlow, & Pimentel, 2011; Ghoshal et al., 2012; Magge & Lembo, 2012; Pimentel, 2016). Nevertheless, dysfunction in the GBA and subsequent changes in the gut microbiota have been suggested to cause further detrimental changes that are commonly observed in IBS patients. These can include changes in intestinal motility and secretion, thereby contributing to visceral hypersensitivity and cellular alterations of the entero-endocrine system and immune system (Raskov et al., 2016).

Changes in the gut microbiota have been associated with the development of low-grade inflammation, which can be sufficient enough to alter neuromuscular and epithelial cell functions (Raskov et al., 2016; Ruigómez, Rodríguez, & Panés, 2007). The increased permeability of intestinal epithelial cells causes metabolites from the lumen to leak through the epithelial barrier and into underlying tissue (Napolitano & Covasa, 2020). This state causes the innate immune system to respond and produce pro-inflammatory cytokines. These cytokines induce a state of systemic low-grade inflammation and can cause further damage to the epithelial barrier, resulting in metabolic endotoxemia and an even further reduction of the epithelial barrier (Napolitano & Covasa, 2020). Additionally, a great abundance of mast cells in close proximity to enteric nerve fibres in the gastrointestinal mucosa has been commonly observed in the intestines of IBS patients (Holtmann, Ford, & Talley, 2016; Moloney et al., 2016). This has been associated with immune activation and the onset of abdominal pain (Amundsen, 2016; Moloney et al., 2016).

Several additional features have been suggested to contribute to alterations in bowel movement in IBS patients. The neurotransmitter serotonin is known to play a significant role in the control of gastrointestinal motility, sensation, secretion and pain sensation (Amundsen, 2016; Houghton, Atkinson, Whitaker, Whorwell, & Rimmer, 2003). Altered levels of serotonin are commonly observed in IBS patients; moreover, the alteration is suggested to be specific to the IBS subtype, with high levels commonly observed in IBS-D, and is believed to trigger diarrhoea and cramps (Cremon et al., 2011; Saha, 2014). In addition, altered serotonin levels are believed to contribute to increased sensitivity in pain receptors, thereby contributing to abdominal pain (Berstad, Raa, & Valeur, 2014; Cremon et al., 2011; Saha, 2014). Furthermore, altered concentrations of bile acid in the colon have been suggested to cause symptoms of IBS (Spiller, 2016). Excessive levels of bile acid in the colon can stimulate colonic secretion and increase stool water. Thus, it has been suggested that malabsorption of bile acid can contribute to diarrhoea in a small share of IBS-D patients (Barkun, Love, Gould, Pluta, & Steinhart, 2013). Stress is also a common factor in several gastrointestinal disorders and is believed to play a central role in IBS. Stress is associated with decreased gastric emptying, increased intestinal motility and increased abdominal discomfort (visceral hypersensitivity) (Amundsen, 2016; Cryan & O'mahony, 2011; Greenwood-Van Meerveld, Moloney, Johnson, & Vicario, 2016; Kennedy, Cryan, Dinan, & Clarke, 2014). Moreover, it has been suggested that stress can lead to alterations in epithelial barrier integrity, thus increasing intestinal permeability (Moloney et

al., 2016). Stress has also been suggested to be an important factor in psychiatric conditions that often occur along with IBS, such as anxiety and mood disorders such as depression (Whitehead, Palsson, & Jones, 2002).

Because the gut microbiota is believed to play a central role in the pathophysiology of IBS, it has been suggested that manipulation of the gut microbiota can influence and decrease key symptoms of IBS, including abdominal pain and bowel habits (Ley, Turnbaugh, Klein, & Gordon, 2006). Transplantation of faecal samples from healthy individuals has been found to significantly improve symptoms in nearly half of IBS patients (Padhy, Sahoo, Mahajan, & Sinha, 2015).

1.3 Gut microbiota

1.3.1 Function and definitions

The mammalian gastrointestinal tract is home to a cluster of microorganisms, referred to as the gut microbiota (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). The gut microbiota includes fungi, viruses and archaea, with the majority being bacteria (Lawley & Walker, 2013). The bacteria in the gut microbiota can be attached to the walls of the intestines or floating freely in the lumen and are estimated to be of the same quantity as the total number of cells in the human body (Greenwood-Van Meerveld et al., 2016; Sender, Fuchs, & Milo, 2016). Soon after birth, the gastrointestinal tract is immediately colonised by microbes from the mother's microbiota and the local environment, and the number of these microbes steadily increases as a person ages (von Bieren & Harris, 2016). When a person reaches adulthood, the microbiota becomes complex, personalised and stabilised. The gut microbiota then continues to evolve and change in response to exogenous factors such as one's diet, intake of antibiotics and other drugs and endogenous factors such as one's genetics and immune response (Lawley & Walker, 2013). One's response to exogenous factors is heavily influenced by the personalised structure of the bacteria in the gut microbiota (Salonen et al., 2014). The composition of the gut microbiota is host specific and can evolve to complement host-encoded functions (Sheflin, Melby, Carbonero, & Weir, 2017).

The gut microbiota provides several important functions on which the body is highly dependent (Arpaia et al., 2013; Chung et al., 2016; Hasan & Yang, 2019). These include maintaining intestinal barrier integrity and synthesizing several vitamins, including vitamin K and certain B vitamins such as biotin, folate, riboflavin, thiamine and cobalamin (Yatsunenko et al., 2012). Most importantly, the gut microbiota aids digestion by fermenting carbohydrates and dietary fibres, which are not digested in the small intestine. Although the human genome contains approximately 17 genes encoding digestive enzymes, gut bacteria is rich in carbohydrate-active enzymes (CAZymes) (Cantarel, Lombard, & Henrissat, 2012; Zhang LS & Davies, 2016). Cantarel et al. (2012) found that a single gut microbiota sample contains 15,882 different CAZyme genes. Generally, the more complex a carbohydrate is, the more enzymes are required for its breakdown (El Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013; Martens et al., 2011). Therefore, dietary fibres are resistant to digestion and absorption in the human small intestine and must be partially or completely fermented by bacteria in the large intestine (El Kaoutari et al., 2013). Thus, the gut microbiota has acquired specialised skills and roles that enable it to confer metabolic activities which are not encoded in the human genome (El Kaoutari et al., 2013; Hooper, Midtvedt, & Gordon, 2002).

The fermentation process provides multiple beneficial effects to the host, including increased diversity and growth of beneficial bacteria as well as the provision of short-chain fatty acids (SCFA). SCFAs are believed to be involved in numerous physiological processes (den Besten et al., 2013). The most studied SCFAs, butyrate (C4:0), propionate (C3:0) and acetate (C2:0), have been associated with a reduced risk of cardiovascular disease (CVD) (Canfora et al., 2017; Gao et al., 2009), inflammatory bowel diseases (Segain et al., 2000; Zhang LS & Davies, 2016) and type 2 diabetes (Chambers et al., 2019). Furthermore, butyrate is believed to be a main energy source for colonocytes and has been associated with a reduced risk of colorectal cancer (El Kaoutari et al., 2013; O'Callaghan & van Sinderen, 2016). Because the bacteria in the human gut extract energy by fermenting carbohydrates and dietary fibres, they are central to the survival of prominent members of the gut microbiota (El Kaoutari et al., 2013).

1.3.2 Taxonomic classification

Of all the microbiota in the human body, the gut microbiota is the most complex (Sankar, Lagier, Pontarotti, Raoult, & Fournier, 2015), and knowledge of the gut microbiota has extensively improved in recent years (Sankar et al., 2015). The bacteria of the gut microbiota are divided into several taxonomic classes: phylum, class, order, family, genus and species (Distrutti, Monaldi, Ricci, & Fiorucci, 2016). Additionally, gut bacteria have been found to consist of 17 families, 50 genera and more than 1,000 species (Amundsen, 2016; Rajilic-Stojanovic & De Vos, 2014; Rinninella et al., 2019). The most common phyla of the human gut microbiota are Firmicutes (58–88%) and Bacteriodetes (8.5–28%) followed by Actinobacteria (2.5–5%), Proteobacteria (0.1–8%) and to a lesser extent Verrucomicronia (Falony, Joossens, & Vieira-Silva, 2016; Koç, Mills, Strain, Ross, & Stanton, 2020; Li, Jia, & Cai, 2014). Figure 1.1 illustrates examples of bacteria belonging to each taxonomic class.

Figure 1.1: Examples of taxonomic level of the most common human gut bacteria. (Adapted from: Rinninella et al., 2019)

1.3.3 Gut microbiota in relation to health and disease

To date, the exact definition of a healthy gut microbiota has yet to be elucidated. However, it is generally agreed that diversity, symbiosis and stability in the gut microbiota is important and associated with health benefits (Falony et al., 2016; Koç et al., 2020; Li et al., 2014). Symbiosis is a term used to describe the structure of the gut microbiota, wherein the quantity of beneficial bacteria associated with anti-inflammatory activity dominate that of potentially pathogenic bacteria (Althani et al., 2016; Guinane & Cotter, 2013). Stability is commonly characterised "by the presence of classes of bacteria that enhance metabolism, resilience of infection and inflammation, resistance to cancer or autoimmunity, endocrine signalling, and brain function" (Amundsen, 2016; Hollister, Gao, & Versalovic, 2014, Wu, Tremaroli, & Bäckhed, 2015).

Dysbiosis is a term used to describe the gut microbiota, wherein beneficial microorganisms are decreased, or absent and potentially pathogenic microorganisms are increased and dominating, therefore causing a permanent or temporary imbalance in the gut microbiota (Casen et al., 2015; Hills et al., 2019). Observations of microbial dysbiosis are increasingly associated with a broad range of human diseases (Li et al., 2014). For instance, a high Firmicutes/Bacteriodetes (F/B) ratio, which is considered dysbiotic, has been seen to correlate with the development of numerous non-communicable diseases (Koç et al., 2020; Petersen & Round, 2014) such as obesity (Verdam et al., 2013), type 2 diabetes (Larsen et al., 2010), CVD (Yoshida, Yamashita, & Hirata, 2018) and hypertension (Koç et al., 2020; Yang et al., 2015). It has also been associated with the development of several gastrointestinal disorders including IBS and IBD (Enck & Mazurak, 2018). This is believed to be a result of the reduced abundance of beneficial microorganisms known to produce SCFA, such as *Lactobacillus* and *Bifidobacterium*, and an increase in the prevalence of non-beneficial bacteria, thereby causing inflammation (Koç et al., 2020). As such, a healthy gut microbiota is believed to be essential to the prevention of non-communicable diseases or bowel diseases (Koç et al., 2020).

1.3.4 Gut microbiota and IBS

Several studies have observed significant differences between bacteria found in the gut of healthy individuals and that of IBS patients (Enck & Mazurak, 2018). A dysbiotic intestinal environment, including reduced bacterial diversity and an increased richness of beneficial

bacteria, is believed to be a common feature of IBS and is linked to the pathophysiology of the disease (Kerckhoffs et al., 2009). Patients with IBS have been found to possess a significantly lower abundance of the phyla Bacterioidetes (Enck & Mazurak, 2018) and Actinobacteria (Kassinen et al., 2007) as well as the genera *Alistipe* (Enck & Mazurak, 2018), *Bacteroides* (Balsari, Ceccarelli, Dubini, Fesce, & Poli, 1982), *Faecalibacterium* and *Bifidobacterium* (Enck & Mazurak, 2018; Kerckhoffs et al., 2009). A decrease in *Bifidobacterium* is believed to be a possible microbial signature of dysbiosis in IBS (Kerckhoffs et al., 2009) and is associated with the onset of abdominal pain (Jalanka-Tuovinen et al., 2011). Patients with IBS have also been seen to possess an increased relative abundance of the phylum Proteobacteria and the genera *Ruminococcus spp., Clostridium spp., Dorea spp., Subdoligranulum spp., Dialister spp., Clostridium cluster XIVa., Roseburia spp., Coprococcus spp.* (Jeffery et al., 2012; Rajilic-Stojanovic, 2011; Ringel & Ringel-Kulka, 2015), *Lactobacillus spp*. and *Veillonella spp*. (Amundsen, 2016; Shukla, Ghoshal, Dhole, & Ghoshal, 2015; Tana et al., 2010).

1.4 Dietary fibres

1.4.1 Definitions and recommendations

Dietary fibres are described as either polysaccharides with a minimum of 10 monomeric units (MU) or oligosaccharides containing between three to nine MU (Dhingra, Michael, Rajput, & Patil, 2012; Fraberger et al., 2018; Myhrstad, Tunsjø, Charnock, & Telle-Hansen, 2020; Stephen et al., 2017). Polysaccharides are further classified as non-starch polysaccharides (NSP) and resistant starches (RS), while oligosaccharides are classified as resistant oligosaccharides (RO) (Fraberger et al., 2018; Myhrstad et al., 2020). Dietary fibres as a whole are classified according to their water solubility, viscosity and fermentability (Jones, 2014, Myhrstad et al., 2020). To promote health, the ministry of health recommends a daily fibre intake of at least 25 and 35 grams (g) for women and men respectively (Helsedirektoratet, 2020a). However, the general population continually fails to achieve this recommendation; to date, the average daily fibre intake amongst adults is estimated to be 24 g per day (Helsedirektoratet, 2020b, p. 41). Thus, the ministry of health recommends increasing the consumption of food groups that are rich in dietary fibres, such as whole-grain products, fruits and vegetables (Helsedirektoratet, 2020a).

1.4.2 Characteristics and functionality

The characteristics of dietary fibres determine their functionality in the gastrointestinal tract, including their effects on gut transit time, stool formation and microbial specificity (Jones, 2014). When soluble fibres are mixed with water during digestion, they form a gel-like solution. This is proven to slow down gastric emptying, contribute to distention and enhance the sensation of satiety by increasing the volume of the stomach's contents (Weickert & Pfeiffer, 2008). The increased viscosity of intestinal content has also been seen to improve health by reducing blood glucose levels (Weickert & Pfeiffer, 2008) and improving insulin sensitivity (Robertson, Bickerton, Dennis, Vidal, & Frayn, 2005). Moreover, this increased viscosity has been seen to reduce cholesterol by reducing the glycaemic response, leading to a lower insulin-stimulated hepatic cholesterol synthesis and a reduced re-absorption of bile salt from the large intestine (Gunness & Gidley, 2010; Hartley, May, Loveman, Colquitt, & Rees, 2016; Koç et al., 2020). Insoluble dietary fibres benefit the bowel by being osmotically active, diluting content, promoting regularity in bowel movement, increasing stool bulk and preventing constipation and irregular stool release (Anderson et al., 2009). However, although dietary fibres have been extensively researched, their exact influence on the gut microbiota is still being uncovered. Most dietary fibres are fermented by the gut microbiota, which they are believed to influence (Myhrstad et al., 2020; Schutte et al., 2018; de Faria Ghetti et al., 2019). Epidemiological studies have shown an inverse association between an intake of whole grains that are rich in dietary fibre and the risk of chronic disease (Costabile et al., 2008; Koç et al., 2020; Makki, Deehan, Walter, & Bäckhed, 2018). One mechanism suggested to be contributing to the reduced risk of chronic disease, is the influence that dietary fibres have on the gut microbiota (Costabile et al., 2008; Martínez et al., 2013).

1.4.3 The interaction of the gut microbiota and dietary fibres

The composition of the gut microbiota is strongly affected by several factors, including dietary habits (Hasan & Yang, 2019). Currently, many studies have indicated that a diet high in fibre can influence the gut microbiota in numerous ways, such as by promoting microbiota diversity,

composition and functionality by increasing the abundance and activity of beneficial microorganisms (Koç et al., 2020; Simpson & Campbell, 2015). For instance, increased diversity and richness in the gut microbiota were reported by Ghetti et al. (2019) and Schutte et al. (2018) after an increased intake of fibres or whole grains (Schutte et al., 2018; de Faria Ghetti et al., 2019). Moreover, when comparing the intake of whole grains to that of refined grains, dietary intervention studies have observed an increase in several SCFA-producers, including *Lachnospira, Akkermannsia, Bifidobacterium, Lactobacillus, Ruminococcus, Roseburia, Clostridium, Faecalibacterium*, and *Dorea* in the former (Myhrstad et al., 2020). Additionally, an increased intake of whole grains may decrease the abundance of potentially pathogenic bacteria, potentially restoring symbiosis in the gut microbiota (Myhrstad et al., 2020). Certain types of soluble fibres, including inulin, fructo-oligosaccharides (FOS), beta-glucan and galacto-oligosaccharides (GOS) are considered prebiotic because they have been seen to cause specific changes to the composition and activity of the gut microbiota, thereby causing an increase in beneficial physiological effects in the host (Davani-Davari et al., 2019; Gibson et al., 2017; Holscher, 2017; Koç et al., 2020).

However, a diet that is low in dietary fibres may cause a shift from a stable microbial intestinal environment to one that is temporarily or permanently altered, often characterised by reduced bacterial diversity and richness (Statovci, Aguilera, MacSharry, & Melgar, 2017). A study comparing children in a rural village in Burkino Faso in West Africa who regularly consumed a diet high in fibre to Italian children who consumed a Western diet found a significantly higher level of diversity and richness in the gut microbiota of the Burkino Faso children (De Filippo et al., 2010). Moreover, Firmicutes were found to be almost depleted in the Burkino Faso children, while Bacteroidetes and SCFA were greatly abundant. Specifically, the genera *Xylanibacter* and *Prevotella*, which are known to metabolise cellulose and xylan, were distinctive in the West African children but not identified in the Italian children. Reversely, the potentially pathogenic bacteria Shigella and Escherichia were significantly more abundant in the Italian children compared to the West African children (De Filippo et al., 2010). A low consumption of dietary fibres can potentially cause dysbiosis in the gut microbiota and increase the risk of developing non-communicable diseases and intestinal disorders such as IBS (De Filippo et al., 2010).

1.5 Dietary management of IBS

To date, there is no cure for IBS, and treatment has therefore focused on dietary strategies that aim to improve a patient's symptoms and quality of life (Werlang et al., 2019). A high proportion of patients with IBS associate food intake with the development of abdominal symptoms, and approximately 20% to 65% of patients with IBS have specifically reported problems upon the consumption of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). In particular, fructans have been identified as a main trigger of IBS symptoms (Fraberger et al., 2018). Therefore, the introduction of a diet low in FODMAPs has been common in the dietary management of IBS in the past 10 years (Struyf et al., 2018).

1.5.1 Low FODMAP diet

FODMAPs are short-chain carbohydrates that are not hydrolysed; they are absorbed in the small intestine but pass into the terminal ileum and the proximal colon, where they are fermented by the intestinal microbiota (Mehtab, Agarwal, Singh, Malhotra, & Makharia, 2019; von Bieren & Harris, 2016). Because of their small size, FODMAPs are osmotically active molecules and are rapidly fermented by bacteria (Litleskare et al., 2015). Therefore, they have been shown to cause disadvantageous symptoms such as excessive gas and osmotic diarrhoea. Moreover, as a consequence of a reduced concentration or absence of certain digestive enzymes in the small intestine, malabsorption has been seen to cause intestinal distension and bloating (Staudacher, Irving, Lomer, & Whelan, 2014). While symptoms can be relatively unperceived in healthy individuals, they can be painful for IBS patients. Symptoms mainly occur when one's daily intake of FODMAPs exceeds about 0.3 g/kg body weight or about 15 g/day (Algera, Colomier, & Simrén, 2019; Loponen & Gänzle, 2018).

A low FODMAP diet involves restricting food groups that contain a high proportion of FODMAPs to reduce symptoms. However, many of these foods are of great nutritional value for both the host and the gut microbiota. These include certain plant foods, dairy products, grains and cereals. Therefore, a low FODMAP diet is also typically characterised as being low in dietary fibre (Vandeputte & Joossens, 2020). Similar to dietary fibres, FODMAPs are also

fermented by the gut microbiota and provide multiple beneficial effects, including an increased proportion of beneficial bacteria and an increased diversity and yield of SCFA (Den Besten et al., 2013). The consumption of oligosaccharides such as FOS and GOS has been associated with an enrichment of beneficial bacteria such as *Bifidobacterium* (Davani-Davari et al., 2019). Consequently, a low FODMAP diet has been shown to disturb the gut microbiota, reducing total microbial diversity and richness and causing a disappearance of specific bacterial species that are important for SCFA production in the digestive system (Amundsen, 2016; Logan, Jacka, & Prescott, 2016; Sonnenburg & Bäckhed, 2016). Disadvantageous consequences, including a reduction of bacterial diversity and a significant reduction of *Bifidobacterium*, can be observed within two to three weeks of the introduction of a low FODMAP diet (Dieterich & Zopf, 2019; Halmos et al., 2015). Consequently, recent studies have indicated that the long-term implementation of a low FODMAP diet can be problematic (Hills et al., 2019).

1.5.2 Dietary sources of FODMAPs and dietary fibres

Wheat and its products are the most important sources of fructans in the Western European diet, and they also account for a large proportion of the daily consumption of FODMAPs globally (Fraberger et al., 2018; Verspreet et al., 2013; Verspreet, Dornez, Van den Ende, Delcour, & Courtin, 2015). Most commercially made bread is prepared with wheat, and bread is therefore regularly abstained from in a low FODMAP diet (Fraberger et al., 2018). However, whole-wheat products and bread are essential sources of whole grains and dietary fibre (Helsedirektoratet, 2020b). For example, bread and whole grains contribute to more than 50% of the daily consumption of dietary fibre in Norway (Helsedirektoratet, 2020b). However, during recent years, grain and cereal consumption has been reduced in the Norwegian population. A survey discovered that 34 % out of 956 responders, a majority of whom were female, consumed less bread than they did two years ago (Compete and Polling & Statistics, 2018). The majority of responders chose to refrain from consuming bread and reported several factors as their motivation. Within the group of people who ate bread less than once a week, 17% claimed that is unhealthy, 11% said that it is artificial and 20% claimed it caused them to gain weight. Furthermore, 64 % of the responders believed that bread contains too many carbohydrates and caused them to have gastrointestinal discomfort. Women in particular reported this to be the leading cause of their decision to abstain from eating bread. However,

because of their choice to not consume bread, these individuals are at stake of not consuming the recommended amounts of dietary fibre.

The health benefits and properties of bread are not only dependent on type of flour used but are also influenced by the baking process, including the type of leaven used (Pagliai et al., 2020). The two most commonly used leavening agents are Saccharomyces cerevisiae and sourdough. Currently, the agent that is most frequently used for bread making is *S. cerevisiae*, commonly referred to as baker's yeast (Pagliai et al., 2020). However, during recent years, there has been a change of interest in leavening agents. The uniqueness of the microbial composition of sourdough and its proposed health benefits has created a renewed awareness of sourdough baking. This increased interest has also come from a scientific point of view. In comparison to bread baked with baker's yeast, the sourdough fermentation process is believed to improve bread quality, including its texture, flavour, nutritional content and shelf life and has been seen as a way to replace additives (Dimidi, Cox, Rossi, & Whelan, 2019; Fraberger et al., 2018).

1.6 Sourdough bread

A sourdough starter is made by mixing flour and water, which is then fermented by lactic acid bacteria and yeast that occurs naturally in the flour and general surroundings (Dimidi et al., 2019) On average, it takes a minimum of seven days before the sourdough starter is ready and able to be added to the sourdough base to start the fermentation process in the bread (Dimidi et al., 2019).

Recent investigations have suggested that sourdough bread may confer health benefits through the impact of the sourdough process on the nutritional content of the bread (Dimidi et al., 2019; Loponen & Gänzle, 2018). The microorganisms in the sourdough starter are believed to consume and degrade oligosaccharides during baking and thus lower the bread's content of FODMAPs, specifically fructans and raffinose (Fraberger et al., 2018). Therefore, the FODMAP level in sourdough bread is likely to be reduced compared to that of bread baked with baker's yeast. As such, sourdough bread can potentially be a suitable alternative for those who refrain from consuming bread and may be better tolerated by those who suffer from bowel disorders such as IBS (Laatikainen et al., 2017). However, while sourdough bread appears to have the ability to significantly reduce FODMAPs, whether this is associated with any

gastrointestinal health outcomes or alternations in incidence of discomfort is still undetermined. Presently, the impact of sourdough bread on gastrointestinal symptoms has only been investigated in a few studies, each with different results (Laatikainen et al., 2016; Laatikainen et al., 2017; Raninen et al., 2017).

2.0 Aim of the study

This master project is part of an ongoing research project, the HELFAB-study, where the overall aim is *to investigate possible health effects of consuming sourdough bread in comparison to bread baked with yeast.*

The aim of the present master project is *to investigate gut symptoms and changes in the gut microbiota upon consuming sourdough bread compared to bread baked with yeast, in people who are sceptical towards consuming bread and/or portray mild to moderate gut symptoms upon consumption of bread.*

More specifically, we aim to:

1. Investigate the effect on gut symptoms (GSRS-IBS and BSC) after intake of sourdough bread compared with yeast bread.
2. Investigate the effect on gut microbiota after intake of sourdough bread compared with yeast bread.

3.0 Methods

3.1 Study design

The study is a double-blind randomised control trial (RCT), with a cross-over design (figure 3.1). The study endured for a total of five weeks and started with a two-week run-in where the participants consumed bread baked with baker´s yeast. After run-in they were randomised into two groups, consisting of either sourdough bread (intervention group) or baker´s yeast (control group) for one week. During the preceding (wash out) week all participants consumed baker´s yeast bread, before commencing period two of the study where participants crossed over. Each intervention and control period included two online visits (before and after), adding up to a total of five visits including screening (v0-v4). Data was collected via Case Report Forms (CRF) (Appendix C & D) by project members, and online forms filled out by participants themselves (Bristol stool chart (BSC) (Appendix F) and Gastrointestinal symptom rating scale for Irritable bowel syndrome (GRSR-IBS) (Appendix E) during all five visits. In addition, participants collected their own faecal and blood samples (dried blood spots (DBS) at home. Faecal samples were collected the day before every visit, and blood samples were collected fasting on the morning of each visit (v1-v4), as illustrated in figure 3.1. The participants were communicated not to change their regular diet or their usual level of physical activity during the study, but commence life as normal, within the frames of the study.

All communication with the participants was conducted remotely, and mainly online by forms and zoom-meetings, in addition to phone by request. Screening was conducted through an online zoom-meeting procedure, in which participants received consent forms containing further information regarding dietary restrictions and the aim of the study by email. Health examination was conducted during screening and included recording of pre-existing medical history and chronic diseases, as well as self-reported height and body weight to calculate BMI. Before initiation, all participants were required to sign and return the consent form and complete a food frequency questionnaire (FFQ) designed to register their habitual diet during the past year. During screening and every visit (v0-v4), participants were asked to fill in two online forms, BSC and GSRS-IBS, to define stool classification and severity of discomfort, respectively.

A CRF was filled in during screening and every visit (v0-v4) to record all protocol-required information for each participant. This included anthropometric measurements of height and weight, and follow-up on health status, adherence to protocol, including sample collection, bread consumption and restrictions, as well as responses to the two forms. The bread was distributed by a delivery truck to the participants homes at the start of every week, at 5 time points in total. Packages containing test kit, information sheet and return envelopes was delivered with the bread on two occasions, during visit 1 and visit 3 of the study. All samples were returned by post in ready-made envelopes to OsloMet at Kjeller.

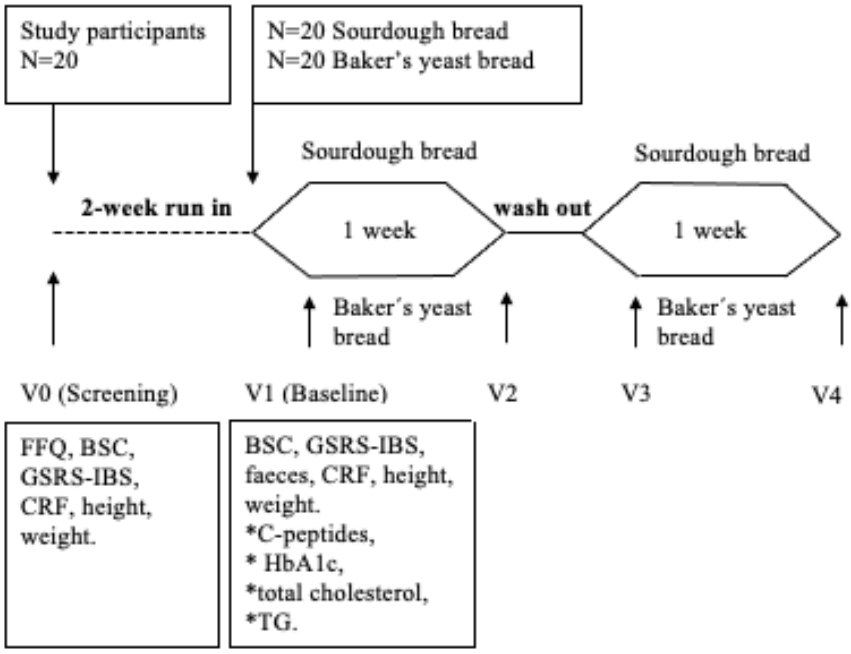

Figure 3.1: Illustration of study design. *Measurement described at baseline are completed during all visits (v1-v4). *Blood measurements only presented as baseline characteristics.*
BSC; Bristol Stool Chart, CRF: Case Report Form, CRP: C-reactive Protein, FFQ; Food Frequency Questionnaire, GSRS-IBS: Gastrointestinal Symptom Rating Scale, IBD; Irritable Bowel Disease, TG: Triglyceride.

3.2 Participants

The study took place from September to December 2020. The participants included in the study were healthy male and females, aged 18-65 years, who were sceptical towards consuming bread and/or portray mild to moderate gut symptoms upon consumption of bread. All participants had to live in the area of Oslo, in addition they were required to have a stable body weight and a normal BMI (18.5 and 27 kg/m2). During participation subjects refrained from consumption of bread other than those provided in the study. In addition, participants had to refrain from using probiotic products (i.e., Biola, Activia), including fermented foods (i.e., Kimchi, Kombucha), dietary supplements, and hormonal treatments (except oral contraceptives) starting four weeks prior to and during the study (Appendix G). Other exclusion criteria included chronic metabolic diseases such as diabetes, food allergies or intolerances towards products in the study, and treatment with antibiotics during the previous three months and throughout the study. Exclusion criteria are presented in table 3.1.

Table 3.1: Exclusion criteria

Not living in Oslo, or surrounding area	
Chronic metabolic diseases	Any type of diabetes, CVD, cancer last six months
Food allergies and Intolerances	Chron´s disease, ulcerative colitis, coeliac disease, IBD
No experience of stomach pain/discomfort	
Pregnant or lactating	
Smokers	
BMI	< 18.5 and > 27 kg/m2
Planned weight reduction and/or 5% weight change previous three months	
Blood donor last two months prior to and/or during the course of the study	
Reluctant to stop using dietary supplements four weeks prior to study start and during the whole study	
Excessive alcohol consumption	> 40 g per day
Antibiotic treatment four weeks prior and during the study	
Hormonal treatment	Except use of oral contraceptives

BMI, body mass, index; CVD, cardiovascular disease, IBD; inflammatory bowel disease.

3.3 Recruitment

Participants were recruited via the Oslo Metropolitan University (OsloMet) website amongst students and employees, and the public. In addition, OsloMet official social media, Facebook, Instagram and Snapchat specifically, was used for recruitment. The internet link to the recruitment website was also shared via personal Facebook pages by project members. The process of recruiting started in August 2020 and continued throughout November the same year. Out of the 140 inquires in total, 73 was screened, whereas 48 was excluded because they found the study too comprehensive, unwilling to follow restrictions, and/or did not meet the requirements of participation. During run-in, four participants dropped out, leaving 21 participants that completed the study. The participants who dropped out after run-in, reported stomach discomfort and pain. One participant was excluded because of BMI above the exclusion criteria, leaving 20 participants to be included in the data analysis.

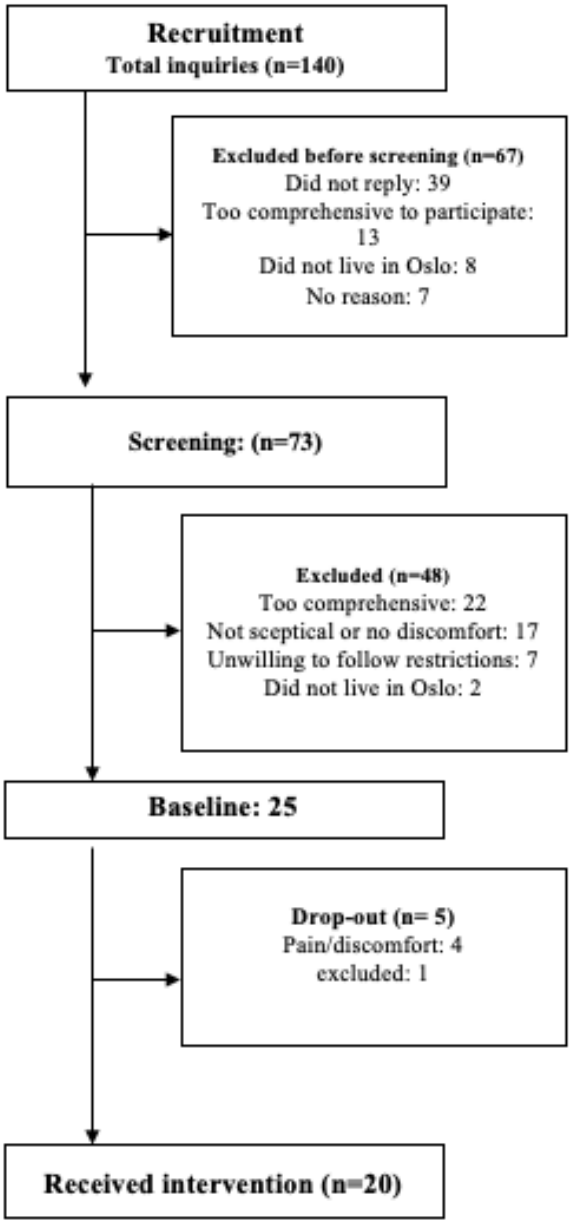

Figure 3.2: Flow chart of the recruitment process.

3.4 Test meal

The intervention was given in the form of sourdough bread, and the control was bread baked with baker's yeast. The recipe of both breads was developed by Mesterbakeren AS bakery. The recipe was developed following certain criteria (salt, fibre and whole-grain content) and consisted of at least 75 % whole grains. Participants were provided with 4-6 frozen bread at the start of each week. Participants were asked to store the bread (pre-sliced) in the freezer and

defrost slices of bread as required. Slices of bread were standardised to a measure of approximately 11 mm and ~40 g of weight. Participants were instructed to consume at least 5 slices of bread per day (a total of 200 g) to ensure a daily intake of 6 g of fibre. They were not given any further restrictions considering spread or topping of the bread. Participants were requested to record adherence to the protocol by registering amount of sliced bread consumed on a form and return it in the ready-made return envelopes. Bread composition was analysed by Eurofins Food & Feed Testing Norway.

Bread loaves were prepared using the following ingredients: water, salt, flour and leavening agent (sourdough or bakers' yeast). The overall formula for the sourdough bread was (in baker's percentages) 40 % water, 30 % flour, 30 % sourdough starter. The dough was covered in plastic film, and bulk fermented in a proving cabinet (Lillonord, Maryland) at 25 °C, and stirred every two hours for a total of 8 hours. The dough was then kneaded in a planetary mixer for 12 minutes on low speed, and 5 minutes on full speed. When the dough reached 27 °C it was left to rest for 10 minutes before portioned into 750 g pieces and left to rest for another 15 minutes. The dough was then shaped and transferred into loaf pans and placed in a proving cabinet holding 24 °C, and 72 % air humidity, for approximately 14 hours. The preparation of the yeast bread was almost identical. However, it excluded the initial fermenting process, and in addition, the yeast bread was proofed at a temperature of 32 °C and for 60 minutes. Both the sourdough bread and the yeast bread were baked in a preheated stone oven, starting with 8 seconds of steam at 250 °C, before immediately dropping the temperature to 230 °C for approximately 40 minutes. Bread was then left to cool down to a core temperature of 25 °C, before sliced into equal parts of 11mm by standardised machine and put in freezer (-20 °C) before delivered and stored at OsloMet, campus Kjeller, at -20 °C ready to be shipped out.

3.5 Measurements of stomach symptoms

In the present study gut symptoms were analysed with the GSRS-IBS and BSC. The GSRS-IBS was developed and validated by Wiklund et al. (2003) and consist of questions outlining gut health and symptoms during the past week (Wiklund et al., 2003). The forms were handed out at each visit with the participant.

Participants were asked to rate themselves on the GSRS-IBS, including 13 questions, asking about abdominal pain or discomfort, including gas, diarrhoea, constipation, as well as symptoms such as urgent need to have bowel movement, trouble emptying bowel, and unusual satiety patterns (Appendix E). Each question included a 7-point Likert scale ranging from no symptoms = "1 point", minor (2 points) or mild discomfort (3 points), then moderate (4 points) and moderately severe discomfort (5 points), and severe = "7 points" and with a total score range of 13–91 points. There are five GSRS-IBS sub-dimensions with respective score ranges: pain (2–14), bloating (3–21), constipation (2–14), diarrhoea (4–28), and satiety (2–14).

The BSC was developed by Lewis and Heaton (1997) and is used to classify stools into seven groups depending on texture and shape to measure passage time (Appendix F) (Lewis & Heaton, 1997). The BSC consist of a picture of the seven stool classification groups including illustrations, and the participants were asked to classify their stool on a scale from 1-7. Bristol type 1-2 are consistent with severe and mild constipation, 3-4 are normal, 5 through 7 are coherent with diarrhoea.

The forms were distributed online by email, after screening, before baseline measures and during every visit. Participants disclosed answers online, and informed to register their individual ID number, before rating themselves on the two scales. All answers were collected and stored in *Services for Sensitive Data* (Tjenester for sensitive data: TSD).

3.6 Faeces sampling

Faecal samples were collected in order to analyse gut microbiota. Participants received the cards hold squares of Whatman® filter paper on which the stool sample is to be applied, a flushable toilet seat cover for stool collection (Fe-Col®, Col-group, Amsterdam, the Netherlands), lollipop stick applicator, gloves and an airtight foil bag (Whatman Foil Bags, item no. 10534321; Whatman Inc.) for storage. Before sample collection participants received both oral and written instructions. In this way, faecal collection occurred participant-friendly with limited contamination by toilet water. Study participants were instructed to collect faecal sample in toilet seat cover with equipment provided, before smearing a thin layer of the faecal sample on the filter using a lollipop stick applicator, and let air-dry for a minimum of 20 minutes, before placing it in the foil bag. Faecal samples were marked with identification

numbers and shipped in ready-made envelopes to the analytical laboratory where they were stored at -20°C before being dispatched to Bio-Me for further management and analysis.

3.7 Analysis of gut microbiota

The analyses were conducted by Bio-Me and are described below.
Three discs of filter paper of 6 mm in diameter were punched out from each card, and microbial DNA was extracted using MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, Massachusetts, US) following the manufacturer's protocols on KingFisher™ Flex (Thermo Fisher Scientific, Waltham, Massachusetts, US). The bacterial cell wall was disrupted using Star Beater (VWR, West Chester, Pennsylvania, US) at 30Hz frequency for 2 minutes. Purified DNA was eluted in 200 µL MagMAX™ Elution Buffer, and DNA was quantified using the Quant-iT™ PicoGreen™ dsDNA Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, US) and an Infinite F200 Fluorescence Microplate Reader (Tecan Group AG, Männedorf, Switzerland).

Faecal microbiota analysis was performed with Bio-Me's Precision Microbiome Profiling platform (PMP™). Consisting of custom-made OpenArray® qPCR panels amplifying 107 bacteria, following the default TaqMan® OpenArray® Real-Time PCR Plates Protocol (Thermo Fisher Scientific, Waltham, Massachusetts, US). Bacterial taxa were quantified in absolute genomic copies per µL, interpolated from standard curves based on quantified reference isolates from DSMZ (Leibniz Institute, Braunschweig, Germany) or ATCC (American Type Culture Collection, Manassas, Virginia, US) [1]. To enable comparison between samples, normalised quantification (number of genomic copies per ng of DNA) was calculated by dividing the absolute quantification for each target by the total DNA concentration in a sample.

3.8 Measurement of habitual diet

3.8.1 Food frequency questionnaire

A food frequency questionnaire (FFQ) was employed to assess habitual diet throughout the year before the study. An FFQ comprises of a list of foods and beverages with response categories to indicate usual frequency of consumption over a given period of time (Gibson, 2005). Categories are ranging from never or less than once a month to 6+ per day. The FFQ is designed to provide descriptive on habitual food- and nutrition intake over a long time period and is useful for measuring intake of specific foods or food components and can also identify food patterns associated with insufficient intakes (Gibson, 2005). The FFQ was delivered in the first package during run-in and returned with the first return-envelope. As such, the FFQ was filled out by the participants before commencing the first interventions week.

3.9 Statistical methods and power calculations

To evaluate BSC, GSRS-IBS, and gut microbiota, the results from each participant were plotted into and processed using Microsoft® Excel for Mac (version 16.45).
Species diversity was calculated from the number of species detected out of the 107 measurable, for each sample. Proportion of phylum was based on the distribution of species at phylum level from all samples pooled together, before and after intervention and control. Species without a detectable level in at least 50% of the samples were discard from further analyses. Of the 107 species, 46 were therefore included in the downstream analyses. The normalised quantification number was thereafter logarithmically transformed with base 2(Log_2) to reduce skewness of original data.

All statistical analyses were performed in IBM SPSS statistic (version 27.0; SPSS, Inc., Chicago, IL, USA). Differences within and between the intervention and control periods was assessed with the non-parametric test Wilcoxon signed-rank test, for results from the GSRS-IBS and BSC forms, and gut microbiota diversity are presented as median values with interquartile range. For the gut microbiota analyses, log2 ratio were obtained by calculating the

ratio post and pre intervention and control (log_2 (post/pre). A parametric paired t-test was used to assess differences between the intervention and control periods based on the log_2 ratio. In addition, a paired t-test was also used to calculate differences within each group based on the log_2 values post and pre intervention and control.

In this pilot study, we want to investigate the possible health effects of consuming sourdough bread compared to baker´s yeast bread. Except leaving agent, the bread will be matched in relation to the content of macro- and micro- nutrients. To the best of our knowledge, no similar studies have been conducted where the primary endpoint is related to gastrointestinal symptoms. Calculations of power strength will therefore be difficult to perform. Based on our and other´s experiences from previous studies with a similar cross-over study design, we estimated that including approximately 20 participants would be sufficient in order to investigate health effects related to metabolic changes. The significance level was set to 5 % $P \leq 0{,}05$.

3.10 Ethical consideration

The study was conducted according to the guidelines in the Declaration of Helsinki and was approved by the Regional Committees for Medical and Health Research Ethics Sør-Øst (REK#96264) and Norwegian Service of Research Data (Norsk Senter for Forskningsdata) (NSD#382297) (Appendix H & I). Eligible participants signed a participation consent form explaining the purpose, risks, expectations and protocol of the study, before any experimental procedures were executed (Appendix A & B). Consent form, CRFs and FFQ was stored in a locked and fire safe cabinet, situated in a locked and restricted area at OsloMet, where only permitted individuals had access. All data were stored in Services for sensitive data (TSD). The TSD (in Norwegian, Service for Sensitive Data) service is designed for storing and post-processing sensitive data in compliance with the Norwegian “Personal Data Act” and “Health Research Act”. The study is registered at ClinicalTrials.org. with reg nr NCT04677881.

4.0 Results

4.1 Characteristics of participants

The baseline characteristics of the participants are shown in Table 4.1. The study involved twenty participants (five men and 15 women) with a median age and IQR of 28 years (21-44). The participants were healthy, had no underlying chronic diseases, and had a median body mass index (BMI) of 22.2 kg/m^2 (20.1-24.6) (Table 4.1)

Table 4.1. Baseline characteristics of participants

Gender	
male	5
Female	15
Age (y)	28 (21-44)
Height (cm)	169 (160-180)
Weight (kg)	65 (60-71.7)
BMI (kg/ m^2)	22.2 (20.1-24.6)
HbA1c (mmol/mol)	32.2 (29.5 – 36.9)
Total cholesterol (mmol/l)	4.6 (3.8 – 5.2)
Triglycerides (mmol/l) *	0.93 (0.68 – 1.41)
C-peptide (pmol/l) *	298.8 (229.5 – 336.6)

Values presented as median (IQR), *Measured in serum (n=19). *cm; centimeter, CRP; C-reactive Protein, kg; kilograms, l; litres, mmol; millimole, y; years*

4.2 Dietary habits

The participants' habitual diets, including their preferred food groups and nutritional intake levels, were measured by FFQ and are presented in Table 4.2. Overall, median daily energy intake and IQR prior to the study was 2203 kcal (1877 – 2687). Carbohydrates, protein, and fat made up 42.2 %, 15.6 %, and 37 % of total energy intake, respectively. The participants had a high median daily fibre intake of 29.5 g. Prior to the study, the habitual diets of the participants included the consumption of an average of 103.6 g of bread.

Table 4.2 Dietary habits measured at baseline

Energy (kcal)	2203 (1877 – 2687)
Carbohydrate (g)	240.5 (194 – 303.9)
Dietary fibre (g)	29.5 (20.1 – 39.1)
Protein (g)	91 (71 - 114)
Fat (g)	86 (74 - 123)
Polyunsaturated fat (g)	16 (13-24)
Saturated fat (g)	33 (26 – 44)
Bread consumption (g)	103.6 (53.4 – 176.8)
Whole grain (g)	53.3 (27.6 – 80.7)

Median (IQR) intake (g/day). g; grams, IQR; interquartile range, kcal; kilocalories.

4.3 Study products

The study products included bread baked with baker's yeast (control) and bread baked with sourdough (intervention). The participants were instructed to consume at least five slices of bread a day (approximately 40 g per slice). Compliance with the study protocol was calculated to be 94% for both the intervention and control. The two types of bread administered to the participants were analysed to determine their total macronutrient content, including their total fibre, insoluble fibre, soluble fibre, and inulin/FOS levels (Table 4.2). Fructose and maltose levels were lower in the intervention bread (0.11 g and 1.23 g, respectively) than the control bread (5.2 g and 1.54 g, respectively), and inulin/FOS content was higher for the intervention bread (0.8 g/100 g) than the control bread (0.4 g/100 g). Between the two breads, no substantial differences in dietary fibre, insoluble fibre, and soluble fibre were measured. Since the participants consumed an average of 200 g of bread each day during the study, bread accounted for approximately 50 % of their overall dietary fibre intake. The composition of the test meals offered to the participants is presented in Table 4.3

Table 4.3. Energy and nutritional composition of test meals.

	Baker´s yeast bread 100 g	Sourdough bread 100 g
Energy (kJ)	924	887
Energy (kcal)	221	212
Fat (total) (g)	1.57	1.05
Saturated fat (g)[1]	0.41	0,25
Monosaturated fat (g)[1]	0.35	0.23
Polyunsaturated fatty acids (g)[1]	0.69	0.49
Protein (g)	9.5	10.1
Total dietary fibre (g)	6.5	6.3
LMWDF fibre (g)[1]	0.9	1.3
Total amount of ISDF (g)[1]	4.3	3.7
Total amount of SDF (g)[1]	1.3	1.3
Inulin/FOS (g)	0.4	0.8
Carbohydrates (g)	38.4	36.8
Sugars (g)[1]	2.83	1.84
Fructose (g)[1]	5.2	0.11
Glucose (g)[1]	0.3	0.36
Lactose (g)[1]	<0.04	<0.04
Maltose (g)[1]	1.54	1.23
Sucrose (g)[1]	0.12	0.14
Galactose (g)[1]	<0.04	<0.04
Salt (g)	1.03	0.98

[1]Listed in grams out of total respective nutrient substance. Analysis conducted by Eurofins AS.
FOS; fructooligosacharides, g; grams, ISDF; Insoluble fibre, kcal; kilocalorie, kj; kilojoule, LMWDF; Low molar weight dietary fibre, SDF; Soluble fibre.

4.4 Effect on gastrointestinal symptoms

4.4.1 Gastrointestinal symptom rating scale for Irritable bowel syndrome

The total GSRS-IBS scores and diarrhoea levels of the two study groups (sourdough bread/baker's yeast bread) varied significantly (P=0.033, P=0.043). Both of these indicators of gut symptoms were reduced in the sourdough group and increased in the baker's yeast group (figure 4.1). Furthermore, a minor variation in pain levels was noticed between the groups (P=0.09), with the participants who consumed sourdough bread reporting less pain than those who consumed baker's yeast bread.

Within groups, the sourdough group's GSRS-IBS was significantly reduced by 5 points (P=0.026), and the baker's yeast group´s score non-significantly increased by 2.5 points (P=0.420). Furthermore, consuming sourdough bread significantly reduced pain (P=0.020) and bloating (P=0.036). Sourdough bread consumption caused a minor and non-significant reduction in diarrhoea, whereas consumption of baker´s yeast bread significantly increased diarrhoea by 2 points (P=0.030).

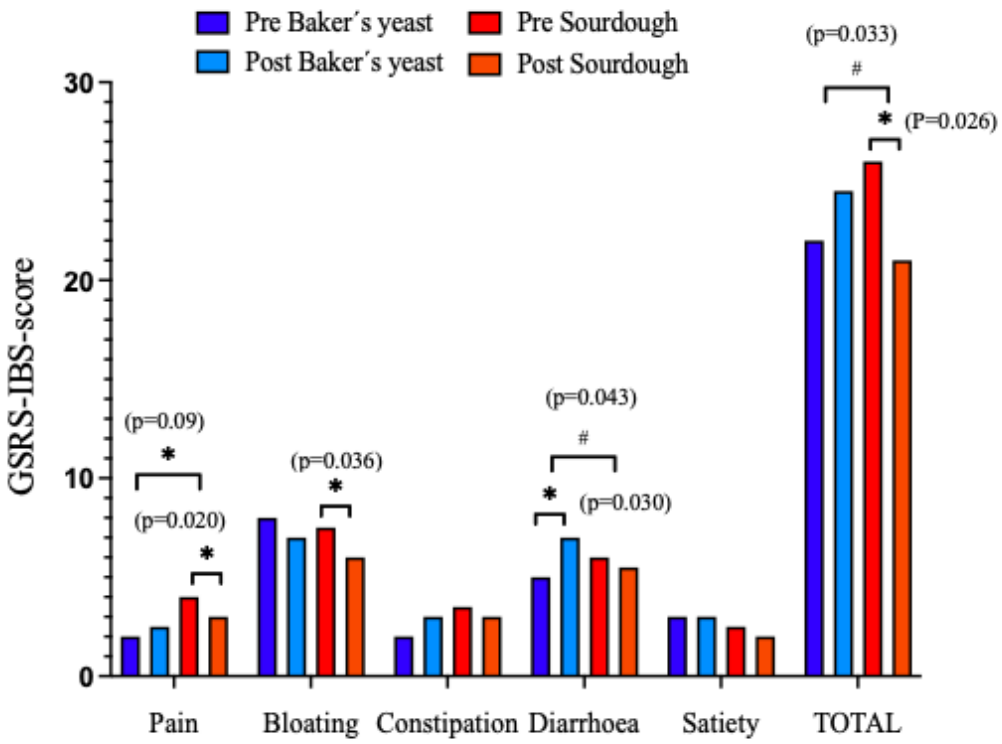

Figure 4.1: GSRS-IBS scores before and after consuming sourdough bread or baker´s yeast bread. *Values presented as median scores (IQR). (Pre sourdough n=19) Differences between and within groups were analysed by Wilcoxon Signed Rank-test. *Significant difference within groups, #Significant difference between groups.*

4.4.2 Bristol stool chart

Overall, the median BSC-score for both the baker´s yeast group and the sourdough bread group remained stable and within the normal range (3.5 (2 – 4.75) vs. 4 (3 – 5). No significant changes were observed between or within either group. However, a trend towards a reduction was seen in the baker´s yeast group (P=0.074) (fig. 4.2).

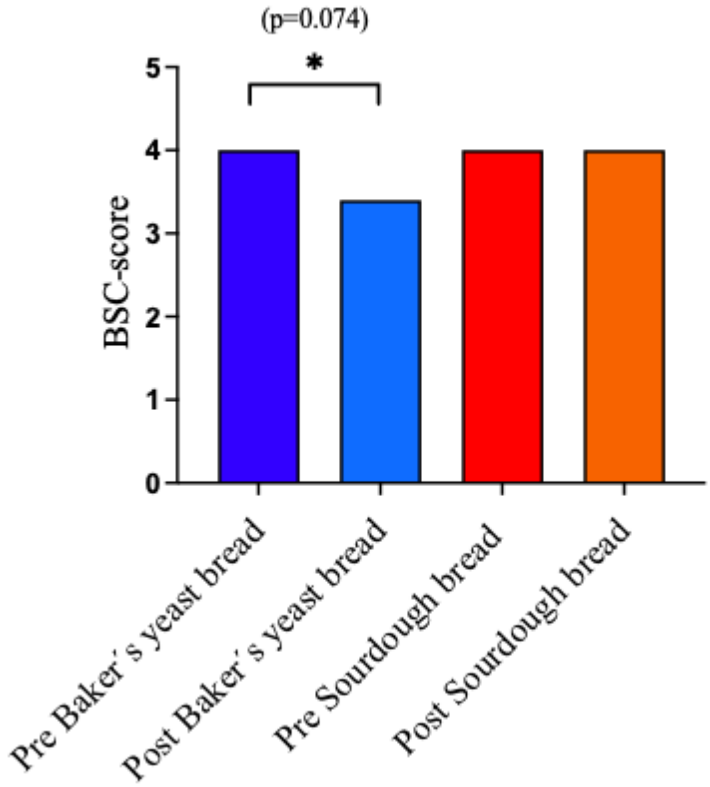

Figure 4.2: BSC-score before and after consuming sourdough bread or baker´s yeast bread. *Values presented as Median scores (IQR) (Pre baker´s yeast & Post sourdough n=19) Differences between and within groups were analysed by Wilcoxon Signed Rank-test. *Significant difference within groups.*

4.5 Effect on Gut microbiota

4.5.1 Distribution of species at phyla level

The 107 species included in the analysis belonged to six different bacterial phyla. Figure 4.3 illustrates the distribution of the observed species at the phylum level, and includes measurements taken before and after intervention and control. Overall, the proportions of phyla remained relatively stable, with only minor differences between groups. Euryarchaeota was the only phyla to significantly differ between groups (P=0.005). Its proportion were significantly reduced by the consumption of sourdough bread (P=0.005), and non-significantly reduced by the consumption of baker´s yeast bread (figure 4.3). The expression of species belonging to the Proteobacteria phylum was significantly increased by 0.9 % in the baker's yeast group. Only a minor increase was observed in the sourdough group (Figure 4.3). The majority of species present in the faecal samples belonged to the phylum *Firmicutes,* followed by *Bacteroidetes*. Together, these phyla constituted approximately 80 % of the total species observed (Fig. 4.3). Other notable phyla included Actinobacteria (10 %), Proteobacteria (6 %), Verrucomicronia (2 %), and Euryarchaeota with (0.9 %).

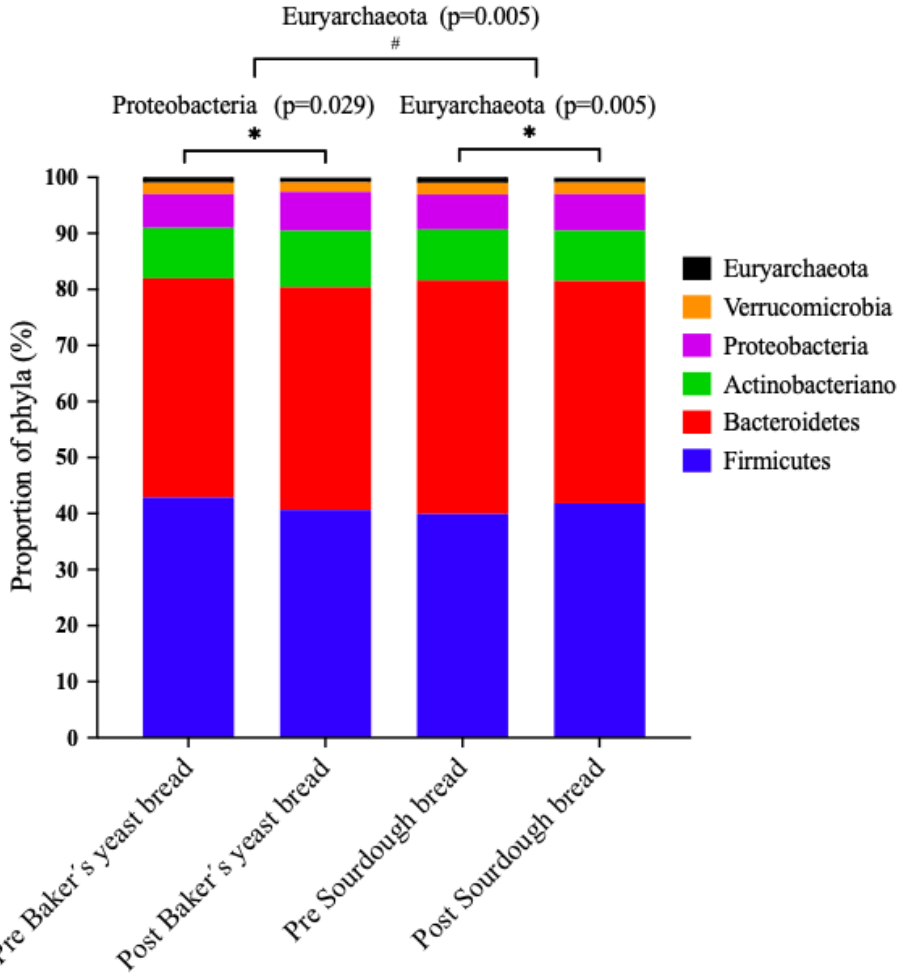

Figure 4.3: Distribution of observed species at phylum level, measured before and after intervention and control. *Proportion of phyla (%) calculated from total expression of species (100%) within all samples. Differences between and within groups were analysed by Wilcoxon Signed Rank-test. *Significant difference within groups, #Significant difference between groups.*

4.5.2 Number of species observed in faecal samples

While a total of 107 bacteria species were measured during the analysis, the median number of species observed in a given faecal sample after consuming the sourdough bread was 39.5. For the baker's yeast bread, this number was 38.5 (fig. 4.4.). Species numbers were found to vary significantly between groups. The samples provided by the participants in the baker´s yeast group had an increase in species variety, whereas the number of species present in the sourdough bread group's samples was relatively unchanged (P=0.012). Furthermore, within the baker's yeast control, the median number of species was significantly increased by a median change of 1.5 (P=0.022). On the other hand, median species numbers for the sourdough bread group changed by 0.

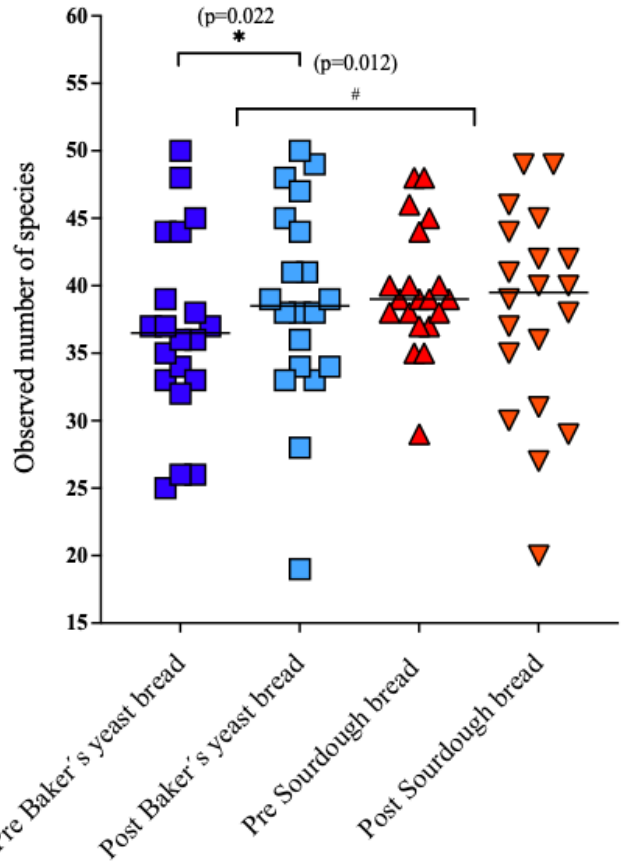

Figure 4.4. Number of *species observed in microbiota*
*Variety in number of species observed before and after consuming sourdough bread or baker´s yeast bread. Squares and triangles illustrate individual number of species observed in the participants. Median score within group presented as a solid line. Differences between groups and within groups were analysed by Wilcoxon signed rank-test. *Significant difference within groups, #Significant difference groups.*

4.5.3 Change in abundance at species level

Of the 107 measured species, 46 were observed in at least 50 % of the samples, and were therefore included in the analyses. In total, 10 species were either significantly different or showed a trend towards significance after the intake of sourdough when compared with the intake of yeast bread (Table 4.4).

The abundance of five species in particular were found to be significantly different between the groups. These five species were increased after the sourdough bread and reduced after the baker´s yeast bread: *Alistipes putredinis (*P=0.015), *Alistipes shahii* (P=0.042), *Bifidobacterium longum subsp. longum* (P=0.027), *Bifidobacterium adolescentis* (p=0.042) and *Bifidobacterium longum* (p=0.012) (Figure 4.5). *Alistipes putredinis* and *Alistipes shahii* were found to be significantly increased after the sourdough bread (P=0.034 and P=0.05, respectively), while *Bifidobacterium longum* (P=0.053) showed a trend towards a significant increase after the sourdough bread. On the other hand, *Bifidobacterium longum* trended towards a significant decrease after the baker's yeast (P=0.065).

Table 4.4: *Change in abundance within specific species before and after consuming sourdough bread or baker´s yeast bread.*

	Baker´s yeast bread				Sourdough bread					
Species	Pre	Post	Post/pre	p-value	Pre	Post	Post/pre	p-value	Sourdough/ Baker´s yeast	p-value*
	Mean (SD)	Mean (SD)	Log2 ratio		Mean (SD)	Mean (SD)	Log2 ratio		Log2 ratio	
Alistipes putredinis	10.55 (6.07)	9.87 (6.25)	-0.67	0.514	9.18 (6.68)	10.49 (6.01)	1.31	**0.034**	1.98	**0.042**
Alistipes shahii	9.21 (4.25)	8.1 (4.96)	-1.11	0.113	8.00 (4.99)	8.63 (5.22)	0.63	**0.005**	1.74	**0.015**
Bifidobacterium adolescentis	5.98 (5.06)	4.90 (5.27)	-1.08	0.103	4.60 (5.27)	5.18 (5.27)	0.57	0.178	1.65	**0.042**
Bifidobacterium longum	9.32 (3.12)	7.83 (4.10)	-1.49	0.065	7.28 (4.59)	8.00 (4.08)	0.72	*0.053*	2.21	**0.012**
Bifidobacterium longum subsp. longum	9.57 (3.12)	8.23 (4.05)	-1.33	0.102	7.60 (4.76)	8.18 (4.55)	0.58	0.098	1.91	**0.027**
Bacteroides cellulosilyticus	7.48 (4.97)	6.34 (5.69)	-1.13	0.156	6.32 (5.68)	6.49 (5.70)	0.17	0.783	1.30	*0.090*
Collinsella aerofaciens	10.49 (1.30)	9.16 (4.07)	-1.32	0.129	8.81 (3.97)	9.00 (4.00)	0.19	0.394	1.51	*0.085*
Parabacteroides merdae	8.10 (5.04)	7.16 (5.51)	-0.94	0.199	6.84 (5.35)	7.28 (5.12)	0.43	0.201	1.37	*0.085*
Paraprevotella clara	3.29 (5.30)	3.46 (5.13)	0.16	0.773	3.14 (5.15)	4.49 (5.32)	1.3	0.065	1.14	*0.099*
Sutterella wadsworthensis	5.94 (5.82)	5.33 (5.87)	-0.61	0.449	5.27 (5.70)	6.57 (5.47)	1.29	0.103	1.90	*0.061*

Values are presented as mean and standard deviation. Values are log2 transformed and differences within groups and between groups are presented as Log2 ratio (post/pre) or Log2 ratio (sourdough/yeast bread), respectively. *Differences between and within groups were analysed by paired t-test. Significant difference within and between groups marked as bold. Trends towards significance within and between groups marked as cursive. The total list of 46 log-transformed species is listed in appendix H, in addition to total list of original values of all 107 species are listed in appendix I, SD; standard deviation.*

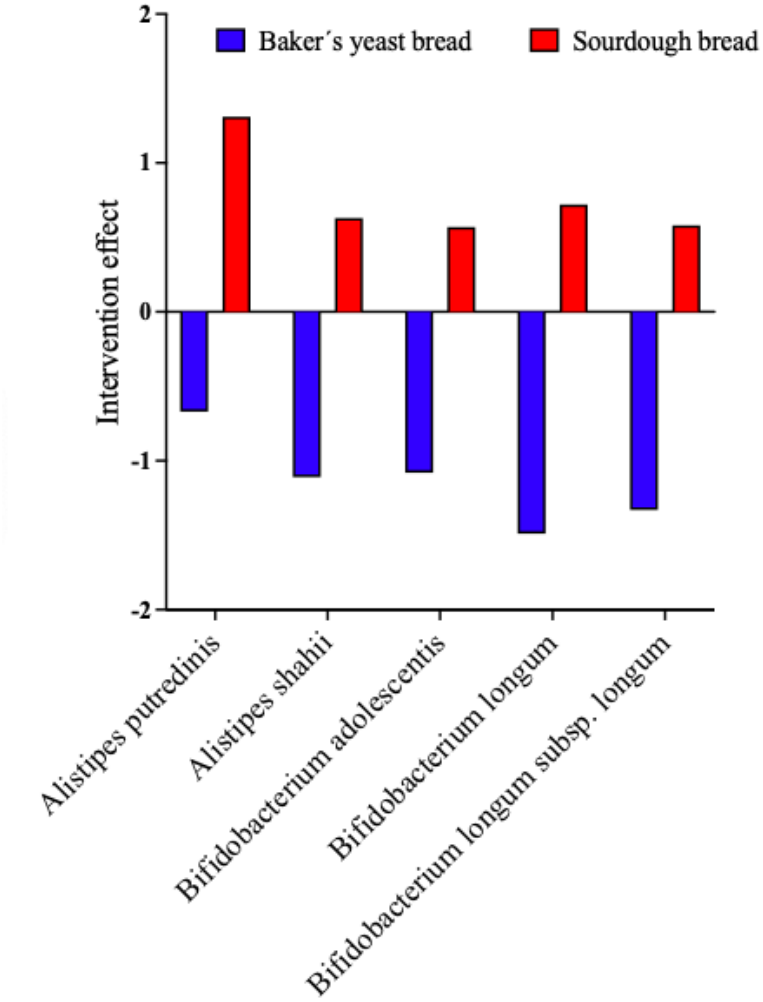

Figure 4.5: *Log2 ratio in species significantly different between groups.*
All values presented as mean. *Difference between groups were analysed by a paired t-test and p-values presented in table 4.4.*

5.0 Discussion

5.1 Method discussion

5.1.1 Study design and sample

This study was an RCT with a crossover design that aimed to investigate the health effects of different types of bread. RCT is a prospective study design which measures the effectiveness of new interventions or treatments and is considered a rigorous tool used to examine the cause-and-effect relationship between an intervention and outcome (Hariton & Locascio, 2018; Stefanopoulou et al., 2020). The employment of an RCT has several benefits. In addition to randomising when participants are given different treatments, an RTC balances the participant characteristics between groups (Hariton & Locascio, 2018). Neither the participants nor the researcher can choose groups on the basis of characteristics. Finally, because they remove and reduce as many sources of bias as possible, RCTs are considered a gold standard within medical research.

Because this study was double-blind and both participants and researchers were unaware of the order of randomisation, it reduced the risk of systematic bias and the placebo effect (Misra S, 2012). However, because this was a dietary intervention study that included two types of bread (sourdough or baker's yeast), it was limited in how blinded it could be. It was possible for some participants to identify the type of bread they were consuming, causing them to be influenced by any prejudice or expectations they may have toward that bread. For instance, it was possible for participants who are familiar with the characteristic smell and taste of sourdough bread to be influenced by a belief that their symptoms should be alleviated. Similarly, it was possible for a recognition of the baker's yeast bread to increase participants' awareness of their symptoms, which could in turn translate to a higher score.

The utilisation of a crossover design has several benefits, but it also has limitations. Because each participant acts as their own control, the impact of confounding variables outside the control of the study is reduced, thus limiting between-subject variability (Hilderley, Fehlings, Lee, & Wright, 2016; Senn S, 2002). Crossover designs are also more efficient than standard

parallel designs or repeated measure designs because they require fewer participants (Hilderley et al., 2016; Louis, Lavori, Bailar III, & Polansky, 1984). However, in comparison to parallel studies, crossover studies typically require twice the time and therefore face a higher risk of limited adherence to study protocol as well as challenges in recruitment. Another limitation of crossover studies is the possibility of carry-over effects (Hilderley et al., 2016). By including a wash-out period between interventions, this study reduced the risk of overlapping intervention effects.

Although RTCs are considered a gold standard they do have several disadvantages. These include being costly in terms of time, effort and money and problems with generalisability and loss to follow-up (Hariton & Locascio, 2018). Additionally, because RCTs often require participants to be physically present at research facilities, they limit the individuals who are able to participate to those who have time to travel or those who live in the surrounding area. Because participants voluntarily participate in an RCT, there is also a great chance that the included sample will consist of people with an above-average interest in health and those who live a relatively healthy lifestyle (Drageset & Ellingsen, 2009). Hence, there is a risk that the participants who volunteer are not representative of the entire population. However, because this study was conducted remotely and samples were collected by the participants in their own house and shipped by post, it can be assumed that a broader range of participants was included. Additionally, because recruitment was conducted on the official university website and on social media, this study was able to reach individuals outside OsloMet. The majority of the participants were women, which is consistent with surveys showing that more women than men engage in health studies (Antonsen, 2009; Bugge, 2012). Normally, this would be considered a weakness; however, because more women than men have been reported to experience gastrointestinal symptoms and have been reported to consume less bread, this can increase the transferability of the results for the study population (Compete and Polling & Statistics, 2018).

All faecal samples were collected by the participants themselves at their homes following the distribution of instructions. A thin layer of stool sample was dispatched on cards with squares of Whatman® filter paper and was then allowed to dry. Similar to the study by Abrahamson, Hooker, Ajami, Petrosino, and Orwoll (2017), the collection process for this study was reported to be tolerable, and all obtained samples were adequate for analysis (Abrahamson et al., 2017). This sampling process is less stressful for the participants because the samples can be issued on their own time and with consideration to their privacy, as they are not under supervision. The

process of air drying the sample allows for minimal bacteria growth and minimal contamination of the sample, ensuring that there is no difference in the profiles of bacteria at the time of collection and when the sample is analysed (Fellows, 2009). Moreover, this process also allows the samples to be delivered by post. With consideration to all of these factors, the self-collection of stool samples for microbiota analysis has been proven to be a successful method.

5.1.2 Questionnaires

The present thesis analysed variables related to gut symptoms with a GSRS-IBS and BSC with the purpose of tracing changes during the study. The employment of questionnaires in research has several benefits. Questionnaires are a method that most people are familiar with; therefore, they require limited supervision and can be easily administrated. Additionally, they are both cost and time efficient and non-invasive in nature. Questionnaire forms have been previously tested and validated in similar studies, demonstrating their strength in accurately measuring gut symptoms (Lewis & Heaton, 1997; Wiklund et al., 2003).

The GSRS-IBS is a validated questionnaire which is well documented as being able to identify specific symptoms that are dominant in patients and as being able to document their responses to change in clinical trials (Wiklund et al., 2003). According to Wiklund et al. (2003), one important attribute of measurement tools such as the GSRS-IBS is their ability to detect changes in a patient's condition over time. The ability of the GSRS-IBS to detect responsiveness to change suggests that this questionnaire is a validated instrument and can be used in clinical trials to establish the most effective therapies for IBS (Wiklund et al., 2003). Thus, the GSRS-IBS is widely used and acknowledged within nutritional research. The BSC is a stool form scale used to monitor changes in intestinal function. Lewis and Heaton (1994) investigated and confirmed the validity of the stool form scale as a guide to gut transit time; they also demonstrated that the scale can be used to monitor changes in intestinal function (Lewis & Heaton, 1994). Although the questionnaires are validated and tested, problems may still occur in relation to patients' understanding and interpretations of the questions. For instance, participants may have difficulties with the questionnaires but fail to clarify these difficulties, causing some answers to be guessed or estimated. While such an issue could easily be resolved when questionnaires are conducted under supervision, it is a particular limitation of conducting

remote research. Additionally, the participants in this study were asked to answer the questionnaire during the last day of the intervention week. As such, some participants may have forgotten about the symptoms they experienced during week and may therefore have failed to record the full spectrum of symptoms they experienced.

Despite the aforementioned limitations, there are several benefits to employing digital questionnaires rather than physical questionnaires (Eberhard-Gran & Winther, 2017). Digital questionnaires are easily accessible, and participants can answer the questions on their own time and with privacy. The forms for this particular study were designed to ensure that there was no missing data and that the questionnaires could not be submitted unless all questions were answered. In this way, the participants were forced to answer every question. To ensure that the participants answered the correct questionnaire, a new link was sent to the participant on the day they were required to answer it. Because all forms were submitted online, the researchers were able to monitor the forms and ensure that the correct number was handed in at the correct points in time.

The digitalisation of the form introduced some technical issues. During submission, all answers were stored in TSD. Therefore, the researchers could only monitor the quantity of submitted forms and ensure that this quantity was correct during each visitation; they were not able to follow up on missing questionnaires. Consequently, a total of three forms were not submitted during the study: one GSRS-IBS and two BSCs. In hindsight, it would have been beneficial to request that participants email a submission confirmation to the research team, as this would have made it easier to follow up on missing questionnaires. Furthermore, an error in the online questionnaire enabled the participants to choose multiple answers in response to each question, despite the questions only requesting one answer. To correct the multiple registered values, the team calculated and recorded the mean value for each question. Within the BSCs, participants were asked to register one value to classify their stool for the entire week. However, especially for patients with IBS, it is not unlikely to experience a wide range of stool changes across multiple days or even within the same day (Raskov et al., 2016). In retrospect, the BSC form should have been distributed daily to record changes in stool classification on a daily basis. This could possibly explain the limited findings in the BSCs.

5.2 Discussion of results

The current study found FODMAP content, specifically fructose, to be lower in the sourdough bread compared to the baker's yeast bread. Furthermore, intake of sourdough bread was observed to decrease overall gut symptoms and increase the abundance of several beneficial gut bacteria to a greater degree than baker's yeast bread.

5.2.1 Application of different types of bread in reducing FODMAP content

Recent studies investigating the potential of different yeast strains to reduce FODMAPs during baking have suggested that, in comparison to baker's yeast bread, sourdough bread might have the ability to degrade FODMAPs to a level that is acceptable to IBS patients (Fraberger et al., 2018; Loponen & Gänzle, 2018). The current study found that FODMAP content was high in the baker's yeast bread, while the sourdough bread presented lower levels of FODMAPs, especially fructose. Previous studies investigating the application of baker's yeast in reducing FODMAPs in bread have shown rather inconsistent results. Ziegler et al. (2016) reported a 77–90% reduction in FODMAPs after four hours of yeast-fermentation; however, other studies have failed to support this finding (Ziegler et al., 2016). Despite this, Ziegler et al.'s (2016) conclusion suggests that *S. cerevisae* may have the ability to degrade FODMAPs but that it does so inconsistently and only to a limited extent. This may explain why the baker's yeast bread in our study was found to have more fructose than the sourdough bread but less fructans.

Compared to the baker's yeast bread, the sourdough bread in this study had a lower content of FODMAPs, particularly fructose, while the baker's yeast bread had less fructans. In addition to *S. cervisae,* which is found in baker's yeast bread, sourdough bread includes a multitude of yeast strains. Two strains that are commonly identified in sourdough, *Kluyveromyces marxianus* and *Torulaspora delbrueckii*, have demonstrated a great capacity to reduce FODMAPs. Struyf et al. found that *kl.marxiuanus* is able to degrade 90% of fructans and fully degrade raffinose (Struyf et al., 2018; Struyf, Laurent, Verspreet, Verstrepen, & Courtin, 2017). In addition, Fraberger et al. (2018) found that both *kl.marxianus* and *T. delbrueckii* are able to completely degrade fructans after 72 hours of fermentation (Fraberger et al., 2018). These yeast strains have also been seen to degrade fructose (Fraberger et al., 2018; Loponen & Gänzle, 2018). Thus, these findings recognise the bacterial composition of sourdough as a central

element in the successful reduction of FODMAPs. Because we did not analyse the composition of the sourdough culture used in this study, it is only possible to speculate whether this particular sourdough benefitted from the abilities of these yeast strains. While a high content of fructans suggests limited breakdown by yeast, the low levels of fructose suggest some activity by either *T. delbruckeii.* or *kl. marxianus* or potentially another yeast. Because the previously mentioned studies were all conducted on the isolated capacities of yeast, it is possible that the synergistic effect is what separates the effect of sourdough from that of baker's yeast.

While T. delbruckeii and kl. marxianus have demonstrated the ability to degrade fructans, this process is only completed after 72 hours (Fraberger et al., 2018). This indicates that fermentation time is an important factor in degrading FODMAPs. The consistent fermentation time of 22 hours implicated in this study was potentially insufficient in degrading FODMAPs, particularly fructans. This suggests that a fermentation time of longer than 22 hours must be considered to possibly reduce all FODMAPs. Moreover, because bacterial composition has been suggested to influence degradation, it is possible that a sourdough that has obtained several yeast strains may benefit from the synergistic effects of these strains and thus require a reduced fermentation time to completely degrade all FODMAPs.

5.2.2 The effect on gut symptoms after intake of sourdough bread compared with yeast bread

This study found the total GSRS-IBS score to be significantly reduced after the intake of sourdough bread and significant different between groups. Previously, fructans have been identified as a potential main trigger of gut symptoms in IBS (Fraberger et al., 2018). For instance, Skodje et al. (2018) found that fructans alone can increase GSRS-IBS by 5 points in individuals with self-reported gluten sensitivity (Skodje et al., 2018). The present study also found that fructans induce a higher score for pain and diarrhoea compared to both placebo and gluten. This is contradictory to the results showing that sourdough, which contains more fructose than baker's yeast bread, reduced GSRS-IBS by 5 points and also reduced pain. The baker's yeast bread increased the total score in GSRS-IBS by 2.5 points and increased diarrhoea. Overall, the sourdough bread contained less FODMAPs, primarily due to the low content of fructose. This suggests that the overall content of FODMAPs may cause a reduction in gut symptoms. Furthermore, these findings could suggest that fructose plays a role similar to that of fructans in the development of gastrointestinal symptoms. Similar to the findings of the

present study, Polese et al. (2018) found abdominal discomfort to be significantly reduced in healthy individuals when consuming sourdough croissants in comparison to brewer's yeast croissants (Polese et al., 2018). This suggests that sourdough containing less FODMAPs may cause fewer symptoms than baker´s yeast.

A previous study of 26 IBS patients who consumed either sourdough bread or baker's yeast bread did not find the sourdough bread to induce a beneficial effect in gut symptoms. Symptoms such as bloating, diarrhoea, constipation and pain as well as total gastrointestinal scores consistently remained equal between the sourdough bread and the baker's yeast bread (Laatikainen et al., 2017). However, the sourdough bread provided by Laatikainen et al. (2017) was only fermented for approximately 12 hours, and the authors did not consider FODMAP content (Laatikainen et al., 2017). Therefore, the FODMAP content of the two breads may have been too similar to alleviate symptoms and cause any differences. In another study by Laatikainen et al. (2016), a low-FODMAP sourdough bread was compared to a traditionally made sourdough with a higher content of FODMAPs. The IBS patients in the study reported significantly milder flatulence, cramps and rumbling when consuming the low FODMAP sourdough bread compared to the traditionally made sourdough bread (Laatikainen et al., 2016). This indicates that overall FODMAP content may indeed be important in alleviating IBS symptoms. The overall FODMAP content in the low-FODMAP sourdough bread provided by Laatikainen et al. (2016) was lower than the FODMAP content of both breads tested in the present study (Laatikainen et al., 2016). Reversely, the traditional sourdough bread in Laatikainen et al.'s (2016) study contained more FODMAPs than the sourdough bread in the present study but less FODMAPs than the bread containing baker's yeast. Therefore, it is possible that a further reduction of FODMAPs in the sourdough bread in the present study could have caused a greater reduction of gut symptoms. However, because Laatikainen et al. (2016) conducted their study on IBS patients, they may have needed an even lower FODMAP content to alleviate symptoms. Thus, the FODMAP content in the sourdough bread provided in the present study may not have been sufficiently reduced enough to alleviate IBS symptoms.

5.2.3 The effect on gut microbiota after intake of sourdough bread compared with yeast bread.

The present study found that baker's yeast bread significantly increased bacterial diversity and that this increase was significantly higher than the increase observed after the sourdough bread intervention. Several previous studies have found that a diet rich in fibre or whole grain can induce benefits related to diversity in the gut microbiota (Myhrstad et al., 2020; Schutte et al., 2018; de Faria Ghetti et al., 2019). The FFQ carried out during screening in the present study found that the participants consumed 100 g of bread and 29 g of dietary fibres daily: more dietary fibres than the general population. Because the participants were told not to change their dietary habits during the study period, it is conceivable that the fibre intake increased in connection with the 100 g increase provided by the increased bread intake. Although baker's yeast bread and sourdough bread have a similar content of dietary fibres, the study noted that the baker's yeast bread significantly increased diversity of bacteria, while the sourdough bread did not. Previous literature is conflicted in regard to the influence of sourdough bread on the gut microbiota. A study by Korem et al. (2017) found no difference in gut microbiota diversity between a white wheat bread providing 8.8 g of fibre daily and a whole wheat sourdough bread providing 17.4 g daily (Korem et al., 2017). Despite the high content of dietary fibre in the sourdough bread, Korem et al. (2017) found that it only caused a small increase in certain bacteria (Korem et al., 2017). Therefore, the limited increase in diversity observed in the present study may also be explained by the fact that sourdough bread increases beneficial bacteria in small amounts. However, further research is required in this area, as other factors such as individual differences could have influenced the outcome.

Certain types of soluble fibre, including inulin and FOS, are more likely to increase the abundance of SCFA-producing bacteria, rather than the diversity of bacteria. (Reimer et al., 2017; Wang et al., 2016). Therefore, it is possible that the differences observed between the groups are a result of differences in the dietary fibre composition of the two types of bread. While the significantly higher increase in bacteria diversity that was observed in the baker's yeast bread can be explained by its greater content of insoluble fibres, the sourdough bread contained more inulin and FOS. This may indicate that the sourdough bread increased the abundance of SCFA-producing bacteria rather than increasing its diversity.

The participants in this study had limited restrictions related to their intake of carbohydrates and dietary fibres. With the exception of other sources of bread, the participants were free to

consume other sources of dietary fibres and carbohydrates. Previous findings have suggested that an intake of a variety of different types of dietary fibres and FODMAPs is most beneficial in increasing diversity (Klimenko et al., 2018; Koç et al., 2020). Therefore, if the participants were consuming more dietary fibre and carbohydrates during the baker's yeast control, this could possibly account for a share of the observed rise in bacterial diversity relative to the sourdough group. Diet registration, such as a 24-hour recall, during the study period could have allowed for the team to record whether the participants made any changes in their diets and reveal the extent to which this may have contributed to the increase in diversity.

5.2.4 The influence of different types of bread on specific bacteria of the gut microbiota

5.2.4.1 Changes in *Alistipes spp.*

The current study found that *Alistipes putredinis* and *Alistipes shahii* were significantly increased in the sourdough bread group; moreover, this increase was significantly different from the corresponding decrease observed in the baker's yeast control. Previous findings have demonstrated that dietary fibres *(Chambers et al., 2019)*, specifically both soluble and insoluble fibres, can increase the growth of *Bacteroides spp.* and *Alistipes spp.* (Li, Hullar, Schwarz, & Lampe, 2009; Salonen et al., 2014). For instance, *A.putredinis* in particular has been associated with cruciferous vegetable intake (Li et al., 2009). As such, because the insoluble dietary fibre content was matched in the baker´s yeast bread and the sourdough bread, the significant increase observed in the sourdough group may be explained by the higher content of inulin. Moreover, the lack of increase in the baker's yeast control may suggest that the inulin and FOS content was not sufficient enough to increase *A. putredinis* and *A. shahii*.

Previous studies have found *Alistipes spp.* to be a prominent genus in a healthy gut microbiota (Parker, Fonseca, & Carding, 2020) and have specifically observed *A. putredinis* and *A. shahii* to be beneficial bacteria. This suggests that the increase in these bacteria in the present study can be beneficial and potentially a sign of health. *A. putredinis* has previously been demonstrated to produce acetate and propionate (Kiewiet et al., 2020), while *A. shahii* has been proven to produce butyrate (Parker et al., 2020). Hence, they are both believed to be associated with an increase in SCFA. This suggests that an increase in *A. putredinis* and *A. shahii* may be

linked to an increase in SCFA. Because the present study did not measure SCFA, it is only possible to speculate whether *A. putredinis* and *A. shahii* do indeed increase SFCA. Future studies could investigate whether this is true. It has also previously been hypothesised that *A. putredinis* and *A. shahii* can induce an anti-inflammatory effect as a result of their metabolic properties (Parker et al., 2020). For instance, El-Salhy et al. (2020) demonstrated that *Alistipes spp.* is inversely correlated with the overall score on the irritable bowel severity scoring system, another instrument frequently used to quantify IBS symptoms (El-Salhy et al., 2020). Although the present study did not test these correlations, it is possible that this was also the case in the present study. The observed increase in *A. putredinis* and *A. shahii* could potentially have induced an anti-inflammatory effect, which could be linked to the reduction in pain, bloating and total score observed in the sourdough bread group.

5.2.4.2 Changes in *Bifidobacterium spp.*

This study found significant differences between the groups in relation to three species of the genus *Bifidobacterium,* specifically *B. adolscentis, B. longym* and *B. longum subsp longum.* In the sourdough group, all of these species increased, with *B. longum* and *B. longum subsp longum* showing trends towards significance. In contrast, these species were reduced in the baker's yeast control, with a trend toward significance for *B. longum.* Several previous studies have documented changes in *Bifidobacterium spp*. in relation to changes in dietary intake (Costabile et al., 2008; Halmos et al., 2015; McIntosh et al., 2017; Staudacher et al., 2012). For instance, it has been suggested that certain types of soluble fibre, such as FOS and inulin, are most beneficial in stimulating the growth of *Bifidobacterium spp.* (So et al., 2018; Wang et al., 2020). Similar to the present study's findings for *Alistipes spp.*, variation in *Bifidobacterium spp.* between the groups in this study could be explained by differences in the composition of inulin and FOS. While the amount of overall soluble fibre was similar between both breads, the sourdough bread had a higher content of inulin and FOS compared to the baker's yeast bread, possibly contributing to the increase in *Bifidobacterium spp*. Furthermore, *Bifidobacterium spp.* is suggested to be an important bacterium in cross-feeding other bacteria (Canfora et al., 2017). Therefore, the observed increase across all *Bifidobacterium spp.* in the sourdough group may suggest that the dosages of FOS and inulin were substantial enough to result in cross-feeding between members of *Bifidobacterium spp.*

Although both the baker's yeast bread and the sourdough bread provided in the present study contained fructans, the level of *Bifidobacterium spp.* only increased in the intervention with sourdough bread. The low content of FOS and inulin in the baker's yeast bread was expected to cause a small increase in *Bifidobacterium*. However, the level of *Bifidobacterium spp.* remained relatively stable in the baker's yeast control, with only a small reduction being observed, especially in *B. longum subsp longum*. Previous studies have found that a low FODMAP diet can significantly reduce the abundance of the genus *Bifidobacterium* and the species *B. adolscentis* and *B. longum* (Halmos et al., 2015; McIntosh et al., 2017; Staudacher et al., 2012). Thus, the reduction observed in the baker's yeast control could in part be explained by the lower content of inulin and FOS. Furthermore, Costabile et al. (2014) investigated the insemination of faecal samples with sourdough bread that had been fermented for eight hours and found that faecal samples collected from IBS patients did not see an increase in *Bifidobacterium spp.*, while faecal samples collected from healthy individuals did (Costabile et al., 2014). However, similar to the present study, the inulin and FOS content in the sourdough bread may not have been substantial enough cause an increase in *Bifidobacterium spp.* in IBS patients. Thus, the small reduction in *B. longum subsp longum* may be caused by the fermentation of inulin and FOS by another *Bifidobacterium spp.*

Although the present study did not investigate correlations between *Bifidobacterium* and gut symptoms, other studies have. These studies have demonstrated that the administration of some probiotics, particularly *Bifidobacterium spp.*, successfully moderates symptoms in patients with IBS, predominantly abdominal pain, diarrhoea and bloating (Amundsen, 2016; Kajander, Hatakka, Poussa, Färkkilä, & Korpela, 2005; O'Mahony et al., 2005; Saggioro, 2004; Whorwell et al., 2006). For instance, supplementation with *B. longum subsp. longum* has been proven to significantly reduce and prevent incidents of diarrhoea (Chenoll et al., 2015; Corrêa, Péret Filho, Penna, Lima, & Nicoli, 2005; O'Callaghan & van Sinderen, 2016). Therefore, it can be speculated that the trend toward significant reduction in *B. longum subsp longum* that was observed in the baker's yeast control in our study may have been linked to the observed increase in diarrhoea. Additionally, an inverse relationship between *Bifidobacterium* and abdominal pain has been demonstrated in previous studies, where participants who experienced pain had less *Bifidobacterium* compared to those without pain (Jalanka-Tuovinen et al., 2011). This suggests that the increase in *Bifidobacterium spp*. and the significant decrease in pain observed in the sourdough bread group in our study, could be linked. However, because the present study did not test correlations between the two, it is only possible to speculate whether this is true.

6.0 Conclusion

Overall, the sourdough bread in this study was found to contain less FODMAPs, specifically fructose. In comparison to baker's yeast bread, sourdough bread caused significantly less gut symptoms in healthy individuals. Additionally, the observed changes in the gut microbiota, namely the increase in *Bifidobacterium spp*. and *Alistipes spp*., suggest that the sourdough bread had a beneficial effect on gastrointestinal health. Therefore, in comparison to baker's yeast bread, it appears that sourdough bread is indeed a better option for those who are sceptical of consuming bread because of gut symptoms. The beneficial effect may be linked to reduced content of FODMAPs in the sourdough bread.

7.0 Future directions of research

A future intervention study comparing sourdough bread and baker's yeast bread should aim to include a greater sample size and consider recruiting IBS patients. In doing so, any outcomes can be transferable to both this population and those with minor stomach symptoms. It would also be relevant to collect and analyse a larger proportion of bacteria in the faecal samples than the 107 analysed in this study. Additionally, an examination of SCFA could shed light on whether the increase in intestinal bacteria is associated with an increase in SCFA and whether this translates to any metabolic changes. Furthermore, it would be beneficial to examine correlations between the gut microbiota, SCFA and gut symptoms. It may also be beneficial to collect data on gut symptoms on several occasions during the intervention week to map variations in the symptoms throughout the intervention. In addition, it may be advantageous for participants to report their dietary intake throughout the study, as this would allow for an exclusion of other potential factors that may affect the outcome. The sourdough bread provided in a future study should be analysed for bacteria and FODMAP content before and after fermentation. Moreover, the protocol should include a fermentation time above 22 hours, which is similar to the fermentation time in other studies which have found a successful degradation of most FODMAPs.

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9.0 APPENDICES

Appendix A: Information sheet

FORESPØRSEL OM DELTAKELSE I FORSKNINGSPROSJEKTET

HELSEEFFEKTER OG FORBRUKERASPEKTER VED INNTAK AV FORSKJELLIGE BRØDTYPER (HELFAB-STUDY)

Dette er et spørsmål til deg om å delta i forskningsstudien «Helseeffekt og forbrukeraspekter ved inntak av forskjellige brødtyper (HELFAB-study)».

Fiber er kjent å ha mange positive helseeffekter, og brød er den viktigste kilden til fiber i det norske kostholdet. Tradisjonelt har brød blitt bakt med surdeig, men i moderne brødproduksjon benyttes gjær som hevemiddel. Surdeig inneholder bakteriekulturer som gjennom fermentering er vist å bidra med positiv effekt på blodsukkerregulering. Individer som reagerer med ubehag i mage/tarm på inntak av brød som inneholder mye ikke-fordøyelige fiber av typen oligosakkarider, disakkarider, monosakkarider og polyoler (FODMAP), er vist å få mindre ubehag etter inntak av surdeig. Det er også antatt at tarmfloraen vår vil påvirkes av type brød vi spiser, men det er begrenset med studier på mennesker som undersøker helseeffekter av surdeigsbrød sammenlignet med brød bakt med gjær.

Hensikten med prosjektet er å undersøke om surdeigsbrød sammenlignet med brød bakt på gjær kan påvirke helseeffekter, som mage- og tarmsymptomer, blodsukker og fettstoffer i blodet, og om dette kan relateres til endringer i tarmflora. Vi ønsker videre å undersøke forbrukers holdninger til brød og magehelse.

HVA INNEBÆRER PROSJEKTET?

All deltagelse i studien vil foregå via telefon/internett og forsendelser vil skje via post/budbil. Kostintervensjonen består i å spise brød bakt med surdeig eller gjær hver dag i 1 uke. Før og etter intervensjonen samler deltagerne selv inn avføringsprøve og blodprøve (blod fra fingerstikk på et trekkpapir) som de sender i posten til OsloMet i ferdigfrankerte konvolutter. I tillegg vil vi spørre om din vekt og høyde, om ditt normale kosthold og be deg fylle ut noen spørreskjemaer.

Hele studieperioden vil foregå over 5 uker. I en run-in periode på 2 uker vil deltagerne spise brød bakt med gjær (kontroll). Etter run-in perioden vil deltagerne bli randomisert til enten å spise surdeigsbrød eller fortsette på kontrollbrødet (gjær) i 1 uke. Deretter vil det være en utvaskingsperiode på 1 uke der alle deltagerne spiser kontrollbrød (gjær). Så vil det være en siste periode på 1 uke der deltagerne igjen spiser surdeigsbrød eller kontrollrød (gjær). Alle brødene som inngår i studien vil bli utlevert. Deltagerne vil få en oversikt over restriksjoner. Deltakerne må være villige til å slutte med bruk av probiotiske melkesyrebakterier (eks. Biola, Activia etc.) og kosttilskudd 4 uker før studiestart og gjennom hele studieperioden. Utover testbrødene og gitte restriksjoner skal deltakerne spise og leve som normalt. Du vil også bli spurt om å delta i individuelle intervjuer ved starten og slutten av prosjektet. I disse intervjuene vil du få spørsmål om dine opplevelser av deltagelsen i studien og produktene du spiste. Intervjuene vil gjennomføres via zoom. Intervjuene transkriberes og lagres uten direkte personidentifiserbare opplysninger.

Mesterbakeren er samarbeidspartner i prosjektet, som vil si at de deltar i diskusjoner og bidrar med gjennomføringen av studien, i tillegg til at de har ansvar for produktene som inngår. Det er forskerne som har det endelige ansvaret for studien og som håndterer alle prøvesvar og er i kontakt med deltagerne. Resultatene fra studien vil bli publisert uavhengig av utfall.

I prosjektet vil vi innhente og registrere opplysninger om deg. Vi vil be deg om å avgi blodprøver i form av fingerstikk før og etter intervensjonsperioden på 4 uker. Vi vil også be om at deltagerne avgir avføringsprøve, vekt og svarer på spørreskjema om tarmsymptomer før og etter intervensjonsperioden. Deltagerne vil få utlevert alle intervensjonsproduktene. Blodprøver og avføringsprøver skal sendes i posten, mens spørreskjema skal besvares elektronisk. Deltagerne vil bli fulgt opp jevnlig via telefon, sms og/eller mail.

MULIGE FORDELER OG ULEMPER

Som deltager i prosjektet vil du få informasjon om egne blodverdier. Det vil alltid være noe usikkerhet i målingene, og det er derfor mulig at noen feilaktig kan få målt høye blodverdier uten å ha det. Dersom du måler høye verdier vil du bli anbefalt videre oppfølging av fastlegen. Noen kan oppleve ubehag ved blodprøvetaking. Utover dette er det ingen kjente ulemper eller ubehag ved å delta.

FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE SITT SAMTYKKE

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte Vibeke Telle-Hansen, 930 48 873, vtelle@oslomet.no.

HVA SKJER MED OPPLYSNINGENE OM DEG?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet i hensikten med prosjektet. Så lenge deltagerne kan identifiseres i datamaterialet, har du rett til å få:

- innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert
- slettet personopplysninger om deg
- utlevert en kopi av dine personopplysninger (dataportabilitet)

Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene.

Alle opplysningene vil bli behandlet avidentifisert, det vil si at navn og fødselsnummer (eller andre direkte gjenkjennende opplysninger) oppbevares adskilt fra målingene (f.eks. alder, vekt, blodprøveverdier). Det er kun medlemmer i prosjektgruppen som vil ha tilgang til de avidentifiserte data. En kode knytter deg til dine opplysninger gjennom en kodeliste. Det er kun Vibeke Telle-Hansen, Mari Myhrstad, Ellen Raael som har tilgang til denne listen.

Opplysningene om deg vil bli innhentet via elektronisk spørreskjema fra UiO. Opplysningene vil bli lagret i databaser hos Tjenester for Sensitive Data (TSD).

Opplysningene om deg vil bli oppbevart ved OsloMet (behandlingsansvarlig institusjon) under prosjektperioden og i inntil 5 år etter prosjektslutt (2025) av dokumentasjonshensyn etter vilkår fra Regionale komiteer for medisinsk og helsefaglig forskningsetikk.

HVA SKJER MED PRØVER SOM BLIR TATT AV DEG?

Prøvene som tas av deg skal oppbevares i en forskningsbiobank tilknyttet prosjektet. Blodprøver og avføringsprøver vil bli oppbevart i en biobank (HELFAB-study), som er lokalisert på OsloMet, Kjeller. Ansvarshavende for biobanken er prosjektleder og førsteamanuensis Vibeke Telle-Hansen.

Biobanken opphører ved prosjektslutt.

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. Det vil ikke være mulig å spore resultatene i studien tilbake til deg når disse publiseres.

En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg.

Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. Informasjon om deg vil bli anonymisert eller slettet senest fem år etter prosjektslutt.

FORSIKRING

Dersom et uhell eller en komplikasjon skulle inntreffe, er deltagerne forsikret gjennom pasientskadeforsikringen i prosjektperioden.

ØKONOMI

Deltagere som gjennomfører studien vil få en «goodiebag» med assorterte produkter fra Mesterbakeren til en verdi av ca. 200 NOK.

GODKJENNING

Regional komité for medisinsk og helsefaglig forskningsetikk har vurdert prosjektet, og har gitt forhåndsgodkjenning (saksnummer hos REK: 96264).

Etter ny personopplysningslov har behandlingsansvarlig OsloMet - storbyuniversitetet og prosjektleder Vibeke Telle-Hansen et selvstendig ansvar for å sikre at behandlingen av dine opplysninger har et lovlig grunnlag. Dette prosjektet har rettslig grunnlag i EUs personvernforordning artikkel 6 nr. 1a og artikkel 9 nr. 2a og ditt samtykke. På oppdrag fra OsloMet - storbyuniversitetet har NSD – Norsk senter for forskningsdata AS vurdert at behandlingen av personopplysninger i dette prosjektet er i samsvar med personvernregelverket.

Du har rett til å klage på behandlingen av dine opplysninger til personvernombudet ved OsloMet - storbyuniversitetet eller Datatilsynet.

KONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet kan du ta kontakt med Vibeke Telle-Hansen, 930 48 873, vtelle@oslomet.no.

Personvernombud ved institusjonen er ingrid.jacobsen@oslomet.no.

JEG SAMTYKKER TIL Å DELTA I PROSJEKTET OG TIL AT MINE PERSONOPPLYSNINGER OG MITT BIOLOGISKE MATERIALE BRUKES SLIK DET ER BESKREVET

Appendix B: Consent form

Sted og dato

Deltakers signatur

Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet.

Sted og dato

Signatur

Rolle i prosjektet

Appendix C: Case Report Form (Screening)

Helseeffekter og forbrukeraspekter ved inntak av forskjellige brødtyper

Kort tittel: HELFAB-study

Periode: Høst 2020

ID nummer: _____

Visitt: Screening

Navn på personen som intervjuer: _____

ID nr:	
Dato:	

Antropometri:

	Ja	Nei
Vekt kg		
BMI		

Kartlegging:

	JA	**NEI**
Har du fått informasjon om hva studien går ut på?		
Har du signert samtykket?		
Har du gjort endringer i bruk av medisiner, inkludert antibiotika eller hormonbehandling, i løpet av de siste 4 ukene? Hvis ja, når og hvilken type?		
Planlegger du å gjøre endringer i kostholdet ditt, inkludert endre vekt i nærmeste fremtid? Hvis ja hva går de ut på? Beskriv kort:		
Er du villig til å opprettholde restriksjonene gjennom hele studieperioden, og ellers spise som normalt?		
Er du villig til å opprettholde normalt nivå av fysisk aktivitet gjennom hele studien?		
Er du villig til å slutte med kosttilskudd fom 4 uker før studiestart og gjennom hele studien?		

Laboratorieprøver:

	JA	NEI

Har du fått informasjon om hvordan ta blodprøve med DBS?		
Har du fått informasjon om hvordan ta feces prøve?		

Spørreskjema og intervju:

	JA	NEI
Er GSRS-IBS skjema fylt ut?		
Er BSC fylt ut?		
Har du fått informasjon om utfylling av FFQ?		
Er du informert om mulighet til å delta i kvalitativt intervju?		
Har du signert samtykke for kvalitativt intervju?		

Videre deltagelse:

	JA	NEI
Har du fått utlevert informasjon om kostrestriksjoner/alternativer?		
Har du fått informasjon om testproduktene, hvor de hentes og instruks om hvordan de skal spises?		
Har du fått informasjon om prøvetaking med feceskit og DBS til V1?		
Har du fått informasjon om hvordan sende inn/levere inn feces- og blodprøver til oss?		

Er det avtalt tid for neste telefonvisitt; V1
Dato:

Appendix D: Case Report Form (Baseline: Visitation 1)

Helseeffekter og forbrukeraspekter ved inntak av forskjellige brødtyper

Kort tittel: HELFAB-study

Periode: Høst 2020

ID nummer: _____

Visitt: Baseline V1

Navn på personen som intervjuer: _____

ID nummer:	
Dato:	

Antropometri:

	Ja	Nei
Vekt kg		
BMI		

Kartlegging:

	JA	**NEI**
Har du hentet og spist de utleverte produktene?		
Hvor mange brødskiver har du spist til sammen (per dag)?:		
Har du gjort endringer i bruk av medisiner, inkludert antibiotika eller hormonbehandling, i løpet av perioden fra screeningvisitten? Hvis ja, hvilke endringer er gjort?		
Har du vært syk i perioden fra forrige visitt? Hvis ja, har du brukt noen medisiner? Navn på medikament: I hvilken periode benyttet du disse medisinene? Dato (f.o.m og t.o.m):		
Har du gjort endringer i kostholdet ditt annet enn fiber og probiotikarestriksjoner de siste 2 ukene? Hvis ja hva går de ut på? Beskriv kort:		
Har du gjort endringer i fysisk aktivitet? Type og mengde fysisk aktivitet: _____		
Bruker du fortsatt ikke kosttilskudd og matvarer med probiotika?		

Forberedelse før blodprøvetaking:

	JA	NEI
Var du fastende ved blodprøvetaking?		
Har du drukket alkohol 24 timer før blodprøvetaking?		
Trente du med høy intensitet i går?		

Laboratorieprøver:

	JA	NEI
Har du tatt fastende blodprøve i dag? (ja/nei)		
Har du tatt avføringsprøve?		
Har du sendt inn blodprøve og fecesprøve? Ønsker du å avlevere/at noen skal hente prøvene på avtalt sted? I så fall, hvor og når?		

Spørreskjema og intervju:

	JA	NEI
Har det vært gjennomført kvalitativt intervju?		
Er GSRS-IBS skjema fylt ut?		
Er BSC fylt ut?		
Er FFQ fylt ut?		

Videre deltagelse:

	JA	NEI
Har du fått utlevert informasjon om kostrestriksjoner/alternativer?		
Har du fått informasjon om henting av testproduktene og instruks om hvordan det skal spises?		
Har du fått utlevert feceskit og DBS til V2?		

Er det avtalt tid for neste telefonvisitt; V2
Dato:

Appendix E: Gastrointestinal Symptoms Ratings scale

THE GASTROINTESTINAL SYMPTOM RATING SCALE (GSRS) IRRITABLE BOWEL SYNDROME (IBS)-VERSJON

Les dette først:

Undersøkelsen inneholder spørsmål om hvordan du har følt deg og hvordan du har hatt det DE 3 SISTE DAGER. Sett kryss (X) ved det alternativet som passer best på deg og din situasjon.

1. Har du i løpet av de siste tre dagene vært plaget av MAGESMERTER?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

2. Har du i løpet av den siste tre dagene vært plaget av SMERTER ELLER UBEHAG I MAGEN SOM GIR SEG NÅR DU HAR HATT AVFØRING?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

GSRS-IBS (Norwegian)(031217)

IDKode_____

3. Har du i løpet av den siste tre dagene vært plaget av OPPBLÅSTHET?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

4. Har du i løpet av den siste tre dagene vært plaget av LUFTAVGANG?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

5. Har du i løpet av den siste tre dagene vært plaget av FORSTOPPELSE (problemer med å tømme tarmen)?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

IDKode_____

6. Har du i løpet av den siste tre dagene vært plaget av DIARÉ (hyppig avføring)?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

7. Har du i løpet av den siste tre dagene vært plaget av LØS AVFØRING?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

8. Har du i løpet av de siste tre dagene vært plaget av HARD AVFØRING?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

IDKode_____

9. Har du i løpet av den siste tre dagene vært plaget av TVINGENDE AVFØRINGSBEHOV (plutselig behov for å gå på toalettet for å tømme tarmen)?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

10. Har du i løpet av de siste tre dagene vært plaget av en FØLELSE AV UFULLSTENDIG TØMMING AV TARMEN ETTER AVFØRING?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

11. Har du i løpet av den siste tre dagene vært plaget av at du FØLER DEG METT LIKE ETTER AT DU HAR BEGYNT PÅ ET MÅLTID?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

IDKode_____

12. Har du i løpet av den siste tre dagene vært plaget av at du FØLER DEG METT SELV LENGE ETTER AT DU ER FERDIG MED Å SPISE?

- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

13. Har du i løpet av den siste tre dagene vært plaget av at MAGEN ER SYNLIG OPPBLÅST?

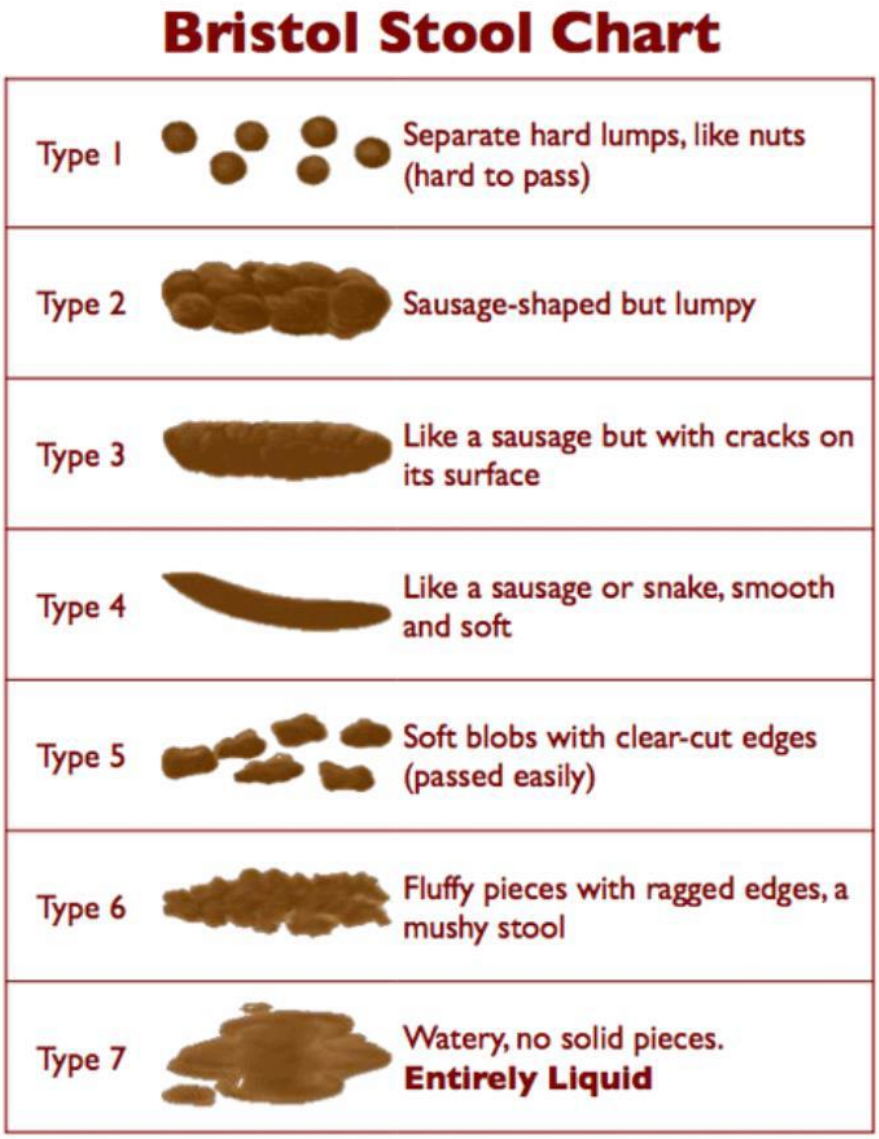
- ☐ Ingen plager i det hele tatt
- ☐ Ubetydelige plager
- ☐ Milde plager
- ☐ Moderate plager
- ☐ Ganske alvorlige plager
- ☐ Alvorlige plager
- ☐ Meget alvorlige plager

KONTROLLER AT ALLE SPØRSMÅLENE ER BESVART!

TAKK FOR DIN MEDVIRKNING.

IDKode_____

Appendix F: Bristol Stool Chart



Reference: Lewis SJ & Heaton KW, 1997, 'Stool form scale as a useful guide to intestinal transit time'. *Scandinavian Journal of Gastroenterology*, vol.32, no.9, pp.920 - 924.

Appendix G: Overview of dietary restrictions

Ikke ta noe form for kosttilskudd

Unngå probiotiske melkesyrebakterier

Unngå produkter som:
- Biola
- Kefir
- Activia

Unngå brødprodukter, andre enn de du får utdelt

Brød/rundstykker kjøpt i butikk, hjemmelaget brød

Brød utlevert av oss

Opprett ellers ditt normale kosthold gjennom hele studien

Appendix H: Regional committes for Medical and Health Research Ethics Sør-Øst Approval

Alle skriftlige henvendelser om saken må sendes via REK-portalen
Du finner informasjon om REK på våre hjemmesider rekportalen.no

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst A	Tove Irene Klokk	22845522	01.04.2020	96264
			Deres referanse:	

Vibeke Telle-Hansen

96264 HELFAB-study

Forskningsansvarlig: OsloMet - storbyuniversitetet

Søker: Vibeke Telle-Hansen

Søkers beskrivelse av formål:

Fiber har mange gunstige helseeffekter, inkludert bedre blodsukkerregulering og lavere kolesterolverdier, som igjen bidrar til redusert risiko for å utvikle metabolske sykdommer. Brød er den viktigste kilden til fiber i det norske kostholdet. Dagens produksjon av brød er endret sammenlignet med tradisjonell brødbaking som ble gjort med surdeig. Bruk av gjær (Saccharomyces cerevisiae) i brødbaking dominerer som hevemiddel i dagens industrielle brødproduksjon. Surdeig inneholder kulturer av melkesyre- og eddiksyrebakterier, som naturlig gir heving av brødet. Bakteriene som naturlig finnes i surdeig frigir flere komponenter som man ikke finner i moderne brødbaking der gjær benyttes. Det er blant annet vist at surdeig har positive effekter på blodsukkerregulering. Hensikten med denne studien er å få bedre kunnskap om mulige positiv helseeffekter av å spise surdeigsbrød sammenlignet med brød bakt på gjær.

REKs vurdering

Vi viser til tilbakemelding i ovennevnte forskningsprosjekt. Tilbakemeldingen ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst A) i møtet 12.03.2020. Vurderingen er gjort med hjemmel i helseforskningslovens § 10.

Saksgang

Søknad om forhåndsgodkjenning ble behandlet av komiteen i møte 13.02.2020. Det ble besluttet å utsette vedtak i saken. Følgende inngikk i komiteens vurdering jf. brev av 02.03.2020:

«Formålet med studien er å få kunnskap om mulige helseeffekter av surdeigsbrød sammenliknet med brød bakt på gjær.

Studien er en randomisert, kontrollert, dobbeltblindet, overkrysset kostintervensjon. Det skal inkluderes 20 deltakere som skal randomiseres 1:1 til å spise surdeigsbrød eller brød bakt på gjær over en periode på 5 uker. Deltakerne er friske frivillige av begge kjønn, med stabil kroppsvekt og i alderen 18-65 år. Rekruttering vil skje via sosiale medier og nettsider.

Deltakerne skal først spise brød bakt på gjær i 2 uker. Deretter skal halvparten spise surdeigsbrød og halvparten gjærbrød i 1 uke. Etter nok en uke med gjærdeigsbrød skal gruppene bytte slik at de som først spiste surdeigsbrød 1 uke nå spiser gjærdeigsbrød og omvendt i 1 uke. Før og etter studien skal deltakerne sende inn blodprøver og fecesprøver, samt besvare spørreskjema om tarmsymptomer. Deltakerne vil også bli spurt om å delta i individuelle intervjuer før og etter intervensjonen.

Deltakerne skal ikke innta mat med probiotiske melkesyrebakterier fire uker før studiestart og under hele studieperioden, og heller ikke kosttilskudd. Utover testmåltidene og gitte restriksjoner skal deltakerne spise og leve som normalt. Studien er samtykkebasert.

Testproduktene skal ha samme grunnoppskrift og samme grovhetsgrad, og de skal være pakket nøytralt. Alle produktene leveres av Mesterbakeren.

Biologiske prøves som samles inn skal lagres i en forskiningsspesifikk biobank med prosjektleder som ansvarshavende, med samme varighet som prosjektet. Det skal ikke gjøres genetiske undersøkelser.

Komiteen mener dette kan være et nyttig prosjekt som vil kunne gi ny kunnskap om potensielle helsegevinster ved å spise surdeigsbrød. Komiteen anser også prosjektet som forsvarlig å gjennomføre. Komiteen har likevel noen spørsmål til prosjektet som bes besvares før det kan fattes et vedtak i saken:

1. *Komiteen ber om at prosjektleder redegjør for hvilken rolle selskapet Mesterbakeren spiller i utforming av design og gjennomføring av prosjektet. Denne informasjon må i tillegg tydelig fremkomme i informasjonsskrivet.*
2. *Prosjektleder bes redegjøre for hvilken rolle de ulike medarbeiderne har i prosjektet, og om det er noen med medisinsk kompetanse i prosjektgruppen.*

Prosjektleder bes komme med en tilbakemelding på komiteens merknader, og samtidig sende inn reviderte informasjonsskriv i henhold til komiteens kommentarer.»

Prosjektleder har sendt tilbakemelding mottatt 05.03.2020. Revidert informasjonsskriv var vedlagt tilbakemeldingen.

Ny vurdering

I tilbakemeldingen svarer prosjektleder utfyllende på komiteens merknader. Informasjon om Mesterbakerens rolle i prosjektet er klargjort, og informasjonsskrivet inneholder nå adekvat informasjon om dette. Det er også klargjort hvilken rolle de ulike medarbeiderne i prosjektet har.

Komiteen godkjenner derfor prosjektet slik det nå foreligger.

Vedtak

Godkjent

REK har gjort en helhetlig forskningsetisk vurdering av alle prosjektets sider. Prosjektet godkjennes med hjemmel i helseforskningsloven § 10.

Vi gjør samtidig oppmerksom på at etter ny personopplysningslov må det også foreligge et behandlingsgrunnlag etter personvernforordningen. Det må forankres i egen institusjon.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknad og protokoll, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Godkjenningen gjelder til 30.04.2025.
Komiteens avgjørelse var enstemmig.

Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Opplysningene skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en datafil. Opplysningene skal deretter slettes eller anonymiseres.

Klageadgang
Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. helseforskningsloven § 10 tredje ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst A. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.

Vennlig hilsen

Knut Engedal
Professor dr. med.
Leder REK sør-øst A

Tove Irene Klokk
Seniorrådgiver
REK sør-øst

Kopi til forskningsansvarlig institusjon(er) og medbruker(e).

Sluttmelding
Søker skal sende sluttmelding til REK sør-øst A på eget skjema senest seks måneder etter godkjenningsperioden er utløpt, jf. hfl. § 12.

Søknad om å foreta vesentlige endringer
Dersom man ønsker å foreta vesentlige endringer i forhold til formål, metode, tidsløp eller organisering, skal søknad sendes til den regionale komiteen for medisinsk og helsefaglig forskningsetikk som har gitt forhåndsgodkjenning. Søknaden skal beskrive hvilke endringer som ønskes foretatt og begrunnelsen for disse, jf. hfl. § 11.

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst A	Tove Irene Klokk	22845522	12.08.2020	96264
			Deres referanse:	

Vibeke Telle-Hansen

96264 HELFAB-study

Forskningsansvarlig: OsloMet - storbyuniversitetet

Søker: Vibeke Telle-Hansen

REKs vurdering

Vi viser til søknad om prosjektendring mottatt 09.08.2020 og ettersendt dokument 10.08.2020 for ovennevnte forskningsprosjekt. Søknaden er behandlet av leder i REK sør-øst på delegert fullmakt fra REK sør-øst A, med hjemmel i helseforskningsloven § 11.

Grunnet covid-19 pandemien vil man ikke invitere deltakere til OsloMet. Det er ønske om å gjennomføre studien i sin helhet via telefon/mail/zoom, og innhenting av blodprøver via post/budbil. Det vil derfor innhentes samtykke elektronisk, og kvalitative intervjuer vil bli gjennomført ved bruk av zoom. Kapillære blodprøver tatt på trekkpapir vil bli sendt i posten. Det er ikke mulig å innhente venøse blodprøver som planlagt, så noen av de opprinnelig planlagte analysene vil derfor utgå. Disse er erstattet med andre analyser.

Alle endringene er inkludert i revidert forskningsprotokoll, og det er utarbeidet nytt informasjonsskriv for deltakerne, samt informasjon om gjennomføring av intervjuer på zoom. Det er også gjort mindre endringer i intervjuguiden.

Komiteens leder har vurdert søknaden og forstår nødvendigheten av å endre prosjektet for å tilpasse seg nåværende situasjon. Endringene anses som forsvarlig å gjennomføre og innenfor formålet med studien. Komiteens leder har derfor ingen innvendinger mot at endringene gjennomføres som beskrevet i søknad om prosjektendring og revidert protokoll.

Vedtak

Godkjent

Komiteen godkjenner med hjemmel i helseforskningsloven § 11 annet ledd at prosjektet videreføres i samsvar med det som fremgår av søknaden om prosjektendring og i samsvar med de bestemmelser som følger av helseforskningsloven med forskrifter.

Vi gjør samtidig oppmerksom på at etter ny personopplysningslov må det også foreligge et behandlingsgrunnlag etter personvernforordningen. Det må forankres i egen institusjon.

Godkjenningen gjelder til 30.04.2025.

Dersom det skal gjøres ytterligere endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende ny endringsmelding til REK.

Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Opplysningene skal oppbevares avidentifisert, dvs. atskilt i en nøkkel- og en datafil. Opplysningene skal deretter slettes eller anonymiseres.

Prosjektet skal sende sluttmelding til REK, se helseforskningsloven § 12, senest 6 måneder etter at prosjektet er avsluttet.

Vennlig hilsen

Knut Engedal
Professor dr. med.
Leder REK sør-øst A

Tove Irene Klokk
Seniorrådgiver
REK sør-øst

Kopi til forskningsansvarlig institusjoner(er) og medbruker(e).

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK sør-øst A. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst A, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag (NEM) for endelig vurdering.

Appendix I: Norwegian Service of Research Data Approval

NSD NORSK SENTER FOR FORSKNINGSDATA

NSD sin vurdering

Prosjekttittel

HELFAB-study

Referansenummer

382297

Registrert

04.05.2020 av Vibeke Telle-Hansen - vtelle@oslomet.no

Behandlingsansvarlig institusjon

OsloMet - storbyuniversitetet / Fakultet for helsevitenskap / Institutt for sykepleie og helsefremmende arbeid

Prosjektansvarlig (vitenskapelig ansatt/veileder eller stipendiat)

Vibeke Telle-Hansen, vtelle@oslomet.no, tlf: 93048873

Type prosjekt

Forskerprosjekt

Prosjektperiode

31.12.2019 - 30.04.2025

Status

20.05.2020 - Vurdert

Vurdering (1)

20.05.2020 - Vurdert

BAKGRUNN Prosjektet er vurdert og godkjent av Regionale komiteer for medisinsk og helsefaglig forskningsetikk (REK) etter helseforskningsloven (hfl.) § 10 (REK sin ref: REK sør-øst A 96264. I vedtaket har REK godkjent opprettelsen av en spesifikk biobank til forskningsprosjektet. Det er NSD sin vurdering at behandlingen også vil være i samsvar med

personvernlovgivningen, så fremt den gjennomføres i tråd med det som er dokumentert i meldeskjemaet datert 20.05.2020 med vedlegg, samt i meldingsdialogen mellom innmelder og NSD. Behandlingen kan starte. MELD VESENTLIGE ENDRINGER Dersom det skjer vesentlige endringer i behandlingen av personopplysninger, kan det være nødvendig å melde dette til NSD ved å oppdatere meldeskjemaet. Før du melder inn en endring, oppfordrer vi deg til å lese om hvilke type endringer det er nødvendig å melde: https://nsd.no/personvernombud/meld_prosjekt/meld_endringer.html Du må vente på svar fra NSD før endringen gjennomføres. TYPE OPPLYSNINGER OG VARIGHET Prosjektet vil behandle særlige kategorier av personopplysninger om helseopplysninger og alminnelige kategorier av personopplysninger frem til 30.04.2025. Data med personopplysninger oppbevares deretter internt ved behandlingsansvarlig institusjon frem til 30.04.2030, dette grunnet dokumentasjonshensyn. NSD bemerker at biobanken opphører ved prosjektslutt. LOVLIG GRUNNLAG Prosjektet vil innhente samtykke fra de registrerte til behandlingen av personopplysninger. Vår vurdering er at prosjektet legger opp til et samtykke i samsvar med kravene i art. 4 nr. 11 og art. 7, ved at det er en frivillig, spesifikk, informert og utvetydig bekreftelse, som kan dokumenteres, og som den registrerte kan trekke tilbake. Lovlig grunnlag for behandlingen vil dermed være den registrertes uttrykkelige samtykke, jf. personvernforordningen art. 6 nr. 1 bokstav a, jf. art. 9 nr. 2 bokstav a, jf. personopplysningsloven § 10, jf. § 9 (2). PERSONVERNPRINSIPPER NSD vurderer at den planlagte behandlingen av personopplysninger vil følge prinsippene i personvernforordningen om: - lovlighet, rettferdighet og åpenhet (art. 5.1 a), ved at de registrerte får tilfredsstillende informasjon om og samtykker til behandlingen - formålsbegrensning (art. 5.1 b), ved at personopplysninger samles inn for spesifikke, uttrykkelig angitte og berettigede formål, og ikke viderebehandles til nye uforenlige formål - dataminimering (art. 5.1 c), ved at det kun behandles opplysninger som er adekvate, relevante og nødvendige for formålet med prosjektet - lagringsbegrensning (art. 5.1 e), ved at personopplysningene ikke lagres lengre enn nødvendig for å oppfylle formålet DE REGISTRERTES RETTIGHETER Så lenge de registrerte kan identifiseres i datamaterialet vil de ha følgende rettigheter: åpenhet (art. 12), informasjon (art. 13), innsyn (art. 15), retting (art. 16), sletting (art. 17), begrensning (art. 18), underretning (art. 19), dataportabilitet (art. 20). NSD vurderer at informasjonen som de registrerte vil motta oppfyller lovens krav til form og innhold, jf. art. 12.1 og art. 13. Unntak fra retten til sletting etter helseforskningsloven § 16 tredje ledd, og personvernforordningen art. 17 nr 3 bokstav d: I utgangspunktet har alle som registreres i forskningsprosjektet rett til å få slettet opplysninger som er registrert om dem. Etter helseforskningsloven § 16 tredje ledd vil imidlertid adgangen til å kreve sletting av sine helseopplysninger ikke gjelde dersom materialet eller opplysningene er anonymisert, dersom materialet etter bearbeidelse inngår i et annet biologisk produkt, eller dersom opplysningene allerede er inngått i utførte analyser. Regelen henviser til at sletting i slike situasjoner vil være svært vanskelig og/eller ødeleggende for forskningen, og dermed forhindre at formålet med forskningen oppnås. Etter personvernforordningen art 17 nr. 3 d kan man unnta fra retten til sletting dersom behandlingen er nødvendig for formål knyttet til vitenskapelig eller historisk forskning eller for statistiske formål

i samsvar med artikkel 89 nr. 1 i den grad sletting sannsynligvis vil gjøre det umulig eller i alvorlig grad vil hindre at målene med nevnte behandling nås. NSD vurderer dermed at det kan gjøres unntak fra retten til sletting av helseopplysninger etter helseforskningslovens § 16 tredje ledd og personvernforordningen art 17 nr. 3 d, når materialet er bearbeidet slik at det inngår i et annet biologisk produkt, eller dersom opplysningene allerede er inngått i utførte analyser. Vi presiserer at helseopplysninger inngår i utførte analyser dersom de er sammenstilt eller koblet med andre opplysninger eller prøvesvar. Vi gjør oppmerksom på at øvrige opplysninger må slettes og det kan ikke innhentes ytterligere opplysninger fra deltakeren. Vi minner om at hvis en registrert tar kontakt om sine rettigheter, har behandlingsansvarlig institusjon plikt til å svare innen en måned. FØLG DIN INSTITUSJONS RETNINGSLINJER NSD legger til grunn at behandlingen oppfyller kravene i personvernforordningen om riktighet (art. 5.1 d), integritet og konfidensialitet (art. 5.1. f) og sikkerhet (art. 32). Nettskjema og TSD er databehandler i prosjektet. NSD legger til grunn at behandlingen oppfyller kravene til bruk av databehandler, jf. art 28 og 29. For å forsikre dere om at kravene oppfylles, må dere følge interne retningslinjer og eventuelt rådføre dere med behandlingsansvarlig institusjon. OPPFØLGING AV PROSJEKTET NSD vil følge opp underveis (hvert annet år) og ved planlagt avslutning for å avklare om behandlingen av personopplysningene er avsluttet/pågår i tråd med den behandlingen som er dokumentert. Lykke til med prosjektet! Kontaktperson hos NSD: Mathilde Hansen Tlf. Personverntjenester: 55 58 21 17 (tast 1)

Appendix J: Overview of log2 transformed Species

Log2 Ratio	Sourdough bread				Baker´s yeast				
	Pre	Post			Pre	Post			
Species	Mean (SD)	Mean (SD)	Log2 Ratio	p-value	Mean (SD)	Mean (SD)	Log2 Ratio	P-value	*P-value
Akkermansia muciniphila	7,52 (5,57)	7,20 (5,74)	-0,31	0.427	8,17 (5,45)	8,17 (5,45)	-0,91	0.128	0.265
Alistipes putredinis	9,18 (6,68)	10,49 (6,01)	1,30	***0.034***	10,55 (6,07)	*9,87 (6,25)*	-0,679	0.514	***0.042***
Alistipes shahii	8,00 (4,99)	8,63 (5,22)	0,63	***0.005***	9,21 (4,25)	*8,1 (4,96)*	-1,11	0.113	***0.015***
Anaerobutyricum hallii	2,86 (4,60)	4,18 (4,84)	1,31	0.320	4,29 (5,01)	3,01 (4,79)	-1,27	0.240	0.179
Anaerostipes hadrus	8,46 (3,37)	8,93 (3,39)	0,47	0.213	9,04 (2,93)	8,57 (3,68)	-0,47	0.497	0.236
Bacteroides caccae	6,80 (5,92)	7,34 (5,64)	0,53	0.405	8,18 (4,97)	7,77 (4,466)	-0,41	0.605	0.343
Bacteroides cellulosilyticus	6,32 (5,68)	6,49 (5,70)	0,175	0.783	7,48 (4,97)	6,34 (5,69)	-1,13	0.156	0.090
Bacteroides dorei	9,59 (5,02)	9,45 (5,11)	-0,13	0.556	9,97 (4,52)	9,14 (5,45)	-0,82	0.241	0.314
Bacteroides fragilis	4,90 (5,23)	4,85 (5,14)	-0,05	0.921	5,52 (4,87)	5,90 (4,82)	0,37	0.311	0.489
Bacteroides ovatus	9,05 (4,43)	9,56 (3,91)	0,50	0.189	10,46 (3,36)	9,84 (4,08)	-0,62	0.436	0.197
Bacteroides thetaiotaomicron	8,15 (4,66)	7,70 (4,42)	-0,45	0.417	8,31 (3,96)	8,25 (4,59)	-0,06	0.895	0.621
Bacteroides uniformis	13,69 (4,07)	13,29 (4,84)	-0,40	0.326	14,65 (1,87)	13,39 (4,96)	-1,25	0.201	0.325
Bacteroides vulgatus	10,41 (5,09)	10,12 (5,37)	-0,29	0.402	11,64 (4,52)	10,41 (5,54)	-1,22	0.216	0.374
Bacteroides xylanisolvens	4,28 (5,07)	4,49 (4,49)	0,20	0.746	5,36 (4,81)	4,19 (4,90)	-1,16	0.108	0.140
Barnesiella intestinihominis	7,29 (5,65)	7,37 (5,77)	0,07	0.312	8,12 (5,22)	7,19 (5,68)	-0,92	0.150	0.125
Bifidobacterium adolescentis	4,60 (5,27)	5,18 (5,27)	0,57	0.178	5,98 (5,06)	4,90 (5,27)	-1,08	0.103	***0.042***
Bifidobacterium longum	7,28 (4,59)	8,00 (4,08)	0,71	0.053	9,32 (3,12)	7,83 (4,10)	-1,49	0.065	***0.012***
Bifidobacterium longum subsp. longum	7,60 (4,76)	8,18 (4,55)	0,58	0.098	9,57 (3,12)	8,23 (4,05)	-1,33	0.102	***0.027***
Bifidobacterium pseudocatenulatum	4,03 (4,15)	3,63 (4,60	-0,40	0.429	4,98 (5,01)	4,27 (4,56)	-0,71	0.430	0.763
Bilophila wadsworthia	6,53 (4,13)	6,39 (4,35)	-0,132	0.672	7,45 (3,43)	6, 35 (4,38)	-1,10	0.114	0.201
Clostridium leptum	3,03 (2,93)	3,25 (2,81)	0,21	0.763	3,68 (2,60)	3,15 (2,23)	-0,52	0.380	0.524
Collinsella aerofaciens	8,81 (3,97)	9,00 (4,00)	0,19	0.394	10,49 (1,30)	9,16 (4,07)	-1,32	0.129	0.085

Coprococcus catus	7,91 (3,52)	7,45 (3,91)	-0,46	0.327	8,49 (3,03)	7,37 (3,88)	-1,11	0.121	0.415
Coprococcus comes	7,83 (3,06)	7,90 (3,02)	0,076	0.721	8,71 (2,42)	8,10 (3,20)	-0,60	0.225	0.218
Dorea formicigenerans	7,40 (2,00)	6,52 (2,87)	-0,87	0.114	7,43 (1,95)	7,20 (2,67)	-0,22	0.601	0.163
Dorea longicatena	8,79 (3,12)	8,72 (3,12)	-0,06	0.702	9,63 (2,45)	8,85 (3,31)	-0,77	0.207	0.272
Eubacterium eligens	5,37 (5,13)	5,76 (4,99)	0,39	0.620	5,70 (4,97)	6,82 (4,96)	1,11	0.392	0.621
Eubacterium rectale	11,34 (3,48)	11,26 (3,31)	-0,08	0.761	12,43 (2,32)	11,10 (4,32)	-1,23	0.136	0.193
Eubacterium siraeum	6,18 (4,42	5,35 (4,67)	-0,82	0.429	6,12 (4,29)	5,29 (5,03)	-0,82	0.305	0.997
Eubacterium ventriosum	4,82 (4,73)	3,74 (4,25)	-1,08	0.160	5,37 (4,83)	4,77 (4,67)	-0,60	0.470	0.632
Faecalibacterium prausnitzii	8,30 (5,02)	8,78 (4,74)	0,47	0.304	9,30 (4,30)	8,71 (4,71)	-0,58	0.455	0.253
Odoribacter splanchnicus	8,86 (3,89)	8,93 (3,90)	0,07	0.589	9,42 (3,27)	9,32 (4,23)	-0,10	0.873	0.787
Parabacteroides distasonis	7,72 (4,71)	7,94 (4,86)	-457,00	0.100	8,67 (4,01)	7,97 (4,84)	-0,70	0.215	0.112
Parabacteroides merdae	6,84 (5,35)	7,28 (5,12)	0,43	0.201	8,10 (5,04)	7,16 (5,51)	-0,94	0.199	0.085
Paraprevotella clara	3,14 (5,15)	4,49 (5,32)	1,34	0.065	3,29 (5,30)	3,46 (5,13)	0,16	0.773	0.099
Prevotella copri	7,52 (6,14)	6,47 (5,93)	-1,05	0.434	7,19 (6,92)	6,58 (6,28)	-0,60	0.467	0.797
Roseburia hominis	6,74 (4,69)	7,13 (4,28)	0,38	0.678	9,39 (1,47)	8,82 (2,60)	-0,56	0.333	0.395
Roseburia intestinalis	6,43 (4,26)	5,80 (4,21)	-0,62	0.127	6,99 (4,45)	5,60 (4,74)	-1,38	0.093	0.453
Roseburia inulinivorans	7,20 (4,21)	6,95 (4,33)	-0,25	0.699	7,62 (4,36)	7,54 (4,27)	-0,08	0.928	0.869
Ruminococcus bromii	5,71 (5,84)	5,46 (5,85)	-0,248	0.599	6,00 (6,24)	5,32 (6,09)	-0,70	0.319	0.597
Ruminococcus torques	4,39 (5,13)	3,78 (4,58)	-0,612	0.483	4,50 (4,80)	4,48 (4,96)	-0,01	0.977	0.575
Streptococcus thermophilus	2,13 (3,48)	2,13 (3,63)	-0,001	0.999	2,30 (3,36)	1,91 (3,15)	-0,39	0.685	0.802
Sutterella wadsworthensis	5,27 (5,70)	6,57 (5,47)	1,29	0.103	5,94 (5,82)	5,33 (5,87)	-0,61	0.449	0.061

Values are presented as mean and standard deviation. Values are log2 transformed and differences within groups are presented as Log2 ratio (post/pre). Signiciant differences are marked with bold, and trend towards differences marked with kursiv.

Appendix K: Overview of all spices analysed

Original values	Sourdough bread			Baker´s yeast bread		
Species	Pre	Post	Delta	Pre	Post	Delta
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Acidaminococcus intestini	0(0-0)	0(0-0)	0	0(0-0)	0(0-0)	0
Akkermansia muciniphila	284,460 (10.68 - 10872.9)	259.025 (3-8731.66)	0	555.218 (11-8920.106)	278.679 (0-10996.913)	0
Alistipes finegoldii	0 (0-81.746)	0 (0-200.77)	0	0 (0-263.683)	0 (0-34.432)	0
Alistipes onderdonkii	0 (0-0)	0 (0-0)	0	0 (0-0)	0 (0-0)	0
Alistipes putredinis	9968.80 (0-33189,91)	12605,15 (210,87-40824,31)	149.02	22463,18 (206,41 - 38471,03)	7100.54 (255.64-38500.1)	-422,6
Alistipes shahii	865.12 (120.4 - 2745.25)	1781.72 (354.43 - 4370.54)	387.97	1063.77 (560.41 - 4233.17)	1041.29 (270.012 - 3013.73)	0
Anaerobutyricum hallii	0 (0-491.17)	0 (0 - 493.16	0	0 (0 - 940.14)	0 (0 - 382.0)	0
Anaerostipes hadrus	588.41 (210.70 - 1946.62)	1172.45 (326.61 - 2532.92)	167.599	997.89 (276.20 - 2526.13)	1153.16 (125.26 - 1919.05)	28.36
Bacteroides caccae	710.62 (0 - 3923.23)	1351.42 (0 - 3779.733)	0	1844.47 (103.74 - 3077.21)	2062.36 (0 - 3152.03)	0
Bacteroides cellulosilyticus	241.60 (0 - 2634.43)	253.46 (0 - 2449.46)	0	302.91 (19.68 - 2835.90)	177.79 (0 - 4114.36)	0
Bacteroides coprocola	0 (0 -0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bacteroides dorei	2194.56 (105.00 - 10301.64)	2937.04 (135.44 - 7834.81)	0	1690.87 (218.69 - 10306.86)	1964.49 (166.22 - 7158.95)	-16.028
Bacteroides eggerthii	0 (0 - 227.56)	0 (0 - 448.08)	0	0 (0 - 585.03)	0 (0 - 473.76)	0
Bacteroides finegoldii	0 (0 - 31.58)	0 (0 - 0)	0	0 (0 - 10.70)	0 (0 - 26.54)	0
Bacteroides fragilis	0 (0 - 1212.55)	0 (0 - 661.23)	0	101.71 (0 - 793.67)	100.29 (0 - 848.37)	0
Bacteroides intestinalis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bacteroides massiliensis	0 (0 - 2043.30)	0 (0 - 1901.12)	0	0 (0 - 2195.47)	0 (0 - 4540.94)	0
Bacteroides nordii	0 (0- 49.09)	0 (0 - 82.29)	0	0 (0 - 81.17)	0 (0 - 45.27)	0
Bacteroides ovatus	1133.95 (230.30 - 2748.39)	1294.92 (293.30 - 3771.19)	0	2238.15 (645.74 - 4331.88)	2172.80 (310.77 - 4173.4)	60.88
Bacteroides plebeius	0 (0 - 35.97)	0 (0 - 535.82)	0	0 (0 - 292.44)	0 (0 - 0)	

Bacteroides pyogenes	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bacteroides stercorirosoris	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bacteroides stercoris	0 (0 - 0)	0 (0 - 152.49)	0	0 (0 - 0)	0	0
Bacteroides thetaiotaomicron	578.72 (68.08 - 2910.55)	527.58 (64.06 - 1411.48)	-51,14	663.03 (139.78 - 1473.65)	1130.54 (49.25 - 1796.49)	88,82
Bacteroides uniformis	37992.23 (6705.68 - 76748.41)	33380.23 (10122.37 - 54551.41)	0	37773.75 (10532.55 - 66160.25)	40165.67 (5722.88 - 62699.18)	-662,21
Bacteroides vulgatus	3923.51 (854.66 - 20982.45)	4667.90 (1041.75 - 14184.6)	0	12337.02 (1966.33 - 19128.59)	8649.88 (1300.82 - 22302.89)	0
Bacteroides xylanisolvens	0 (0 - 591.23)	36.96 (0 - 804.46)	0	73.22 (0 - 917.21)	0 (0 - 566.17)	0
Barnesiella intestinihominis	2501.16 (0 - 4393.8)	3073.82 (0 - 4280.84)	0	2440.84 (18.61 - 4372.87)	1389,324903	0
Bifidobacterium adolescentis	0 (0 - 993.53)	10.84 (0 - 896.91)	0	40.81 (0 - 1126.06)	0 (0 - 1054.12)	0
Bifidobacterium angulatum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium animalis subsp. lactis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium bifidum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium breve	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium catenulatum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium longum	519.19 (65.68 - 1395.96)	908.80 (95.59 - 2028.78)	8,87	731.65 (342.20 - 3184.29)	531.91 (49.23 - 1966.20)	-201,74
Bifidobacterium longum subsp. infantis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Bifidobacterium longum subsp. longum	733.23 (81.56 - 2133.58)	1158,42 (95.35 - 3132.42)	0	1109.89 (350.45 - 3429.91)	634.12 (110.24 - 2452.17)	-380,48
Bifidobacterium pseudocatenulatum	11.48 (0 - 69.97)	0 (0 - 154.77)	0	12.85 (0 - 511.10)	7.94 (0 - 355.75)	0
Bilophila wadsworthia	418.99 (12.48 - 688.61)	430.82 (0 - 645.83)	0	391.45 (138.34 - 841.26)	405.95 (0 - 688.54)	0
Blautia hydrogenotrophica	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Butyrivibrio crossotus	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Christensenella minuta	0 (0 - 0.91)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Citrobacter koseri	0 (0 - 0)	0 (0 - 1534.61)	0	0 (0 - 0)	0 (0 - 130.93)	0

Clostridium bolteae	0 (0 - 0)	0 (0 - 19.38)	0	0 (0 - 5.72)	0	0
Clostridium butyricum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium citroniae	0 (0 - 0)	0 (0 - 10.28)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium leptum	7.74 (0 - 40.94)	6.60 (0 - 37.53)	0	17.54 (2.54 - 36.78)	8.91 (0 - 25.48)	0
Clostridium nexile	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium perfringens	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium scindens	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium sporogenes	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Clostridium symbiosum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Collinsella aerofaciens	1079.25 (519.64 - 2399.70)	1330.77 (636.09 - 2326.89)	0	1635.08 (952.23 - 2386.55)	1542.02 (856.83 - 2827.46)	-136,55
Collinsella intestinalis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Coprococcus catus	606.60 (305.31 - 929.46)	563.46 (252.83 - 929.76)	-75,3	640,3755222	526,1306465	
Coprococcus comes	258.80 (150.29 - 902.50)	482.61 (178.16 - 827.96)	-15	648.34 (253.90 - 972.67)	440.99 (118.80 - 741.69)	-118,72
Desulfovibrio piger	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Dorea formicigenerans	232.15 (151.21 - 332.97)	179.00 (109.56 - 262.82)	-76,92	182.88 (135.13 - 338.38)		0
Dorea longicatena	956.20 (391.93 - 1171.11)	869.97 (374.40 - 1164.19)	-69	854.29 (597.84 - 1704.52)	686.42 (362.96 - 1659.85)	-157,03
Eggerthella lenta	854.29 (597.84 - 1704.52)	0 (0 - 0)	0	0 (0 - 10.44)	0 (0 - 81.43)	0
Enterococcus dispar	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Enterococcus faecalis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Enterococcus faecium	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Enterococcus hirae	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Erysipelatoclostridium ramosum	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Escherichia coli	0 (0 - 26,71)	0 (0 - 9,82)	0	0 (0 - 27,97)	0 (0 - 78.70)	0
Eubacterium eligens	150.91 (0 - 1257.66)	323.99 (0 - 1262.32)	0	209.59 (0 - 983.16)	364.98 (0 - 1809.91)	125.582
Eubacterium rectale	4974.26 (1246,72 - 7588.37)	3424.00 (1295.24 - 10765.48)	-374,2	4754.95 (2103.76 - 17033.88)	4015.71 (1456.38 - 13877.03)	-141,19
Eubacterium siraeum	204.36 (0 - 762.84)	117.09 (0 - 766,17)	0	71.36 (0 - 728.56)	75.30 (0 - 1256.50)	0

Eubacterium ventriosum	73.72 (0 - 651.42)	0 (0 - 130.77)	0	213.33 (0 - 1113.94)	102.86 (0 - 523.19)	0
Faecalibacterium prausnitzii	1476.27 (256.92 - 3042.90)	1410.67 (188.32 - 3682.42)	0	1578.11 (595.89 - 3269.56)	2014.58 (160.01 - 3228.98)	0
Fusobacterium varium	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Haemophilus parainfluenzae	0 (0 - 286.14)	0 (0 - 282.13)	0	40.21 (0 - 163,53)	0 (0 - 204.15)	0
Hafnia alvei	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Holdemanella biformis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Klebsiella variicola	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus acidophilus	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus animalis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus brevis	0 (0 - 0)	0 (0 - 46.92)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus paracasei	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus reuteri	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Lactobacillus ruminis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Methanobrevibacter smithii	0 (0 - 328.31)	0 (0 - 719.87)	0	0 (0 - 583.03)	0 (0 - 426.11)	0
Morganella morganii	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Mycoplasma hominis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Odoribacter splanchnicus	1360.43 (0 - 635.46)	1336.70 (812.17 - 2142.02)	0	1528.86 (811.94 - 1764.50)	1676.42 (678.66 - 2375.21)	0
Parabacteroides distasonis	814.49 (150.94 - 1508.20)	1042.40 (190.55 - 2057.79)	0	1181.30 (328.34 - 1768.13)	1116.43 (208.60 - 1835.44)	-13,39
Parabacteroides goldsteinii	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Parabacteroides gordonii	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Parabacteroides merdae	784.50 (0 - 3610.25)	873.23 (0 - 2744.94)	0	1178.70 (77.63 - 4437.78)	715.09 (0 - 3994.59)	0
Paraprevotella clara	0 (0 - 1721.76)	0 (0 - 1641.81)	0	0 (0 - 863.85)	0 (0 - 1389.74)	0
Prevotella copri	139.69 (2.21 - 14955.73)	105.88 (0 - 9364.86)	0	79.02 (0 - 44178.75)	69.67 (0 - 34776.64)	0
Prevotella stercorea	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Proteus mirabilis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0

Roseburia hominis	643.88 (0 - 1748.61)	668.8 (245,01 - 995.84)	0	791.14 (389.30 - 1267.01)	563.26 (230.74 - 1325.73)	6,95
Roseburia intestinalis	82.32 (3,85 - 629.96)	103.49 (0 - 574.52)	-7,109	187.39 (3.77 - 1066.60)	41.60 (0 - 472.43)	-13,94
Roseburia inulinivorans	370.65 (53.61 - 1033.87)	332.62 (45.14 - 941.95)	-47,43	722.07 (79.23 - 1851.87)	575.54 (84.29 - 1668.55)	109,67
Ruminococcus albus	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Ruminococcus bromii	54.58 (0 - 3428.85)	36.44 (0 - 4838.50)	0	1037.31 (0 - 5793.91)	0 (0 - 3399.23)	0
Ruminococcus gnavus	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 9.76)	0
Ruminococcus torques	0 (0 - 816.36)	0 (0 - 381.66)	0	0 (0 - 768.25)	14.91 (0 - 354.67)	0
Streptococcus sanguinis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Streptococcus thermophilus	0 (0 - 48.53)	0 (0 - 26.37)	0	0 (0 - 49.58)	0 (0 - 37.27)	0
Subdoligranulum variabile	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Sutterella wadsworthensis	35.23 (0 - 1745.37)	282.83 (0 - 2236.84)	0	92.20 (0 - 2417.50)	11.37 (0 - 1916.15)	0
Turicibacter sanguinis	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0
Veillonella atypica	0 (0 - 0)	0 (0 - 0)	0	0 (0 - 0)	0 (0 - 0)	0

Values are presented as median and IQR. Differences within groups are presented as delta (post/pre).