# Health effects of ancient wheat species compared to modern wheat varieties

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Publication number 2018-015 VG

27 pages

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Louis Bolk Institute: Research and advice to advance sustainable agriculture. nutrition and health

# Preface

This report describes three studies carried out by the Louis Bolk Institute concerning differences between ancient and modern grain species and their impact on human health.

For the financing we would like to express our gratitude to Ekhagastiftelsen and bakery Tom van Otterloo and their employees and volunteers. We also thank all participants who participated in one of the studies.

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# Summary


## Introduction

Nowadays, it seems that more and more people report health complaints like Non-Celiac Gluten sensitivity and Irritable Bowel Syndrome, that are possibly related to their grain, wheat and/or gluten intake. Modern wheat products often get blamed as possible causes of these complaints. Wheat qualities have been changed during the last 50 years, as breeding of wheat was aimed to increase grain yield and on traits to optimise the mechanised bread baking process. Which was in itself changed from an artisan to an industrial process which, for example, requires much more (resilient) gluten. As a side effect of this selective cultivation modern cereal also contain more so-called FODMAPS, which consists of short chain carbohydrates that are poorly absorbed in the small intestine. FODMAPS are linked to abdominal complaints in sensitive individuals. Ancient wheat species, such as einkorn, spelt and emmer, might contain more protein and micronutrients, fibres, less resilient gluten and less FODMAPS, while having better glycaemic index compared to modern wheat species. Therefore, ancient wheat species might be "healthier" and might have certain benefits for people at risk for gluten and or FODMAP sensitivity. The objectives of this research were (1) to explore what motivation people have for consuming ancient wheat products, (2) to map the scale and infrastructure ancient wheat varieties are grown, produced and processed in the Netherlands, (3) to develop a diet intervention program based on ancient wheat products, and (4) to perform a day-to-day, real life study on that diet intervention program to compare health effects between ancient and modern wheat products using so-called 'Reported Outcome Measures' (ROM's).

## Methods

For objective 1, an online questionnaire (Reported Outcome Measures) was developed to ask participants about their experience with different wheat species, whether they have any health or physical related complaints when consuming them, what their reasons were to stop or switch to consume a certain wheat variety and whether this led to a change in health-related complaints. This questionnaire has been send to people over a period of 8 weeks in different locations in the Netherlands. For objective 2, growing trials were conducted under organic conditions, on clay soil. Sowing was done in October and harvesting in July-August a year later. Protein content, 'Zeleny', wet gluten content, total dietary fibre and fructan content analyses were conducted separately for each season by the certified lab of the University of Gent on flour or ground air dried bread. For objective 3 and 4, an observational study was performed with a single semi-blind randomized crossover design among women in the Eastern part of the Netherlands. The participating 46 women were randomly assigned to test a different bread each week over a period of five weeks. In total five different types of bread were tested: three of them were based on ancient grains spelt (baked with sourdough or yeast) and emmer (sourdough) and two control breads made from the modern grain variant: whole grain (sourdough) and white (yeast) bread. Each week, for two days the participants were asked to consume only this bread for breakfast and lunch, followed by a five-day washout period.

## Results

The study on motivation of people to consume ancient wheat varieties (objective 1) showed that almost half of the participants experienced health related complaints when consuming cereal products, with the modern cereal as most reported wheat type that was associated with

discomfort. More than a third of the participants eliminated this wheat type and 27% of those who experienced complaints switched to consume the ancient grain spelt, of whose 71% reported an improvement in their health complaints. These results indicated an improvement of reported health related complaints after the participants changed and/or eliminated a wheat species.

The study on differences between wheat species (objective 2) showed that the gluten index tended to be higher for the modern species, indicating a shift in type of gluten: a higher percentage of gluten are resilient gluten. Also, the modern species had the highest Zeleny values. On the other outcome measures, including Fructan content, no clear differences between the species were found. Due to constraint in material all the products were tested in mono.

In the study on comparing health effects of consumption of ancient wheat and modern wheat varieties (objective 3 and 4), minor significant improvements with the sourdough breads (emmer, spelt and modern cereal) were seen on different aspects of general mood, such as feeling energetic and healthy. Besides, consumption of the spelt sourdough bread showed a decrease in stomach ache and flatulence, and together with emmer sourdough in bloating, whereas the other breads didn't show clear differences. In the onset of hunger, a difference was found between the ancient and modern grains, with 36% of the participants reporting a quicker than usual rise in hunger with the modern cereal white yeast, versus 0-11% with the ancient grains. Furthermore, participants were in general positive on most aspects of all breads, but were either being very positive or very negative about the taste and overall experience with the sourdough breads.

## Conclusion

This research showed that some people indicate to experience health complaints with consuming modern wheat products in the Netherlands, with some of these people experiencing less complaints when switching to ancient wheat species. Although only minor differences between ancient and modern wheat breads were found within the laboratory analyses and the randomized intervention study, a shift to more resilient gluten in the modern species was found compared to spelt and emmer sourdough breads, which seemed to reduce some abdominal related complaints. Also, these ancient grains based breads seemed to delay onset of hunger compared to modern white cereal. Therefore, these results indicate that consumption of ancient grains, especially as sourdough breads, such as spelt and to a less extent emmer, might reduce health complaints. More research should be conducted to confirm these results.

# 1 Introduction

Nowadays, independently of the gluten intolerance celiac disease, it seems that more and more people report health complaints possibly related to their gluten and/or wheat intake. Modern wheat products often get blamed as possible causes of chronic abdominal complaints concerning people with the Irritable Bowel Syndrome (IBS) or reflux esophagitis. Next to this, the prevalence of the so-called Non-Celiac Gluten sensitivity has increased and a gluten free diet could be considered popular. As well in popular literature like “Bread Belly” by William Davis as in peer-reviewed literature these concerns seem to be discussed and gradually confirmed (Van den Broeck et al. 2010). Wheat qualities have been changed dramatically during the last 50 years. Above all, breeding of wheat was aimed to increase grain yield. In large breeding programs wheat varieties were crossed (hybridized) followed by selection of the best genotypes. It is well known that in this selection process, traits were included like harvest index (grain-straw ratio) and resistance to abiotic stress conditions (drought) and biotic stress conditions as fungal diseases -and selection took place on traits to optimise the bread baking process that has changed from an artisan to an industrial process over the past 50 years. A higher and more resilient gluten content improves loaf structure and volume (Osman et al. 2012). For industrial baking, more and more resilient gluten is required compared to artisan baking. Reasons that more resilient gluten are necessary are: 1) during the shortened time of the modern baking process to achieve faster risening of the dough; 2) during mechanical kneading a part of the gluten is damaged; and 3) to make the bread more fluffy what is ‘preferred’ by most people.



## 1.1 Wheat

The development of cultivated wheat has a complex history that started about 10.000 years ago Purseglove, 1971). The first cultivated wheat is einkorn wheat (*Triticum monococcum*) from which emmer wheat (*Triticum dicoccum*) has developed. Together einkorn and emmer wheat are considered ancient wheat. Various scenarios exist on how spelt wheat (*Triticum spelta*), bread wheat (*Triticum aestivum*), pasta wheat (*Triticum durum*) and other species like kamut (*Triticum turgidum*) have developed from emmer wheat. Over the past 50 years much breeding has been done on bread and pasta wheat and to a limited extent on spelt wheat. It seems that emmer is grown in the Netherlands only at very small scale at the moment. In contrast, spelt wheat has been cultivated on a larger scale.

Ancient wheat species have different protein qualities compared to modern species (Van den Broeck et al., 2010). They contain more protein while having a less resilient gluten and a lower gluten index. In addition, they have a better glycaemic index caused by less rapidly degradable starch chains and may have higher value of micronutrients (Sofi et al. 2014). ‘Zeleny’ is a measure of protein quality and indirectly of gluten percentage (Osman et al. 2012). Research under organic conditions in the Netherlands indicate that modern bread wheat varieties contain Zeleny of 40-50 ml compared to ancient bread wheat varieties with Zeleny of 10-20 ml and commonly cultivated spelt wheat contains Zeleny of 10-30 ml (Nuijten, unpublished). These findings are in accordance by findings from Van den Broeck et al. (2010). This indicates the effect breeding has had on the increase and change of gluten content in bread wheat. In addition, breeding has had a clear effect on a reduction of a range of micro-nutrients (Ca, Cu, Mg, Mn, P and Se) in bread wheat (Murphy et al. 2008). Research on the history of spelt and bread wheat indicates that the gliadin (able to trigger allergic reactions) of spelt and bread wheat differs (Blatter et al. 2004). Moreover, the

composition of modern wheat varieties might have a higher rise in blood sugar and insulin and lower satiating effects.

## 1.2 Celiac Disease, Non-Celiac Gluten sensitivity and Irritable Bowel Syndrome

In Celiac disease the body has a specific autoimmune response on gliadine, one of the components of gluten. Today it is known that the expression of this pathology is multifaceted and has an association with other autoimmune diseases (Gasbarrini et al. 2014). The search of naturally detoxified or less toxic ancient grains is of great interest for their potential use in the general diet to prevent disease in those individuals at high risk to develop gluten intolerance (Lamacchia et al. 2014). Per example, einkorn was studied by Hidalgo et al. (2014) and showed weaker toxic reactions than other *Triticum* species, although it appeared not suitable for coeliacs.
Non-Celiac Gluten Sensitivity (NCGS) was originally described in the 1980s and recently a 're-discovered' disorder characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing food in subjects that are not affected with either celiac disease or wheat allergy(Aufiero et al 2018; Catassi et al 2015). An overlap between the irritable bowel syndrome (IBS) and NCGS has been detected (Eswaran et al. 2013; Catassi et al. 2013).

IBS is a chronic condition characterized by abdominal discomfort or pain, altered bowel habits, and often bloating and abdominal distention (Choung et al 2011). This causes lower quality of life, impaired health status, mood disturbances and interference with daily activity. The prevalence of IBS among adults in the Netherlands is estimated around 9%. In general, the efficacy of drug therapies is poor. People with IBS believe diet plays a significant role as more than 60% report worsening of symptoms following food ingestion, especially wheat or dairy products, although the role of various food groups/nutrients are not yet clear and dietary advice is mostly based on the known effects of nutrients on gut physiology rather than on controlled trials (Oberndorff et al 2005

## 1.3 FODMAP

An exclusion diet that has received attention in relation to abdominal pain recently is the FODMAP diet (Barrett, 2012). The term FODMAP is an acronym, derived from 'Fermentable, Oligo-, Di-, Mono-saccharides And Polyols'. These are short carbohydrates that are poorly absorbed by the small intestine and can cause abdominal complaint within sensitive individuals. Although FODMAPs are naturally present in a lot of food types and average the human diet, FODMAP restriction has been found to improve symptom control in people with IBS and other functional gastrointestinal disorders (FGID). The FODMAP hypothesis was first proposed by Gibson in 2005 from the Monash University. The Monasch University remains the leading standard for FODMAP research. A pilot study by Ong et al. (2010) found that the consumption of FODMAP rich products like bread significantly increased IBS related symptoms. Several types of cereals, breads and gluten-free products were tested for FODMAP content. The type of FODMAP short carbohydrate that was found to be present in most cereal were Fructans (in the form of fructo-oligosaccharides and Inulin). This also showed that most FODMAPS were removed in the process of making a product gluten free as well as that ancient grains showed to naturally contain less FODMAPs (Biesiekierski J. R., 2011 ) (table 1). A recent study from Laatikainen (2017) showed that yeast fermentation of modern cerceals yielded a higher FODMAP content versus slow sourdough fermentation. An Italian research by Sofi (2014) showed that replacing modern wheat with Kamut (ancient wheat emmer variety in Italy) strongly improved the relieved the complaints in participants with IBS.

*Table 1. Different cereal products tested on amount of fructans (Biesiekierski J. R., 2011)*

| | **Total fructans g/100gram** |
|---|---|
| Spelt (25% spelt) | 0.14 |
| Gluten free | 0.19 |
| Spelt | 0.2 |
| Modern cereal whole wheat | 0.48 |
| Modern Cereal, multigrain | 0.56 |
| Modern wCereal , white | 0.68 |
| Modern wCereal, whole grain | 0.69 |
| Rye | 1.05 |
| Rye, sourdough light | 1.07 |
| Rye, dark | 1.42 |
| Average | 0.65 |

# 2 Aims and objectives

The objectives of this study were:

1. To make an inventory and understand which reasons people have (with or without specific complaints) consuming various products from ancient wheat varieties in the Netherlands and to get insight in possible health effects in healthy subjects and patients with IBS, and their experiences, with a focus on consuming emmer wheat or spelt wheat.
2. To map information about the scale and infrastructure ancient wheat varieties grown, produced and processed in the Netherlands, with a focus on emmer and spelt wheat, and how this knowledge could be used in a dietary intervention concerning ancient compared to modern wheat varieties.
3. To develop a 'real life' dietary intervention program based on ancient wheat and gathering specific data on the feasibility of such a diet intervention in subjects with IBS.
4. To perform a study to compare Reported Outcome Measures on health effects and grain-specific laboratory parameters of a dietary intervention program based on ancient wheat and modern wheat varieties based on the guidelines for a healthy diet on parameters of IBS.

# 3 Materials and methods

## 3.1 Research setup objective 1: Understanding what motivates people to consume ancient wheat varieties

The study on objective 1 is carried out by a student and the results of this thesis have been previously reported in 2016 (An inventory of reasons for people to consume products from ancient grain varieties: Is this symptom driven? Verburgh 2015). An online questionnaire (Reported Outcome Measures) was developed to ask, participants about their experience with different wheat species and whether they have any health or physical related complaints when consuming

them. Furthermore, they were asked what their reasons were to stop or switch to consume a certain wheat variety and whether this led to a change in health-related complaints and symptoms. This questionnaire was complete by 356 participants over a period of 8 weeks in different locations with consumers close to Driebergen (such as a bakery, supermarket and organic food store). Besides, the questionnaire link was also published on the website of the Dutch Organization for IBS patients. There was also a press release and the research link had been published on different websites such as 'bakkerswereld.nl' and 'veeteelt.nl'. Finally, the research link was shared on social media (Facebook and Twitter).

## 3.2 Research setup objective 2: Analysis of grain, flour and bread

### Agricultural trials

All growing trials were conducted under organic conditions. Sowing was done in October a year earlier and harvesting was conducted in late July- early August (depending on speed plant development and ripening). To avoid lodging the wheat species were cultivated under low input conditions. Manure was only applied before sowing of the trials. The trials conducted in 2010 and 2011 were situated on clay soil in Wilp, and the trials conducted in 2016 and 2017 were situated on clay soil in Bemmel. Protein content and quality analyses were conducted separately for each season by the same laboratory, using the same protocols.

### Zeleny, gluten index, fibre, protein, and fructan analysis

All analyses were performed by the certified lab of the University of Gent on flour or ground air dried bread. All tests were done in mono due to material constraints. The kernels were ground into flour on a Perten Lab mill 3100. Zeleny was performed on flour according the ICC method 116.1. and 118 (ICC, 1994). Wet gluten index was determined through the ICC method 155 (ICC, 1994). Total Dietary Fiber was determined through by the AOAC 991.43 standard method (Cunniff P., 1995): dry matter results after drying at70°C. Protein content was determined with the ISO 16634-1 (factor 5.7) according to the Dumas principle (ISO, 2008). The Fructan content was determined according the AOAC 999.03 protocol (Mccleary, 2000).

## 3.3 Research setup objective 3 and 4: observational study for ancient grains with randomized crossover design

An observational study was performed through a single semi-blind randomized crossover design. Participants were randomly assigned to test a different bread each week over a period of five weeks. The bread was provided by the Dutch organic baker Tom van Otterloo who specializes in baking organic yeast and slow fermented sourdough bread with modern and ancient local grains. In total five different types of bread were tested: three of them were based on ancient grains spelt (*Triticum spelta)* (sour dough and yeast version) and emmer (*Triticum dicoccum)* (sourdough) and two control breads made from the modern grain variant (*Triticum aestivum)*. The control breads consisted of whole grain (sourdough based) and white (yeast based) bread. These two controls were chosen in order to differentiate between the sourdough and the yeast baking process, as well and taking into account the difference in fibre content between whole grain bread and the white bread. The loafs of breads were distributed to the participants in neutral white plastic bags. All breads were made to look identical although some difference in colour and structure still could be observed. For two days the participants were asked to consume only this bread for breakfast and

lunch (as is common in Dutch food culture) with a topping for their choice. This was followed by a five-day washout period (the rest of the week), after which they received the next bread.

**Study population**

The bakery 'Tom van Otterloo', a bakery with two locations, became the main project partner because they already had experience with baking bread with local ancient grain varieties. They also used a traditional sourdough fermentation that averagely allows the sourdough bread to ferment for 16 hours, as well as baking standard yeast baking techniques.

Women in the Eastern part of the Netherlands were invited to participate to the study via flyers, social media and the website of the Dutch Organisation for IBS patients. Inclusion criteria were female, no Celiac disease or any active inflammation of the gastrointestinal, being between 18 and 80 years old, willing to eat bread for 5 weeks and being able to visit the pick bread pick up points. In total 54 women responded and 46 (85%) women agreed to participate. Main reasons for not participating included 'not being able to pick up the bread every week during the 5 week period' or 'being on vacation' . As the study design was similar to a consumer study and measurements were not invasive (based on questionnaires only), approval from a local ethical committee was not deemed necessary. All participants provided written informed consent.

**Questionnaires**

Baseline questionnaire

Information on gender, education, and employment status as well as habitual consumption of grain products, timing of bread consumption (i.e. breakfast, lunch, evening meal), location where bread was mostly purchased, type of bread that was consumed, changes in bread consumption over the past 12 months (including motivation), food related complaints was collected at baseline.

Intervention questionnaire

Each week participants completed 2 online questionnaires. A pre-test questionnaire inquired about general mood, perceived health and grain consumption of the past days. After which the test bread was consumed for 2 days. Followed by a post-test questionnaire inquiring about the test bread consumption (compliance), general mood, perceived health, onset of hunger, abdominal pain, energy dips and grain consumption of the past days. Modern grains were cultivated to have desirable traits for the baking process, while ancient grains can give a different sensation in taste, airiness and mouthfeel. In order to determine how the ancient grans compared to the modern ones several positive traits were scored by the participants on a Likert scale 1-7 scale ranging from 1 'strongly disagree' to 7 'strongly agree.' The results were not normally distributed, often showing a strong lovers and hates group of each bread. Therefore, the median and the range were reported rather than the mean and SD. Participants were asked to score their different facets of their general mood on an Osgood scale ranging from 1- totally not agree to 5- totally agree pre- and posttest bread consumption.

**Statistical analyses**

Results were processed by using statistical analyses done with SPSS version 24. Descriptive statistics, including frequency statistics, means and standard deviations (SDs) for normally distributed data were used to provide insight into the results. If data was not normally distributed,

medians and interquartile ranges were presented. A Wilcoxon sign rank test was used for non-normally distributed data. A P-value ≤ 0.05 was considered to indicate statistical significance.

# 4 Results

## 4.1 Motivation of people to consume ancient wheat varieties

The results of objective 1 were performed by a student, show that 156 of the 356 participants (contacted randomly outside stores and approached through the IBS patient website) (44%) experienced health related complaints when consuming a wheat specie. The most frequent reported complaints were stomach-ache, bloating and tiredness. The most reported wheat species that was associated with the discomfort seemed to be common wheat (*Triticum aestivum*) as 57 (37%) eliminated this specie. 42 (27%) of the participants who experienced complaints switched to consume spelt (*Triticum spelta*). Participants experiencing complaints when consuming a certain wheat species gave themselves a significant lower health quantification on a 1-10 scale (6.8) than participants who did not experience any pain (7.7). Participants with IBS gave themselves a 6.3 as health quantification. Of the participants12% had an official IBS diagnose; 65% of the participants with complaints were not diagnosed. These results indicate an improvement of reported health related complaints after the participants changed and/or eliminated a wheat species. Of the 42 participants with complaints who switched to consume spelt, 30 participants reported an improvement in their health complaints.

## 4.2 Analysis of grain, flour and bread

**Production and cultivation of ancient wheat varieties**

Next to the commonly grown (bread) wheat, three species of wheat are cultivated in the Netherlands: spelt, emmer and einkorn. Some of the reasons for their limited cultivation are low yield, tendency for lodging and the need of dehulling. Another factor might be their lack of adaptation to modern baking processes, and particularly in the case of emmer and einkorn, late ripening and lack of responsiveness to nitrogen fertilizer. Farmers who would want to cultivate these ancient species are recommended to have their own storage and drying facilities, and ideally are also in possession of cleaning and harvesting equipment.

The last few years the cultivation of spelt wheat has shown a clear increase. This is exemplified by the fact that certified seed is now sold on the market, and that in the case of organic farming, farmers have to use organically multiplied seeds. Although it is still a niche, the cultivation of spelt wheat is gradually becoming part of mainstream farming. It is not only cultivated by organic farmers, but also by conventional farmers. Two varieties of spelt are most commonly grown: Franckenkorn and Oberkulmer rotkorn. Frankenkorn is the result of a cross between spelt and soft wheat and has a higher yield compared to Oberkulmer rotkorn. Oberkulmer rotkorn is said to be one of the real ‘pure’spelt varieties (next to Ebner rotkorn). Whether, and to what extent, Oberkulmer rotkorn can be more easily digested compared to Franckenkorn is not clear.

Emmer and einkorn are only cultivated by a few farmers in the Netherlands. Compared to spelt these two species have lower yield, and ripen later, which increases the risk of harvesting for the farmer. Emmer has two varieties that are known to have been cultivated in the Netherlands: white and red emmer. Einkorn has only one variety known to be grown in the Netherlands. Likely less than ten farmers are cultivating these two species in the Netherlands. The organic farmer Piet van

Zanten seems to play an important role in the cultivation and distribution of seed to other farmers, both organic and conventional. To exemplify the status of emmer and einkorn as crops is that no certified seed is sold on the market, and that organic farmers can use non-treated conventional seed. In addition to emmer and spelt, a few farmers have an interest in cultivating landraces and old varieties of soft wheat. These varieties are typically lower in yield compared to modern varieties. In terms of baking quality they are more similar to spelt than to modern wheat varieties. The protein quality of the landraces and old varieties of soft wheat are similar to that of spelt, shown by the low values for Zeleny sedimentation.

**Gluten index and Zeleny analysis**

Samples have been grouped according to species, genome constitution and breeding intensity. For samples belonging to a group without much variation, average values are shown (table 2). One exception is rivet wheat of which two populations show clearly different values for Zeleny and gluten index. Wet gluten percentage shows a large variation across the groups. Overall, there are no clear differences between the species for wet gluten content. However, one group that may be different is einkorn, for which gluten analysis did not yield any results. The emmer flour also did not yield any results.The gluten index tends to be higher for modern material (based on flour data and data from other projects), indicating a shift in type of gluten: a higher percentage of gluten are resilient gluten.

For Zeleny large differences among certain groups are visible. The largest differences are shown between varieties of bread wheat: modern material has clearly the highest Zeleny values, whereas the old material of bread wheat has Zeleny levels similar to spelt, emmer and rivet wheat. When looking at material with no or little breeding intensity, the diploid species einkorn seems to differ clearly from the other species for baking quality, whereas the tetraploid species (emmer and rivet wheat) do not clearly differ from the hexaploid species (spelt and modern cereal ). An explanation for the observation that there is no clear difference between the tetraploid and hexaploid species is, amongst others, the variation within species for protein quality. Other factors are a limited data set, and variation in weather conditions.

*Table 2. Grain and flour gluten index and Zeleny analysis*

| | # | Type of cultivar | Zeleny* | Gluten index (%) | wet gluten (%) | dry gluten (%) | Water-binding (%) |
|---|---|---|---|---|---|---|---|
| Whole grains | 1 | Rivet wheat | 13 | | | | |
| | 2 | Rivet wheat | 23 | 63.8 | 24.1 | 7.5 | 16.6 |
| | 3 | Rivet wheat | 11 | 31.2 | 20.7 | 6.5 | 14.2 |
| | 4 | Einkorn | 4 | | | | |
| | 5 | Einkorn | 3 | | | | |
| | 6 | Einkorn | 3 | | | | |
| | 7 | Einkorn | 3 | | | | |
| | 8 | Einkorn | 4 | | | | |
| | 9 | Emmer | 13 | 5.8 | 29.9 | 10.3 | 19.6 |
| | 10 | Emmer | 18 | 7.1 | 31.2 | 10.7 | 20.5 |
| | 11 | Spelt | 29 | 45.0 | 30.0 | 9.3 | 20.6 |
| Flour | 12 | Emmer Flour | 29 | | | | |
| | 13 | Spelt Flour | 26 | 95.5 | 27 | 9.4 | 17.6 |
| | 14 | Modern Cereal whole wheat flour | 23 | 95.3 | 26.4 | 9.3 | 17.1 |
| | 15 | Modern cereal white flour | 38 | 83.6 | 29.1 | 9.7 | 19.4 |

*For the results of former studies, see Appendix 1.*

### Grain, flour and bread protein, fibre and FODMAP analysis

The fibre content of white wheat spelt and wheat modern cereal was similar (11.9 and 11.4) while whole wheat Emmer flour scored lower with 8.4 and white modern cereal had the lowest value of 3.5. The bread contents follow a similar pattern but the white modern cereal shows an increase in fibre. Fructan content (% of air dried sample) showed variation between the whole grain sample of 1.5% for Einkorn and 1-1.1% for Emmer. Within the flour group Spelt and modern cereal whole wheat flour scored similar and Emmer and modern cereal white flour scored lower. Within the bread sample no difference was found. Protein content was similar for all samples with some variation between samples of the cultivar.

*Table 3. Fibre, fructans and protein analysis*

| # | | Type of cultivar | fibre | Fructans | Protein |
|---|---|---|---|---|---|
| 1 | Whole grains | Rivet wheat | | | 11.9 |
| 2 | | Rivet wheat | | | 10.6 |
| 3 | | Rivet wheat | | | 9.4 |
| 4 | | Einkorn | | | 10.5 |
| 5 | | Einkorn | | | 11.2 |
| 6 | | Einkorn | | | 11.7 |
| 7 | | Einkorn | | 1.5 | 10.8 |
| 8 | | Einkorn | | | 10.7 |
| 9 | | Emmer | | 1.1 | 12.3 |
| 10 | | Emmer | | 1.0 | 13.8 |
| 11 | | Spelt | | | 12.1 |
| 12 | Flour | Emmer Flour | 8.4 | 1.0 | 13 |
| 13 | | Spelt Flour | 11.9 | 1.4 | 11.7 |
| 14 | | Modern Cereal whole wheat flour | 11.2 | 1.4 | 11.7 |
| 15 | | Modern cereal white flour | 3.5 | 1.1 | 10.8 |
| 16 | Bread | Emmer bread | 9.9 | 0.3 | 12.8 |
| 17 | | Spelt Sourdough bread | 10.8 | 0.3 | 12.3 |
| 18 | | Spelt Yeast bread | 11.3 | 0.3 | 11.9 |
| 19 | | Modern Cereal whole wheat Sourdough bread | 10.6 | 0.3 | 11.9 |
| 20 | | Modern cereal white flour yeast bread | 5.79 | 0.3 | 11 |

## 4.3 Observational study for ancient grains with randomized crossover design

### Baseline characteristics

The women in this study were 51 years on average and more than half (59%) were higher educated. Most of them lived with a partner and children at home (33%) or with a partner but no children at home (28%). They work on average less than 24 hours a week (32%) or are entrepreneur (22%). (table 4) Of the 53, 48 started the 5 week testing program and 46 women completed the full program.

*Table 4. Demographics of women participating in Bread intervention study (n=54)*

| | n=54 | % or average (SD) |
|---|---|---|
| Age (in years) | 51.1 | 13.5 |
| Education level | | |
| No education | 1 | 2% |
| Lower education | 5 | 9% |
| Lower/higher education | 16 | 30% |
| BSc level | 28 | 52% |
| MSc/Postdoctoral level | 4 | 7% |
| Married/ living together no children | 15 | 28% |
| Married/ living together with children at home | 18 | 33% |
| Married/ living together with children outside home | 5 | 9% |
| Single, no children | 5 | 9% |
| Single, with children living elsewhere | 3 | 6% |
| Single, with children at home | 4 | 7% |
| Living with (grand)parents/ family | 1 | 2% |
| Student dorms/ communal living | 1 | 2% |
| Employment status* | | |
| Paid work < 24h/wk | 7 | 13% |
| Paid work >24h/wk | 17 | 32% |
| Entrepreneur | 12 | 22% |
| Care household/children | 2 | 3% |
| Pension | 3 | 6% |
| Student/scholar | 2 | 4% |
| No paid work due to health problems | 5 | 9% |
| Volunteer work | 9 | 17% |
| Employment seeking | 3 | 6% |
| Other | 6 | 11% |

**Participants can be active in multiple categories, for example pensioner and volunteer therefor making the total count higher than 100%.*

At baseline, bread was consumed mostly with 63% on a daily basis and 33% for 3-6 days a week (Appendix 2). Lunch was the most popular time for bread consumption (93%), followed by breakfast (65%) and dinner (9%). This pattern is in line with the Dutch food habits. The three most consumed bread types were whole grain (65%) (based on 100% whole grain flour by law in the Netherlands), followed by brown bread (46%) and sourdough bread (41%). Most participants bought their bread at either the supermarket (54%) or the (non-organic) bakery (52%) follow by the organic supermarket (22%).

**Reported complaints related to bread consumption prior to the study**

A fifth of the participants (22%) indicated that they had made any changes in their bread and grain consumption prior to the study, with a diverse set of reasons (table 5). The two most common ones were 'negative experience with intake' (9%) and 'after reading or hearing about the subject in the media' (9%).

Within the participants group, 24% suffered from food related complaints. Within the group that suffered from food related complaints, the most common complaints were flatulence (73%),

fatigue (64%) and feeling bloated (55%) (table 5). The majority of these 24% of the participants did not fully understood the origin of their complaint (73%), followed by IBS (27%) and lactose intolerance (18%). Most of them indicated they had not (64%) followed by a diagnosis made by a General practitioner (18%).

*Table 5. Food related complaints*

| | Total n=46 | % |
|---|---|---|
| **Are you currently suffering from food-related complaints?** | | |
| Yes | 11 | 24% |
| No | 35 | 76% |
| | | |
| **What kind of complaints are you currently experiencing** | | |
| Food allergy | 2 | 18% |
| Stomach pain/cramps (not related to menstrual cramps) | 3 | 27% |
| Flatulence | 8 | 73% |
| Bloated feeling | 6 | 55% |
| Abnormally hard stool | 3 | 27% |
| Abnormally loose/watery stool | 3 | 27% |
| Slime with stool | 2 | 18% |
| Sensation of fullness without being able to pass | 0 | 0% |
| Constipation | 1 | 9% |
| Nausea | 0 | 0% |
| Fatigue | 7 | 64% |
| Dips in energy | 2 | 18% |
| Being limited in daily activities by stomach problems | 0 | 0% |

### Compliance of consuming the test breads

The participants indicated in general to have eaten either ‘a few slices’ or ‘most of the loaf’ (table 6).

*Table 6. Compliance to consumption of the test breads during the study.*

| How much of the test bread have you consumed in the past days? | Emmer n=43 | Spelt Yeast n=45 | Spelt Sourdough n=42 | Modern cereal sourdough n=42 | Modern cereal white yeast n=42 |
|---|---|---|---|---|---|
| Just a taste | 2% | 4% | 0% | 0% | 2% |
| A few slices | 33% | 44% | 50% | 36% | 45% |
| Most of the loaf | 61% | 49% | 48% | 60% | 43% |
| The whole loaf | 5% | 2% | 2% | 5% | 10% |

### Bread traits

The participants seem to be divided in two groups, a strong sourdough lovers and sourdough haters group, causing the results to not be normally distributed. The traits ‘good density/airiness’, ‘good texture inside’ and ‘good stickiness inside’ were scored slightly more favourable in general for the modern cereals. Modern cereal sourdough scored highest on the positive traits followed by Spelt sourdough (table 7).

*Table 7. Bread traits scored on a Likert scale ranging from 1 'strongly disagree' to 7 'strongly agree'.*

| Median (interquartile range)* | Emmer n=43 | Spelt yeast n=45 | Spelt sourdough n=42 | Modern cereal sourdough n=42 | Modern cereal white yeast n=42 |
|---|---|---|---|---|---|
| Tasted good** | 6 (6) | 6 (5) | 6 (6) | 6 (5) | 5 (6) |
| Smelled good** | 4 (6) | 6 (5) | 5 (6) | 6 (5) | 5 (6) |
| Looked attractive | 6 (5) | 6 (5) | 6 (5) | 6 (4) | 6 (6) |
| Preserves well | 5 (5) | 5 (6) | 5 (6) | 6 (5) | 6 (4) |
| Easily cut | 6 (4) | 5 (5) | 6 (6) | 6 (6) | 5 (5) |
| Easily to spread with toppings | 6 (3) | 6 (5) | 6 (5) | 6 (4) | 6 (6) |
| Good t thickness of slices | 6 (6) | 6 (4) | 6 (5) | 6 (4) | 6 (5) |
| Good size of slices | 5 (5) | 6 (6) | 6 (5) | 6 (5) | 6(6) |
| Good crunch in crust | 5 (6) | 4 (6) | 5 (6) | 6 (6) | 4 (6) |
| Good density/airiness | 4 (6) | 4 (6) | 4 (6) | 5 (6) | 6 (6) |
| Good texture inside | 4 (6) | 4 (6) | 5 (6) | 6 (4) | 6 (6) |
| Good stickiness inside | 4 (6) | 4 (6) | 5 (6) | 5 (5) | 5 (6) |

**The results were not normally distributed, therefore, the median and the range were reported rather than the mean and SD.*
***Because one participant indicated to have no sense of smell, her answers for the questions' tasted good' and 'smelled good' were counted as missing.*

Modern cereal sourdough was scored the highest overall, followed by Spelt sourdough. The modern cereal white yeast bread was scored least positive (table 8).

*Table 8. Averages of intentions and perceptions of breads scored on a Likert scale ranging from 1 'strongly disagree' to 7 'strongly agree.'*

| | Emmer n=43 | Spelt yeast n=45 | Spelt sourdough n=42 | Modern cereal sourdough n=42 | Modern cereal white yeast n=42 |
|---|---|---|---|---|---|
| I would like to eat it again | 4.0 (2.3) | 4.2 (2.2) | 4.4 (2.1) | 5.0 (1.6) | 3.7 (2.3) |
| Fits into my current nutritional pattern | 4.6 (2.0) | 4.6 (1.7) | 4.6 (1.9) | 5.2 (1.2) | 2.9 (1.9) |
| Expect to be nutritious | 5.4 (1.4) | 5.3 (1.2) | 5.4 (1.4) | 5.4 (1.1) | 2.7 (1.6) |
| Expect to be good for stomach | 5.0 (1.7) | 4.9 (1.4) | 5.0 (1.7) | 5.2 (1.3) | 3.0 (1.6) |

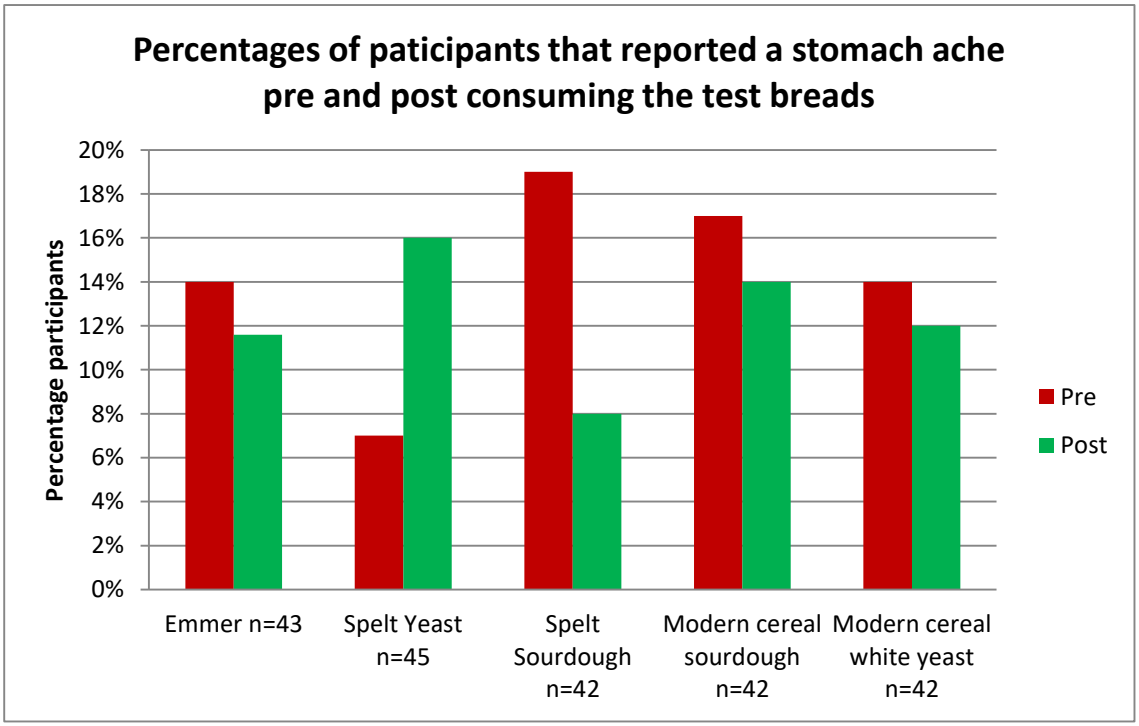
### General mood pre and post consumption test bread

Overall, not much effects were found between pre and post consumption and between the different breads. Emmer sourdough showed a significant increase on feeling energetic, healthy and being comfortable with appearance, and Spelt Sourdough on feeling fresh and energetic after eating and stomach feeling good. Modern cereal sourdough showed a significant increase in feeling comfortable and feeling health (table 9). Regarding energy dips, no notable difference in post consumption compared pre consumption of the test bread were observed (data not shown).

*Table 9. General mood on a 1-5 scale presented as median (range) pre and post bread consumption*

| Participants were asked. 'The last two days I felt' | Emmer Sourdough n=43 | | Spelt yeast n=45 | | Spelt Sourdough n=42 | | Modern cereal sourdough n=42 | | Modern cereal white yeast n=42 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Happy | 4 (3) | 3 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 3 (4) |
| Comfortable | 4 (3) | 4 (3) | 4 (4) | 4 (3) | 4 (3) | 4 (3) | **4 (3)** | **4 (3)*** | 4 (4) | 4 (4) |
| Relaxed | 4 (3) | 3 (4) | 4 (4) | 4 (3) | 4 (4) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 4 (4) |
| Energetic | **3 (4)** | **3 (3)*** | 4 (4) | 3 (4) | 3 (4) | 3 (3) | 3 (4) | 4 (3) | 3 (4) | 3 (4) |
| Fresh and energetic after eating | 3 (4) | 3 (3) | 3 (4) | 3 (3) | **3 (4)** | **4 (3)*** | **3 (4)** | **4 (4)*** | 3 (4) | 3 (4) |
| Concentrate easily | 3 (3) | 4 (4) | 3 (4) | 4 (3) | 4 (3) | 4 (3) | 3 (3) | 4 (3) | 4 (4) | 4 (4) |
| At ease | 3 (3) | 4 (4) | 4 (4) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 3 (4) |
| Healthy | **3 (4)** | **4 (3)*** | 4 (4) | 4 (3) | 4 (3) | 4 (4) | **3 (3)** | **4 (3)*** | 3 (4) | 3 (4) |
| Slept well | 4 (4) | 4 (4) | 3 (4) | 4 (4) | 4 (4) | 4 (4) | 4 (4) | 4 (4) | 3 (4) | 4 (4) |
| Eaten well | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 4 (4) |
| No pain or discomforts | 4 (4) | 4 (4) | 4 (4) | 4 (3) | 4 (4) | 4 (4) | 4 (4) | 4 (4) | 4 (4) | 4 (4) |
| Comfortable with appearance | **3 (3)** | **4 (3)**** | 3 (3) | 3 (3) | 3 (3) | 3 (3) | 4 (3) | 4 (3) | 3 (4) | 3 (4) |
| Stomach felt good | 4 (4) | 4 (4) | 4 (4) | 3 (3) | **3 (3)** | **4 (3)**** | 4 (3) | 4 (3) | 4 (4) | 4 (4) |
| Head felt good | 4 (3) | 4 (3) | 4 (4) | 4 (3) | 4 (4) | 4 (3) | 4 (4) | 4 (3) | 4 (4) | 4 (4) |
| Skin felt good | 4 (3) | 4 (3) | 4 (4) | 4 (4) | 4 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 4 (4) |
| Bowel movement felt good | 4 (3) | 4 (3) | 4 (3) | 3 (3) | 4 (3) | 4 (3) | 4 (3) | 4 (4) | 4 (4) | 4 (4) |

**P-value = <0.050. **P-value = 0.010*




*Graph 1. Percentages of participants that reported a stomach ache pre and post consumption of the test breads.*

**Prevalence of stomach ache pre and post consuming the test breads**

Prevalence of stomach ache was relatively low in general with 7%-19%. Post consumption of the ancient grain Spelt yeast test bread showed an increase in stomach ache of 9%. In post consumption of the Spelt sourdough test bread a decrease of stomach ache of 11% was reported. The other test bread showed no big differences between pre and post consumption (graph 1).

The three most prevalent IBS related complaints were flatulence, bloating and fatigue (table 10 and Appendix 3). Spelt sourdough showed an experienced reduction in all these three complaints, emmer sourdough in bloating and fatigue, and the other breads only in fatigue. The other complaints were only mildly present in the study population and showed no differences between pre and post consumption of test bread.

*Table 10. IBS related complaints pre and post consumption of test breads*

| | Emmer Sourdough n=43 | | Spelt yeast n=45 | | Spelt Sourdough n=42 | | Modern cereal sourdough n=42 | | Modern cereal white yeast n=42 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Flatulence** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| No | 51% | 51% | 47% | 49% | 38% | 62% | 52% | 52% | 51% | 48% |
| A, Little | 35% | 40% | 44% | 42% | 45% | 28% | 38% | 31% | 26% | 36% |
| Yes | 14% | 9% | 9% | 9% | 17% | 10% | 10% | 17% | 23% | 17% |
| **Bloated** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| No | 58% | 67% | 69% | 62% | 57% | 74% | 64% | 67% | 65% | 62% |
| A, Little | 28% | 23% | 22% | 31% | 24% | 18% | 26% | 24% | 28% | 31% |
| Yes | 14% | 9% | 9% | 7% | 19% | 8% | 10% | 10% | 7% | 7% |
| **Fatigue** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| No | 37% | 51% | 33% | 51% | 45% | 64% | 41% | 57% | 41% | 55% |
| A, Little | 33% | 28% | 40% | 27% | 24% | 15% | 29% | 21% | 33% | 29% |
| Yes | 30% | 21% | 27% | 22% | 31% | 21% | 31% | 21% | 26% | 17% |

## Onset of hunger

Around 50% of the participants felt hungry after the same amount of time as usual for all breads, except for the modern cereal sourdough which scored 79%. When trying the Modern cereal white yeast test bread, 36% felt hungry quicker than normal, followed by Spelt yeast with 11% and Emmer with 7%. In the 'less quick than usual' categories the ancient grains all score between 44%-49%, where the modern cereal score lower with 21% for Sourdough whole grain and 14% for white yeast. (table 11).

*Table 11. Onset of hunger*

| Feeling Hungry | Emmer Sourdough | Spelt yeast | Spelt Sourdough | Modern cereal sourdough | Modern cereal white yeast |
|---|---|---|---|---|---|
| | n=43 | n=45 | n=42 | n=42 | n=42 |
| Quicker than usual | 7% | 11% | 0% | 0% | 36% |
| No the same | 47% | 44% | 51% | 79% | 50% |
| Less quick than usual | 47% | 44% | 49% | 21% | 14% |

# 5 Conclusions

The objectives of this study were to understand which reasons and experiences people have consuming various products from ancient wheat varieties, to map information about the scale and infrastructure ancient wheat varieties grown, produced and processed in the Netherlands, and to compare health effects and laboratory parameters between ancient and modern wheat varieties.

The study on differences between wheat species showed no clear differences for wet gluten content. However, one group that may be different is einkorn, for which gluten analysis did not provide results. The gluten index tended to be higher for modern material (based on flour data and data from other projects), indicating a shift in type of gluten: a higher percentage of gluten are strong gluten. For Zeleny large differences among certain groups were visible. The largest differences were seen between varieties of bread wheat: modern material had clearly the highest Zeleny values, whereas the old material of bread wheat had Zeleny levels similar to spelt, emmer and rivet wheat. When looking at material with no or little breeding intensity, the diploid species einkorn seemed to differ clearly from the other species for baking quality, whereas the tetraploid species (emmer and rivet wheat) do not clearly differ from the hexaploid species (spelt and bread wheat). Other factors were a limited data set and limited material, and variation in weather conditions.

The study on motivation of people to consume ancient wheat varieties showed that almost half of the participants experienced health related complaints when consuming a wheat species. The most reported wheat species that was associated with the discomfort seemed to be modern wheat specie. More than a third of the participants eliminated this species and 27% of those who experienced complaints switched to consume spelt, of whose 71% reported an improvement in their health complaints. These results indicated an improvement of reported health related complaints after the participants changed and/or eliminated a wheat species.

In the study on comparing health effects of consumption of ancient wheat and modern wheat varieties, no big differences were found. Minor significant improvements of the sourdough breads (emmer, spelt and modern cereal) were seen on different aspects of general mood, such as feeling energetic and healthy. Prevalence of stomach ache was relatively low in general with 7%-19%, but consumption of spelt yeast bread showed an increase in stomach ache of 9%, whereas consumption of the spelt sourdough bread showed a decrease of stomach ache of 11%. Spelt sourdough reduced the occurrence of energy dips by 15%, but and seem to increase (+13%) the energy dips between 17:00 and 20:00. Also, spelt sourdough showed a reduction in the percentage of participants having flatulence, and together with emmer sourdough in bloating, whereas the other breads didn't show clear differences. Flatulence and bloating were, together with fatigue, also the most prevalent IBS related complaints in this study population. In the onset of hunger, a difference was found between the ancient grains and modern grains, with 36% of the participants reporting a quicker than usual rise in hunger with the modern cereal white yeast, versus 0-11% with the ancient grains. In the contrary, with the ancient grains it was reported that 44%-49% felt hungry less quick then usual versus 21% for modern cereal sourdough and 14% for modern cereal white yeast. Therefore, it seems that consumption of sourdough breads, especially the ancient types, such as spelt and to a less extent emmer, leads to a reduction in overall health complaints. And the consumption of sourdough (ancient grains-based) breads leads to a larger and longer satiating feeling.

The participants seem to be divided over the 'taste and overall experience' qualification. The participants were in general positive on most aspects of all breads. The modern cereal sourdough bread seemed to be judged as most positive and the emmer sourdough least. Participants had the highest intention to consume the cereal sourdough bread again and considered this bread to be most healthy. The white yeast modern cereal bread was regarded as least healthy.

Due to the input from the Dutch Organisation for IBS patients we focussed on the difference in FODMAPS in ancient grains. Namely, fructans which are often present in modern cereals but are removed in the process when making the flour gluten free. Being sensitive to fructans could be one of the reasons that a lot of self-diagnosed 'gluten sensitive' people felt better and experienced less abdominal complaints on a gluten free diet. In the literature FODMAPs content of ancient grains differs to that from modern cereals and are effected by the fermentation process of sourdough bread. Fructan content of emmer and spelt was indicated to be lower than average modern cereals (Biesiekierski J. R., 2011). In the study performed by Biesiekierski (Biesiekierski J. R., 2013), no significant increase in IBS symptoms was found when the self-reported gluten sensitive participants were randomly controlled blindly placed on a low FODMAP diet high gluten diet. Also, when testing grains, flour and bread types for fructan content within this project no notable difference were found in fructan content in either grain type of fermentation process. Due to sample material limitation only one of each sample was tested. A larger sample pool or a more sensitive test may have given a different results and further testing is needed to determine fructan levels in ancient grains.

The original objective was to test an IBS patient versus an healthy population, but two months of recruiting in partnership with the Dutch Organisation for IBS patients did not yield enough participants with an official of unofficial diagnosis of IBS. Perhaps IBS patients who had discovered that they were sensitive to consuming for example bread, gluten or FODMAPS would not apply for a study requiring them to eat bread. Therefore, the study population of the intervention study was a relatively healthy population. A less healthy population might have shown more and/or other differences between breads.

Another limitation was the two-day testing period, because the test breads were baked without any preservatives of bread improvers the bread would go stale after two days. On average most of the loaf was eaten on the two-day period, followed by a few slices. This is enough to trigger an IBS or FODMAP induced attack, but not enough to trigger a long term change gut flora. Besides, this limited the region in which the test breads could be distributed which in turn limited the recruitment of IBS patients and healthy participants. Consumption of the bread over a longer period of time (for example the whole week), might have yielded bigger results and is therefore recommended for a future intervention study.

Finally, the test breads were all of similar shape and size and were offered in a random order to the participants, however they could still distinct differences colour, smell and taste. The control test bread with modern cereal white yeast baked was most distinctive. This could have influenced the answers of the participants on this bread.

**Conclusion**

This research indicated that (some) participants reported health complaints while consuming modern wheat products in the Netherlands. Some of the participants report experiencing better health after switching to ancient wheat species. Although only minor differences between ancient

and modern wheat breads were found in the grain-laboratory analyses and the randomized intervention study, indicating more resilient gluten in the modern species were found. Furthermore, spelt and emmer sourdough breads seemed to reduce some of the most common prevalent abdominal related complaints. Also, these ancient breads seemed to delay onset of hunger. This satiating effect could be a very useful outcome to support efforts to reduce overconsumption of particular macronutrients and processed foods in light of our overweight and obesity epidemic. Therefore, our results indicate that consumption of sourdough breads, especially when using ancient grain varieties, such as spelt and to a less extent emmer, could reduce gut-health related complaints and support efforts in reducing our obesity burden. More research should be conducted to confirm these results.

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# Appendix 1: Previous results ancient grains

| variety type | species | genome constitution | breeding intensity | # accessions | year | protein % dry matter | Zeleny | gluten index (%) | wet gluten (%) |
|---|---|---|---|---|---|---|---|---|---|
| landrace | emmer | AABB | no breeding | 1 | 2015 | 13.6 | 16 | 61.3 | 29 |
| landrace | spelt | AABBDD | no breeding | 1 | 2015 | 10.6 | 16 | 75 | 23 |
| old variety | spelt | AABBDD | little breeding | 1 | 2015 | 14.2 | 17 | 45.2 | 40 |
| landrace | winter wheat | AABBDD | no breeding | 2 | 2015 | 10.9 | <10 | 86.5 | 19 |
| old variety | winter wheat | AABBDD | little breeding | 1 | 2015 | 9.42 | <10 | 62.7 | 19 |
| variety (semi-modern) | winter wheat | AABBDD | intensive breeding | 1 | 2015 | 14.5 | 27 | 67.3 | 30 |
| population (modern) | spring wheat | AABBDD | intensive breeding | 2 | 2015 | 11.6 | 41 | 98 | 24 |
| landrace | winter wheat | AABBDD | no breeding | # | 2011 | 12.1 | 14 | | |
| old variety | winter wheat | AABBDD | little breeding | 3 | 2011 | 11.1 | 11 | | |
| variety (modern) | winter wheat | AABBDD | intensive breeding | 1 | 2011 | 10.9 | 43 | | |
| population (modern) | winter wheat | AABBDD | intensive breeding | 1 | 2011 | 12.9 | 50 | | |
| old variety | spelt | AABBDD | little breeding | 5 | 2010 | 12.9 | 18 | | |
| old variety | winter wheat | AABBDD | little breeding | 1 | 2010 | 11.7 | 12 | | |
| variety (modern) | winter wheat | AABBDD | intensive breeding | 1 | 2010 | 13 | 58 | | |

# Appendix 2: Baseline characteristics for bread consumption

**Baseline consumption of grain products (n=46)**

| | (Almost) never | Once a month | 1-2 week | 3-6 week | Daily |
|---|---|---|---|---|---|
| Bread loaf (all types) | - | - | 4% | 33% | 63% |
| Breakfast cereals | 13% | 13% | 33% | 17% | 24% |
| Crackers (grain based) | 20% | 26% | 39% | 11% | 4% |
| Cookies and cakes | 9% | 24% | 44% | 20% | 4% |
| Beer | 61% | 15% | 22% | 2% | - |
| Readymade meals | 83% | 15% | 2% | - | - |
| Meat replacements | 35% | 13% | 41% | 7% | 4% |
| Pasta | 4% | 13% | 72% | 11% | - |
| Premade sauces and soups | 54% | 33% | 9% | 4% | - |

**Timing and type of bread consumption**

| | Total n=46 | % |
|---|---|---|
| **At which meal times do you eat bread?** | | |
| Breakfast | 30 | 65% |
| Lunch | 43 | 93% |
| Dinner | 4 | 9% |
| | | |
| **What type of bread do you regularly eat?** | | |
| Brown bread | 21 | 46% |
| White bread | 4 | 9% |
| Whole grain bread | 30 | 65% |
| Sourdough bread | 19 | 41% |
| Spelt bread | 17 | 37% |
| Raisin buns | 4 | 9% |
| Gluten free bread | 2 | 4% |
| | | |
| **Where do you regularly buy your bread?*** | | |
| Supermarket | 24 | 52% |
| Whole foods/organic supermarket | 10 | 22% |
| Regular bakery | 25 | 54% |
| Organic bakery | 6 | 13% |
| Market | 3 | 7% |
| Home made | 3 | 7% |
| Other | | |

*Participants can buy their bread regularly at several places therefor making the total count higher than 100%

# Appendix 3: IBS related complaints pre- and posttest bread consumption

**IBS related complaints pre and post consumption of test breads**

| | Emmer Sourdough | | Spelt yeast | | Spelt Sourdough | | Modern cereal sourdough | | Modern cereal white yeast | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n=43 | | n=45 | | n=42 | | n=42 | | n=42 | |
| **Stomach ache** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** | **Pre** | **Post** |
| No | 74% | 79% | 91% | 73% | 76% | 82% | 79% | 76% | 74% | 83% |
| A, Little | 21% | 16% | 7% | 22% | 21% | 18% | 12% | 19% | 23% | 14% |
| Yes | 5% | 5% | 2% | 4% | 2% | 0% | 10% | 5% | 3% | 2% |
| **Fluatulance** | | | | | | | | | | |
| No | 51% | 51% | 47% | 49% | 38% | 62% | 52% | 52% | 51% | 48% |
| A, Little | 35% | 40% | 44% | 42% | 45% | 28% | 38% | 31% | 26% | 36% |
| Yes | 14% | 9% | 9% | 9% | 17% | 10% | 10% | 17% | 23% | 17% |
| **bloated** | | | | | | | | | | |
| No | 58% | 67% | 69% | 62% | 57% | 74% | 64% | 67% | 65% | 62% |
| A, Little | 28% | 23% | 22% | 31% | 24% | 18% | 26% | 24% | 28% | 31% |
| Yes | 14% | 9% | 9% | 7% | 19% | 8% | 10% | 10% | 7% | 7% |
| **Abnormally hard stool** | | | | | | | | | | |
| No | 74% | 91% | 71% | 82% | 76% | 77% | 79% | 81% | 86% | 76% |
| A, Little | 19% | 5% | 22% | 11% | 21% | 15% | 14% | 12% | 7% | 17% |
| Yes | 7% | 5% | 7% | 7% | 2% | 8% | 7% | 7% | 7% | 7% |
| **Abnormally loose/watery stool** | | | | | | | | | | |
| No | 74% | 81% | 98% | 71% | 74% | 82% | 86% | 81% | 81% | 76% |
| A, Little | 14% | 7% | 0% | 27% | 21% | 10% | 7% | 14% | 12% | 19% |
| Yes | 12% | 12% | 2% | 2% | 5% | 8% | 7% | 5% | 7% | 5% |
| **Slime with stool** | | | | | | | | | | |
| No | 98% | 98% | 98% | 98% | 95% | 97% | 98% | 98% | 98% | 95% |
| A, Little | 2% | 2% | 2% | 2% | 2% | 3% | 2% | 2% | 2% | 5% |
| Yes | 0% | 0% | 0% | 0% | 2% | 0% | 0% | 0% | 0% | 0% |
| **Blood with stool** | | | | | | | | | | |
| No | 100% | 100% | 100% | 100% | 100% | 100% | 98% | 100% | 100% | 98% |
| A, Little | 0% | 0% | 0% | 0% | 0% | 0% | 2% | 0% | 0% | 2% |
| Yes | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| **Sensation of fullness without being able to pass** | | | | | | | | | | |
| No | 79% | 91% | 89% | 89% | 88% | 85% | 86% | 93% | 86% | 81% |
| A, Little | 14% | 9% | 9% | 4% | 7% | 15% | 14% | 5% | 9% | 12% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Yes | 7% | 0% | 2% | 7% | 5% | 0% | 0% | 2% | 5% | 7% |
| **Constipation** | | | | | | | | | | |
| No | 84% | 91% | 89% | 87% | 83% | 87% | 88% | 86% | 91% | 86% |
| A, Little | 7% | 5% | 2% | 7% | 12% | 8% | 7% | 7% | 2% | 10% |
| Yes | 2% | 5% | 9% | 7% | 5% | 5% | 5% | 7% | 7% | 5% |
| **Nausea** | | | | | | | | | | |
| No | 95% | 95% | 96% | 93% | 95% | 92% | 93% | 95% | 84% | 95% |
| A, Little | 5% | 2% | 4% | 4% | 5% | 8% | 7% | 5% | 9% | 2% |
| Yes | 0% | 2% | 0% | 2% | 0% | 0% | 0% | 0% | 7% | 2% |
| **Fatigue** | | | | | | | | | | |
| No | 37% | 51% | 33% | 51% | 45% | 64% | 41% | 57% | 41% | 55% |
| A, Little | 33% | 28% | 40% | 27% | 24% | 15% | 29% | 21% | 33% | 29% |
| Yes | 30% | 21% | 27% | 22% | 31% | 21% | 31% | 21% | 26% | 17% |
| **Being limited in daily activities by stomach problems** | | | | | | | | | | |
| No | 95% | 98% | 96% | 100% | 98% | 100% | 93% | 98% | 98% | 95% |
| A, Little | 5% | 2% | 2% | 0% | 0% | 0% | 7% | 2% | 2% | 5% |
| Yes | 0% | 0% | 2% | 0% | 2% | 0% | 0% | 0% | 0% | 0% |