

## **Preview of the 'Well on Wheat?' (WoW) project**

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### **Abstract**

The 'Well on Wheat' (WoW) project aims to generate robust data on the effects of wheat-based food products on gastrointestinal function and metabolism. The 1<sup>st</sup> objective of the project is to obtain in depth analytical data of the composition of whole meal obtained from bread wheat, spelt wheat and emmer wheat as well as the dough and the finally baked bread made thereof. The 2<sup>nd</sup> objective is to study the effects of two alternative fermentation processes: yeast and sourdough fermentation, on compositional changes. Objective 1 and 2 will give insight in the overall effects of food processing on the (bio)chemical composition as defined by proteomics, carbohydrate analysis (carbohydrates, fibers, FODMAPs), phytate, selected micronutrients and pesticide residues. The 3<sup>rd</sup> objective is to study the effects of consuming the various bread types (according to grain type and fermentation type) in individuals with irritable bowel syndrome (IBS), which will be monitored for effects on intestinal function and physiology, including e.g. faecal microbiota/metabolism, and using markers for gut permeability and inflammation as well as measuring subjective perceptions. The project will generate new scientific insights that will be translated into recommendations to food industries and to health professionals and patient/consumer organisations. This project will be carried out in the framework of Health Grain Forum and supported by the ICC (International Association for Cereal Science and Technology) and funded by private and public organisations.

### **Introduction**

During the last decade, a significant movement to the adoption of gluten-free and wheat-free foods has developed in Western societies. The prevalence of wheat intolerance (coeliac disease) and wheat allergy are well known, being ~1% and 0.2% of the general population, respectively. However, in the US, nearly 30% of the adult population has expressed a desire to reduce or eliminate wheat and/or gluten from their daily diet [1] while a recent questionnaire-based study in the Netherlands [2] showed that 6.2% of a cohort of 785 adults reported adverse symptoms after the ingestion of gluten-containing foods. The most widely reported intestinal symptoms were bloating, abdominal discomfort and flatulence, but extra-intestinal symptoms were also mentioned such as fatigue and headache. Symptoms were generally experienced several days a week, starting mostly between one and six hours after consumption and lasting several hours. These self-reporting 'gluten sensitive' individuals were mainly younger females (~80%) living in urban regions with a trend of higher education levels (which confirms previous data of a UK study on self-reported gluten sensitivity [3]). Over one third of the reported symptoms met the consensus criteria for a positive diagnosis, the 'Rome III criteria for IBS', which have been established due to the absence of reliable biomarkers and specific laboratory tests [4].

The reasons why so many people feel more comfortable on a gluten-free diet may extend beyond the food itself. Several popular books [5-7] and many statements on social media have promoted gluten-free ('Palaeolithic') diets, suggesting that wheat consumption has adverse health effects leading to various chronic diseases. Furthermore, it is often claimed that products made from modern bread wheat varieties have negative health effects, but not foods made from so-called 'ancient' wheats such as spelt (which is closely related to modern bread wheat) and emmer (which is more closely related to modern pasta wheat), which are generally cultivated under organic conditions. These messages are, however, in contradiction to ample scientific data that have demonstrated significant health-promoting

effects of whole grain consumption [8-12]. Despite these proven health benefits, the negative messages have resulted in a significant decline in the consumption of breads and other wheat products in Western countries.

In this context, Irritable Bowel Syndrome (IBS) has often been considered by the patients themselves to be associated with food and especially wheat consumption. IBS is the most commonly diagnosed functional gastrointestinal (GI) disorder with a prevalence of 10-20% worldwide, predominantly among women [13]. Structural abnormalities and tissue damage are generally absent, but psychiatric comorbidity is often reported, indicating a psychosomatic component in a subgroup of these patients. Although several factors have been associated with IBS, including e.g. microbial perturbations, altered permeability, motility and visceroperception, the exact pathophysiology is not yet clear. Also markers for mucosal immune activation and inflammatory responses have been reported in a subset of IBS patients that may disappear after elimination of wheat/gluten from the diet [14,15]. This condition is often referred to as 'non-celiac wheat sensitivity' (NWCS) or 'non-celiac gluten sensitivity' [16].

Dietary factors such as FODMAPs (Fermentable, Oligo-, Di-, Mono-saccharides and Polyols) have been recognized as triggers for symptoms in some subjects, by providing substrates for colonic fermentation [16 and refs therein, 17]. It has also been reported that replacing a bread wheat-based diet by whole grain products from 'ancient' wheats such as spelt, has benefits for IBS patients [18,19]. In addition to gluten and FODMAPs, the presence of relatively high quantities of amylase trypsin inhibitors (ATIs) in bread wheat has also been suggested as a potential IBS causing factor [20, 21]. Direct comparative data about the effects of foods obtained from different wheat types and their possible contribution to the pathophysiology of NCWS are, however, still lacking. Here we propose a research strategy to address this issue.

### **Project design**

The Wow project will study the effect of different grains in IBS patients to provide information on the wheat- and disease-related issues at three levels: (1) The biochemical composition of wheat grains and changes during processing steps (milling, fermentation, baking) into consumable food products (bread); (2) The impact of bread consumption on well-being and GI symptoms, gut permeability, immune function and the microbiome; and (3) The impact of the opinions and perception of consumers/patients on wheat consumption or avoidance regarding gastrointestinal symptoms and well-being. The project will be managed by the academic and funding partners in a contractually agreed *pre-competitive* manner.

### **Materials and Methods**

*Grains.* Grains from bread wheat (*Triticum aestivum*) (representing current bread products), spelt wheat (*T. aestivum* ssp *spelta*) and emmer wheat (*T. dicoccum*) (both representing 'ancient' wheat species) obtained commercially will be analysed for biochemically using proteomics (detection of gluten, globulins, albumins, ATIs, lectins, indigestible peptides), fibre (including fructans and other FODMAPs), phytate, phenolics, minerals (such as zinc and magnesium), at the level of grains, flours, fermented (yeast and sourdough) doughs, and breads.

*Cohort and intervention groups.* We aim at measuring the effects of wheat consumption in IBS patients, recruited from a large cohort of well-characterised IBS patients [22] that has been established at Maastricht University Medical Centre. Three groups will be used in the intervention study, including successively: a running-in period (1 week), a free-from diet (2 weeks), a yeast or sourdough bread food challenge (2 weeks), a free-from wash-out diet (2 weeks) and a sourdough or yeast bread food challenge (2 weeks). It should be noted that the first and the second challenge are reversed regarding the yeast and the sourdough breads. The three groups will differ in their challenge: the groups 1, 2 and 3 will be challenged blinded with yeast and sourdough bread from either bread wheat, spelt wheat or emmer wheat.

*Sampling human materials.* At each step in the challenge sequence, patient samples will be taken from (1) the stool to analyse microbiota composition and metabolites from bacterial protein and carbohydrate fermentation ((i.e. short chain fatty acids, branched chain fatty acids, etc.); (2) breath metabolome to identify volatile organic compounds reflecting host and microbial metabolism; (3) blood to determine alkylresorcinols, inflammation markers (C-reactive protein and cytokines); zonulin; and

(4) urine sugar ratios as proxy for gut permeability. Furthermore, validated scores will be applied to measure wellbeing and GI symptoms.

*Yeast versus sourdough fermentation.* Significant differences are expected in the biochemical composition after yeast fermentation as compared to sourdough fermentation [23]. If the results are not significant, the intervention schedule will be adapted accordingly.

*Nocebo effects.* In healthy consumers, nocebo effects related to wheat/gluten avoidance will be determined through a food challenge with a single bread type that will be differently labelled and offered in four categories of emotional perceptivity and acceptability.

*Ethics.* Before starting, the project will be evaluated, commented and approved by a Medical-Ethical Committee.

*Wheat cultivation.* In an extension to the WoW project, the various wheat types (see Grains above) will be compared after growth under different conditions (organic vs standard) to determine effects of environment on composition. This data will may help to explain possible differences, between the grain types obtained from different European countries and cultivation practices.

### **Partners and Sponsors**

The project proposal has been initiated by Maastricht University and further elaborated together with Wageningen University & Research, the Dutch Bakery Centre (all from The Netherlands) and Rothamsted Research (UK). Most research partner organizations are members of Health Grain Forum (HGF) and the project fits into the activities of the HGF working group on 'Cereals and health' and has been included in the general HGF programme. The International Association for Cereal Science and Technology (ICC; Austria) will serve as financially administrative partner for the following sponsoring entities:

- AB-Mauri bakery Ingredients, Made, Netherlands
- CSM innovation Bakery Center, Bingen, Germany
- CYMMIT, Texcoco, Mexico.
- DSM Food Specialties, , Delft, Netherlands
- Fazer Bakeries Helsinki, Finland
- ICC- Intl. Association Cereal Science and Technology, Vienna, Austria
- IWGA- Intl. Wheat Gluten Association, Kansas, USA
- Lantmännen EK , Stockholm, Zweden
- Mondelez , Saclay, France
- Dutch Bakery Center, Wageningen, Netherlands
- Nutrition et Sante, Revel, France
- Puratos BV, Groot Bijgaarden, Belgium
- Sonneveld Group BV, Papendrecht, Nederlands,
- Zeelandia Zierikzee, Netherlands
- Baking Industry Research Trust Howick, Auckland, New Zealand
- Health Grain Forum, Vienna
- The project is in part publicly funded by the Dutch Topsector Agri&Food.

The sponsoring organisations have neither a role in the design and execution of the project, nor in the collection, analyses, interpretation and publication of the data.

### **Expected outcomes**

The project will generate comparative data on the biochemical composition of grains of bread wheat, spelt and emmer, with major focus on those compounds expected to have positive or negative effects on health, such as carbohydrates (starch, fibre, FODMAPs), proteins (gluten, CD-immunogenic gluten peptides, albumins, globulins, lectins, ATIs), phenolic compounds, phytate, and minerals (Zn; Mg). In an extension of the project biochemical analyses will be carried out on grain grown under different conditions.

After milling breads will be made using yeast fermentation and sourdough fermentation and the compositions of the doughs and flours compared. A great challenge is the production of breads from

flours of the different grain types that are visually and organoleptically similar, so that they can be used in the double-blind food challenges.

Administration of the baked products to patients with IBS (i.c. NCWS) will reveal any effects on the aetiology of non-celiac wheat sensitivity

The project will also provide insight into the occurrence of nocebo effects by collecting data obtained about post-consumption gastrointestinal symptoms from a group of healthy volunteers that prefer to avoid gluten.

The results will be published in international scientific journals. New scientific insights will be translated into recommendations to wheat-processing industries, governmental regulatory bodies, health professionals, patients and consumers, and will underpin innovation in the production of wheat-based foods.

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