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Gastrointestinal symptoms in individuals with non-coeliac wheat sensitivity: does type of bread matter?

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Abbreviations

ΔVAS	delta visual analogue scale symptom score, calculated as [score test day] – [average of 3-day run-in period]
ATIs	amylase-trypsin inhibitors
BMI	body mass index
CD	coeliac disease
FD	functional dyspepsia
FODMAPs	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GAD-7	Generalized Anxiety Disorder assessment
GI	gastrointestinal
IBS	irritable bowel syndrome
IgA	immunoglobulin A
IQR	interquartile range
NCGS	non-coeliac gluten sensitivity
NCWS	non-coeliac wheat sensitivity
PHQ-9	Patient Health Questionnaire-9
PHQ-15	Patient Health Questionnaire-15
SF	sourdough fermentation
VAS	visual analogue scale
WA	wheat allergy
YF	yeast fermentation

Abstract

Background: Many individuals reduce their bread intake due to the belief that wheat is the cause of their gastrointestinal (GI) symptoms. Different grains and processing methods may impact tolerability.

Objective: We investigated the effects of six different types of bread on GI symptoms in individuals with self-reported non-coeliac wheat sensitivity (NCWS).

Methods: Two parallel randomised double-blind crossover multicentre studies were conducted. NCWS individuals, in whom coeliac disease and wheat allergy were ruled out, received five slices of (study A, n=20) yeast fermented (YF) or (study B, n=20) sourdough fermented (SF) bread made of bread wheat, spelt or emmer on three separate intervention days. Each test day was preceded by a run-in period of 3 days and separated by a wash-out period of at least 7 days. GI symptoms were evaluated by change in symptom score (test day minus average of the 3-day run-in period) on a 0-100mm visual analogue scale (ΔVAS). Responders were defined as an increase in ΔVAS of at least 15mm for overall GI symptoms, abdominal discomfort, abdominal pain, bloating and/or flatulence.

Results: The overall change in GI symptoms did not differ between breads of different grains (YF p=0.267; SF p=0.144). The number of responders was also comparable for both YF (6 to wheat, 5 to spelt, and 7 to emmer, p=0.761) and SF breads (9 to wheat, 7 to spelt, and 8 to emmer, p=0.761).

Conclusion: The majority of NCWS individuals experienced GI symptoms for at least one of the breads, but on a group level, no differences were found between different grain types for either YF or SF breads.

Clinical Trial Registry: ClinicalTrials.gov, NCT04084470

Keywords

Non-coeliac wheat sensitivity, gastrointestinal symptoms, wheat, spelt, emmer, yeast fermented bread, sourdough fermented bread

Introduction

Wheat is the most important staple food consumed in the Western world. Whole grain wheat products provide a substantial source of carbohydrates, non-starch polysaccharides (dietary fibres), proteins, vitamins, minerals, and phytochemicals, making an important contribution to daily energy intake and a healthy diet (1, 2). Accordingly, based on epidemiological evidence, the consumption of whole grain cereal foods has been associated with, among others, a reduced risk of type 2 diabetes, cardiovascular disease, various types of cancer, and mortality (3-6). Nevertheless, despite these beneficial effects, grain-based foods can also elicit adverse reactions in susceptible individuals, *e.g.* coeliac disease (CD) and wheat allergy (WA) (7-9).

In addition, a substantial proportion of the general population avoids or reduces their wheat intake because of symptoms, even though CD and WA have been ruled out. Initially, this was defined as non-coeliac gluten sensitivity (NCGS) due to gluten as presumed cause. As also amylase-trypsin inhibitors (ATIs) and fermentable carbohydrates (*i.e.* FODMAPs) are considered potential triggers, the term non-coeliac wheat sensitivity (NCWS) is increasingly used (10). NCGWS has an estimated prevalence up to 15% (11-13), although the true prevalence remains unknown due to problems in establishing diagnostic criteria and reliable biomarkers. NCWS generally manifests as gastrointestinal (GI) symptoms such as abdominal discomfort or pain, bloating, and diarrhoea, and to a lesser extent extra-intestinal symptoms such as headache and tiredness (14-16). In general, symptoms occur within 12 hours after wheat intake and ameliorate within a few hours (17). The exact underlying trigger is however unclear. There is conflicting evidence on the role of gluten (18, 19). Additionally, ATIs are potential activators of innate immune responses and intestinal barrier dysfunction, but evidence so far is based on *in vitro* and animal studies (20-22). Furthermore, FODMAPs such as fructans may lead to bloating, abdominal discomfort, and diarrhoea due to osmotic effects and gas production by bacterial fermentation, but concentrations in bread are generally rather low (23, 24). Eliciting the contribution of these components is further complicated by the fact that the biochemical composition differs between grain types and bread processing methods (10).

Many NCWS individuals claim they benefit from consuming ancient grains, such as spelt or emmer, instead of modern varieties like bread wheat products (17, 25). Although spelt is usually included in the definition of ‘ancient grains’, the spelt varieties

that are currently grown appear to be more related to ‘modern’ bread wheat (26). Spelt and emmer contain about 20% more gluten than bread wheat (27), whereas FODMAP concentrations were reported to be comparable between spelt and bread wheat, although with variability among varieties within one species (28). Furthermore, there is conflicting evidence on whether hexaploid (AABBDD) wheats, such as bread wheat and spelt, induce more immune reactivity than tetraploid wheat types (AABB; e.g. emmer) (29, 30). Previous double-blinded intervention studies found inconsistent results for effects of bread from these different grain types on GI symptoms (25, 31).

Whereas yeast fermentation (YF) is the major practice in bread baking, sourdough fermentation (SF) has gained renewed interest because of presumed enhanced nutritional value, improved digestive tolerance and possible related health benefits (32). Experimental sourdough systems, expressing high proteolytic activity, have been found to degrade FODMAPS, gluten and ATIs to a significant extent (> 50%), but are generally not being used by bakers and baking industry (28, 33-36). Moreover, while SF could theoretically result in improved GI tolerability, a pilot study in IBS patients did not confirm this (37).

Currently, the impact of fully characterised breads, including details on composition for different grain types and processing methods, on symptoms in NCWS is not well investigated. Therefore, we aimed to investigate the effects of YF and SF bread made from bread wheat, spelt, and emmer on overall GI symptoms in individuals with self-reported NCWS in two parallel studies. Secondarily, we investigated the effects of these breads on individual GI and extra-intestinal symptoms. We hypothesised that the consumption of YF and SF bread made from tetraploid emmer would cause less GI symptoms than hexaploid wheat and spelt.

Methods

Two randomised, double-blind, cross-over, multicentre studies were conducted at Maastricht University and Wageningen University and Research, both in the Netherlands, between 11 September 2020 and 29 November 2022. The studies were approved by the Medical Ethics Committee of Academic Hospital Maastricht/ Maastricht University, and by the Board of Directors of Wageningen University and Research, and was performed in accordance with the Declaration of Helsinki and Dutch Regulations on Medical Research involving Human Subjects. All participants

gave their written informed consent prior to participation. The studies were registered at ClinicalTrials.gov (NCT04084470).

Participants

Participants were recruited via advertisements on social media, patient association websites, notice boards at the university campus and local public areas, and in local newspapers. After being informed via written and verbal information, interested participants were invited for a screening visit to assess eligibility.

Males and females aged 18-70 years who experience GI symptoms within 12 hours after a single intake of bread (NCWS) were included. Medication had to be stable for at least one month prior to and during the study. Participants were excluded if they had been diagnosed with CD, WA, or other organic GI diseases, any malignancies, or any other disease interfering with GI function, or if they previously had major abdominal surgery or radiotherapy interfering with GI function (uncomplicated appendectomy, cholecystectomy and hysterectomy were allowed if more than six months ago). If CD was not excluded by previous serology or upper GI endoscopy, and participants still consumed gluten or were willing to re-introduce gluten into their diet for at least six weeks, an additional visit was scheduled for serological testing to rule out CD (total immunoglobulin A (IgA) and anti-tissue transglutaminase IgA). Furthermore, use of antibiotics, probiotics or prebiotics, participation in other studies 14 days prior to and during the study, excessive use of alcohol or drugs, and intentional weight-loss during the study period were not allowed. Women could not be pregnant or lactating. Participants had to have sufficient understanding of the Dutch language.

Participants were requested to adhere to a “symptom-free diet”, *i.e.* to replace or avoid food products that they consider to induce GI symptoms. Practical application of this diet varied from replacing their usual bread to following a completely gluten-free diet, depending on what was necessary for the individual participant to obtain a low GI symptom score at baseline. After following the symptom-free diet for at least one week prior to the screening visit, overall GI symptoms had to be minimal, *i.e.* below ≤ 30mm on a 100mm visual analogue scale (VAS). The symptom-free diet was maintained throughout the intervention period.

Medical history, Rome IV criteria for irritable bowel syndrome (IBS) (38) and functional dyspepsia (FD) (39) were assessed by the researcher during the screening visit. After inclusion into the study, but prior to starting the intervention, participants completed the

Generalized Anxiety Disorder assessment (GAD-7) (40), Patient Health Questionnaire-9 (PHQ-9) (41), and the Patient Health Questionnaire-15 (PHQ-15) (42) to assess anxiety, depression, and somatic symptoms, respectively.

Study design

Two parallel randomised double-blind crossover multicentre studies were conducted. Study A tested YF bread made of bread wheat, spelt or emmer, whereas study B tested SF bread, also made of bread wheat, spelt or emmer. Within each study, participants received five slices of these breads (125-150 gram in total) in a randomised order on three separate test days. Participants were unaware of the different bread types under investigation, and the researchers were blinded to the randomisation order. Participants received all three study breads at the end of the screening visit and completed the full test period at home. They were instructed to consume the breads for breakfast and lunch, with 2-3 slices per mealtime. Each test day was preceded by a 3-day run-in period and separated by wash-out period of at least seven days (see Figure 1). Participants received a reminder via text message on the evening prior to each run-in period. For women, run-in periods and test days were not scheduled during the menses phase of their menstrual cycle, thus the wash-out period was prolonged if necessary.

On the evening of each test day and during the three run-in days, participants completed symptom diaries for GI and extra-intestinal symptoms, and the Bristol Stool Scale to assess stool frequency and consistency.

All participants were asked to adhere to their symptom-free diet throughout the study period. Food records were completed during each run-in period and test day to assess compliance to the symptom-free diet, and, combined with photos of the study breads sent on the test day, to assess compliance to the intervention.

Participants who completed study A could also participate in study B.

Study bread

All study breads were manufactured by the Dutch Bakery Center, Wageningen, the Netherlands. Grain for bread wheat (*Triticum aestivum*), spelt (*Triticum aestivum ssp spelta)* and emmer (*Tritordeum dicoccum*) were obtained commercially. Breads made from bread wheat and spelt were chosen to represent modern bread products, whereas emmer represented ancient wheat species. All breads were prepared using

100% food-grade ingredients suitable for human consumption. Additions such as salt and minor processing additives were constant throughout and in accordance with standard commercial bread baking process, with minor adjustments to the addition of water to obtain uniform-looking breads. For the SF breads, the commercial sourdough starter culture 'Mailander Le Chef' (Böcker, Germany) was used.

The breads used in the present study were baked from the same materials according to the processing methods as described by Shewry *et al.* 2022 (43). More details about baking, and analysis of the bread composition are included in Supplemental Material 1.

Primary and secondary outcomes

The primary outcome was the effect of YF bread (study A) and SF bread (study B) made from either bread wheat, spelt or emmer on overall GI symptoms. Secondarily, the effects of these breads on individual GI symptoms (*i.e.* abdominal discomfort, abdominal pain, belching, bloating, constipation, diarrhoea, flatulence, fullness, nausea, urge to empty bowel) and extra-intestinal symptoms (*i.e.* confusion, headache, joint pains, loss of coordination, skin rash, tiredness) were investigated. All symptom scores were measured on a 100mm VAS as part of the symptom diary.

Statistical analysis

Sample size was calculated using G*power version 3.1. Based on a study by Biesiekierski (19), a mean difference in VAS of 10.3mm with standard deviation (SD) of 12.8mm was expected. With a power of 80% and a Bonferroni-corrected alpha of 0.0167, this resulted in a sample size of 20 participants per study. Expecting a drop-out rate of maximum 10%, permission was granted to include two extra participants per study if necessary.

Statistical analyses were conducted using IBM SPSS statistics version 26.0. Study A and B were analysed separately. Normality of data was evaluated using histograms and the Kolmogorov-Smirnov test. Baseline characteristics were presented as mean with SD for normally distributed continuous variables, as median with interquartile range (IQR) for non-normal distributed continuous variables, and as frequencies and percentages for categorical variables.

To assess primary and secondary outcomes, delta VAS symptom scores (ΔVAS) for each bread were calculated per symptom for each bread as [score test day] – [average

of 3-day run-in period], where the average of the 3-day run-in period was considered as baseline. The ΔVAS per symptom was analysed using the non-parametric Friedman test, with post-hoc Wilcoxon test. Missing values for run-in days were imputed per symptom, using the mean of the other days of that run-in period. No values were missing for the test days.
The averages of each 3-day run-in period were compared to check for carry-over effects, and the ΔVAS of each test day to check for an order effect, both using the Friedman test with post-hoc Wilcoxon test.

Because of the large variation observed during each test day, in a post-hoc analysis responders and non-responders were further explored. Responders were defined as participants with an increase of at least 15mm on ΔVAS for overall GI symptoms and/or predominant symptoms abdominal discomfort, abdominal pain, bloating or flatulence. The number of responders for each bread was compared by Cochran's Q test with post-hoc McNemar test.
Exploratively, the effects of dough processing using either yeast- or sourdough fermentation was assessed in the subgroup of participants that completed both study A and B. Again, the Friedman test was used to compare symptom scores, and Cochran's Q test to compare the number of responders.

Results

Study A: YF breads

Fifty-seven potential participants received the study information. Of these, 39 completed the pre-screening and 26 the full screening. Main reasons for ineligibility were that their symptoms were not reported to result from bread (n=6), that CD was not ruled out (n=4), or that symptoms were too high despite following the symptom-free diet (n=2). Twenty participants started and completed study A (see Figure 2).
In study A, mean age was 42.8 ± 2.8 years, mean body mass index (BMI) was 25.6 ± 3.7 kg/m^2, and 15 participants were female (75%). Most participants never smoked (85%) and had an alcohol intake of less than 1 unit (35%) or 1-5 units per week (40%). Fifteen percent (3/20 participants) met de Rome IV criteria for IBS, and 5% (1/20) for FD. For full details, see Table 1 and Supplementary Table 5.

Overall GI symptoms (Figure 3A) were comparable between YF breads made of bread wheat (median ΔVAS 5.7mm [IQR 0-17.8mm]), spelt (median ΔVAS 0mm [IQR -7.6-9.4mm]), and emmer (median ΔVAS 1.3mm [IQR 0-21.3mm], p=0.267). Predominant GI symptoms were abdominal discomfort, abdominal pain, bloating and flatulence. None of the assessed GI symptoms showed significant differences between YF bread types (Figure 3B-K). Also none of the assessed extra-intestinal symptoms showed significant differences between YF breads (Figure 4).

No carry-over effect or order-effect was found for any of the symptoms (for all symptoms p>0.05).

Study B: SF breads

Fourteen participants from study A gave permission to also participate in study B. Additionally, 29 new potential participants received the study information. Eleven completed the pre-screening and nine the full screening. The main reason for ineligibility was insufficient understanding of Dutch (n=5), the other participants were no longer interested in participation. Twenty-two participants started the study, but two participants dropped out after test day 1 because of too severe symptoms, or because they found the study too time consuming. Twenty participants completed study B (see Figure 2).

Of these, 18 were female (85%), mean age was 41.9 ± 12.9 years, and mean BMI was 25.1 ± 4.8 kg/m^2. Most participants never smoked (80%) and had an alcohol intake of less than 1 unit (35%) or 1-5 units per week (40%). Fifteen percent (3/20 participants) met de Rome IV criteria for IBS and 10% (2/20) for FD. For full details, see Table 1 and Supplementary Table 5.

Overall GI symptoms (Figure 5A) were comparable between SF breads made of bread wheat (median ΔVAS 2.1mm [IQR -3.1-31.5mm]), spelt (median ΔVAS 8.5mm [IQR 0-15.3mm]), and emmer (median ΔVAS 0mm [IQR -2.9-9.3mm], p=0.144). Predominant GI symptoms were abdominal discomfort, abdominal pain, bloating, flatulence, and fullness. None of the assessed GI symptoms showed significant differences between SF bread types (Figure 5B-K). Also, none of the assessed extra-intestinal symptoms showed significant differences between SF breads (Figure 6).

No carry-over effect or order-effect was found for any of the symptoms (for all symptoms p>0.05).

Post-hoc analyses

Responders vs. non-responders

On group level, no differences in symptom scores were found between YF breads and SF breads. Nevertheless, we noted a wide range in symptom scores, suggesting inter-individual variation in response. To further explore these individual differences in symptom response, responders were defined as participants with an increase of at least 15mm VAS for overall GI symptoms, or for any of the predominant symptoms abdominal discomfort, abdominal pain, bloating and flatulence.

For study A, the number of responders (Supplementary Table 6) was comparable between YF breads made of bread wheat (n=6), spelt (n=5) or emmer (n=7, p=0.761). Forty percent of participants were considered non-responders. Seven participants (35%) responded to one type of bread, four participants (20%) to two types of bread, and one (5%) to all three breads (Supplementary Table 7).

For study B, the number of responders (Supplementary Table 8) was comparable between SF breads made of bread wheat (n=9), spelt (n=7) or emmer (n=8, p=0.761). Thirty percent of participants were considered non-responders. Seven participants (35%) responded to one type of bread, four participants (20%) to two types of bread, and three (15%) to all three breads (Supplementary Table 9).

Yeast vs. sourdough (n = 13)

Fourteen participants from study A volunteered to also participate in study B. One of these participants dropped out of study B after test day 1, resulting in 13 participants that completed both studies (see Figure 2).

Overall GI symptoms (Supplementary Figure 2A) were comparable between all YF and SF bread types (p=0.396). None of the assessed individual GI symptoms (Supplementary Figure 2B-K) or extra-intestinal symptoms (Figure 8) showed

significant differences between the six bread types. The number of responders (Supplementary Table 10) was comparable between all YF and SF breads (p=0.835).

Discussion

The present study investigated the effects of YF and SF breads made of bread wheat, spelt, and emmer on symptoms in individuals with self-reported NCWS. NCWS was defined as symptom development within 12 hours after bread consumption, while CD and WA were ruled out. When comparing the three grain types within each type of fermentation, we found no differences in GI and extra-intestinal symptoms between YF breads, nor between SF breads. Although on a group level no differences were seen, on an individual level we noted that more than half of the participants responded with GI symptoms to at least one of the breads. Nevertheless, the number of responders was also not different between bread types.

Breads made from bread wheat, spelt and emmer did not result in differences in GI symptoms in our study population. Although several previous studies investigated the effects of gluten (18, 44-52) and/or FODMAPs (19, 33, 53-59) on symptoms in NCGS/NCWS, so far only a few studies investigated the effects of different grains or fermentation methods on symptoms in NCWS. In line with our results, the only previous intervention study using bread wheat and spelt also found no differences between bread types in NCWS individuals (25). Contrary to these findings, an intervention study comparing food products (pasta, bread, crackers, and biscuits) made from ancient and modern durum wheat varieties did find a significant reduction of IBS symptoms with consumption of ancient grain products (31). Similarly, intervention studies investigating tritordeum-based products compared to habitual wheat-containing diet (60) found these reduced IBS symptoms, and just as effective as a low-FODMAP diet (57). However, a comparison to our study population should be done with care, as these studies included IBS patients in whom CD was excluded, but who were not specifically characterised as NCWS (31, 57, 60).

Our study also showed no differences between extra-intestinal symptoms. To our knowledge, this has previously been investigated in only one other human study, which showed a significant improvement of fatigue when eating ancient wheat products (31). Possibly, the longer duration of their intervention (6 weeks) was better suited to investigate extra-intestinal symptoms, as these usually have a longer time until onset (61).

The majority of previous studies investigating the effect of bread used different grain types (62, 63) or different processing methods (33, 34, 64-66) to obtain a difference in one specific component that may potentially trigger symptoms in NCWS, usually FODMAPs or gluten. Nevertheless, these compounds are present in bread in varying amounts (27, 28, 67), making it difficult to attribute effects of different breads to one specific compound. Additionally, it is important to consider that growing conditions such as environments (location and years) and nitrogen fertiliser were found to have more impact on grain fructans content than the type of grain itself (68). Also, for our own study breads, we found different proportions of the main compounds, as shown by Shewry *et al*. (43). However, absolute differences in quantity remain rather low and the clinical relevance thereof is unclear. This may also be illustrated by the fact that in our study, there was large variation between participants to which bread or breads they responded, and no single bread caused the lowest symptoms.

Exploratively, we also compared YF and SF in a subset of our study population, finding no significant differences in GI symptom response. Also, these results should be interpreted with caution as the study was not designed nor powered for this comparison. Our findings are in line with a pilot study by Laatikainen *et al.*, with a parallel 1-week intervention, who also found no differences between YF and SF wheat bread on GI symptoms. However, contrary to our findings, they did show SF resulted in higher extra-intestinal symptom scores, *i.e.* for tiredness, joint pain and decreased alertness (37).

We feel that studies addressing the effects of wheat-based foods “as consumed part of a typical daily human diet” are needed to obtain reliable data that are useful for optimizing appropriate food processing and product development as well as for dietary recommendations to consumers. Participants consumed five slices of study bread per day, based on the Dutch healthy diet guidelines and average daily consumption, therefore considered sufficient to induce GI symptoms, and maintain clinical relevance (69, 70). Since we want to stay as close as possible to commercially available bread and mimic the real-life situation, the provided study breads contained no more gluten, ATIs or other components than is present in normal commercially available bread. As only a few individuals responded to all different breads in our study, this highlights the need for individualised dietary treatment. NCWS individuals in whom CD and WA have been excluded may benefit from trying different bread types.

There was large inter-individual heterogeneity in our study population, which may have contributed to no significant differences on group level. However, a strength of this study was the cross-over design that compared the effects within one person instead of using a different control group, who themselves indicated to develop symptoms after consuming bread. The variation observed may also point to a variety of biological and/or psychological factors that may contribute to symptoms in individuals. Given the fact that GI symptoms generally arise rather fast (*i.e.* within 1-8 hours) and as predominant symptoms are abdominal pain, bloating and flatulence (17), the intestinal microbiota may be a relevant factor in symptom generation (71), although this was not subject to detailed assessment in the current study.

Contrary to previous studies, our intervention period only consisted of one test day. Although we may have missed symptom scores that develop after prolonged intake, previous studies show that most NCWS individuals report symptoms within 12 hours (17). This was also the group included in the current study.

A possible limitation or our study is the small sample size. Although this was considered sufficient based on the sample size calculation, the heterogeneity found in symptom response may require a larger sample size to show differences between intervention. This should be taken into account for future studies.

With a cross-over design, there is always the risk of a carry-over effect, especially since some individuals indicate symptoms can last up to weeks (17). Symptom scores were not significantly different between run-in periods, ruling out a carry-over effect. Furthermore, although participants adhered to a symptom-free diet throughout the study, we found some participants had higher symptom scores during run-in than on the test day. This may be due to the overlap with IBS, which we know is also affected by other factors, such as stress, that were not assessed in our study. In addition, symptoms of NCWS can fluctuate over time. Also, given the high nocebo-response found in the NCGS/NCWS population, an order effect may occur in cross-over studies. We did not find that in our study.

Conclusion

The majority of NCWS individuals experienced GI symptoms for at least one of the breads, but on a group level, no differences were found between different YF or SF breads. Nevertheless, these individual differences confirm that in clinical practice it would be worthwhile to try different bread types.

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Conflict of Interest

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All authors have declared their individual conflicts of interest according to the rules of the International Committee of Medical Journal Editors (ICMJE).

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