De Graaf, M. C. G., Timmers, E., Bonekamp, B., Van Rooy, G., Witteman, B. J. M., Shewry, P. R., America, T., Keszthelyi, D., Brouns, F. J. P. H. and Jonkers, D. M. A. E. 2024. Two randomized crossover multicenter studies investigating gastrointestinal symptoms after bread consumption in individuals with noncoeliac wheat sensitivity: do wheat species and fermentation type matter? *The American Journal of Clinical Nutrition.* https://doi.org/10.1016/j.ajcnut.2024.02.008

The American Journal of Clinical Nutrition xxx (xxxx) xxx

The American Journal of CLINICAL NUTRITION

journal homepage: https://ajcn.nutrition.org/

Original Research Article

Two randomized crossover multicenter studies investigating gastrointestinal symptoms after bread consumption in individuals with noncoeliac wheat sensitivity: do wheat species and fermentation type matter?

Marlijne CG de Graaf [1,2], Emma Timmers [1,2], Bo Bonekamp [1,2], Gonny van Rooy [1,2], Ben JM Witteman [3,4], Peter R Shewry [5], Alison Lovegrove [5], Antoine HP America [6], Luud JWJ Gilissen [7], Daniel Keszthelyi [1,2], Fred JPH Brouns [2,8], Daisy M.A.E. Jonkers [1,2,*]

[1] *Department of Gastroenterology-Hepatology, Maastricht University Medical Center+, Maastricht, The Netherlands;* [2] *NUTRIM School of Nutrition and Translational Research in Metabolism, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands;* [3] *Division Gastroenterology-Hepatology, Gelderse Vallei Hospital, Ede, The Netherlands;* [4] *Division of Human Nutrition, Wageningen University & Research, Wageningen, The Netherlands;* [5] *Rothamsted Research, Harpenden, United Kingdom;* [6] *Business Unit Bioscience, Plant Sciences Group, Wageningen University & Research, Wageningen, The Netherlands;* [7] *Plant Breeding, Wageningen University & Research, Wageningen, The Netherlands;* [8] *Department of Human Biology, Maastricht University, Maastricht, The Netherlands*

A B S T R A C T

Background: Many individuals reduce their bread intake because they believe wheat causes their gastrointestinal (GI) symptoms. Different wheat species and processing methods may affect these responses.

Objectives: We investigated the effects of 6 different bread types (prepared from 3 wheat species and 2 fermentation conditions) on GI symptoms in individuals with self-reported noncoeliac wheat sensitivity (NCWS).

Methods: Two parallel, randomized, double-blind, crossover, multicenter studies were conducted. NCWS individuals, in whom coeliac disease and wheat allergy were ruled out, received 5 slices of yeast fermented (YF) (study A, $n = 20$) or sourdough fermented (SF) (study B, $n = 20$) bread made of bread wheat, spelt, or emmer in a randomized order on 3 separate test days. Each test day was preceded by a run-in period of 3 d of a symptom-free diet and separated by a wash-out period of ≥7 d. GI symptoms were evaluated by change in symptom score (test day minus average of the 3-d run-in period) on a 0–100 mm visual analogue scale (ΔVAS), comparing medians using the Friedman test. Responders were defined as an increase in ΔVAS of ≥15 mm for overall GI symptoms, abdominal discomfort, abdominal pain, bloating, and/or flatulence.

Results: GI symptoms did not differ significantly between breads of different grains [YF bread wheat median ΔVAS 10.4 mm (IQR 0.0–17.8 mm), spelt 4.9 mm (−7.6 to 9.4 mm), emmer 11.0 mm (0.0–21.3 mm), $P = 0.267$; SF bread wheat 10.5 mm (−3.1 to 31.5 mm), spelt 11.3 mm (0.0–15.3 mm), emmer 4.0 mm (−2.9 to 9.3 mm), $P = 0.144$]. 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, $P = 0.761$).

Conclusions: The majority of NCWS individuals experienced some GI symptoms for ≥1 of the breads, but on a group level, no differences were found between different grains for either YF or SF breads.

Clinical Trial Registry: clinicaltrials.gov, NCT04084470 (https://classic.clinicaltrials.gov/ct2/show/NCT04084470).

Keywords: noncoeliac wheat sensitivity, gastrointestinal symptoms, wheat, spelt, emmer, yeast fermented bread, sourdough fermented bread

Abbreviations: ΔVAS, delta visual analogue scale symptom score, calculated as [score test day] – [average of 3-day run-in period]; ATI, amylase-trypsin inhibitor; CD, coeliac disease; FD, functional dyspepsia; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyol; GI, gastrointestinal; IBS, irritable bowel syndrome; IgA, immunoglobulin A; NCGS, noncoeliac gluten sensitivity; NCWS, noncoeliac wheat sensitivity; SF, sourdough fermentation; VAS, visual analogue scale; WA, wheat allergy; YF, yeast fermentation.

* Corresponding author.
E-mail address: d.jonkers@maastrichtuniversity.nl (D.M.A.E. Jonkers).

https://doi.org/10.1016/j.ajcnut.2024.02.008
Received 9 August 2023; Received in revised form 14 February 2024; Accepted 16 February 2024; Available online xxxx

Introduction

Whole grain wheat products provide a substantial source of nutrients, making an important contribution to energy intake and a healthy diet [1,2]. Accordingly, their consumption has been associated with reduced risks of type 2 diabetes, cardiovascular disease, cancer, and mortality [3–6]. Nevertheless, wheat-based foods can elicit adverse reactions in susceptible individuals, such as those with coeliac disease (CD) and wheat allergy (WA) [7–9]. In addition, some people avoid or reduce wheat intake because of symptoms, even though CD and WA have been excluded. Initially, this was defined as noncoeliac gluten sensitivity (NCGS) because of gluten as the presumed cause [10]. As also amylase-trypsin inhibitors (ATIs) and fermentable carbohydrates [that is, fermentable oligosaccharides, disaccharides, monosaccharides and polyol (FODMAP)] are potential triggers, the term noncoeliac wheat sensitivity (NCWS) is increasingly used [11,12], and the Salerno expert's criteria [10], including a gluten elimination and challenge, may need reconsideration. NCWS has an estimated self-reported prevalence $\leq$15% [13–15], generally manifesting with gastrointestinal (GI) symptoms like abdominal discomfort or pain, bloating, and diarrhea, and sometimes extraintestinal symptoms [16–18]. Symptoms mostly occur within 12 h after wheat intake and ameliorate within a few hours [19].

Evidence on the role of gluten is inconsistent [20–28]. Gluten preparations used in previous human studies also contain ATIs [29], potential activators of innate immune responses, although evidence is mostly based on in vitro and animal studies [30–35]. FODMAP like fructans may lead to osmotic effects and gas production [36,37]. Eliciting the contributions of these components is complicated by the biochemical composition differing between wheat species and varieties, environmental, cultivation, and processing conditions [11, 38,39].

NCWS individuals claim experiencing less GI symptoms from consuming "ancient" grains, including spelt and emmer, compared with modern wheat varieties [19,40–43]. Spelt and emmer contain ~20% more gluten than bread wheat [44], whereas FODMAP concentrations are comparable between spelt and bread wheat [38]. Furthermore, there is conflicting evidence on hexaploid (AABBDD) wheats, including bread wheat and spelt, inducing more immune reactivity than tetraploid species (AABB) such as emmer [45,46]. Previous double-blinded intervention studies found inconsistent effects of bread from different wheat types on GI symptoms [40,47].

Whereas yeast fermentation (YF) is the major practice in modern bread baking, sourdough fermentation (SF) has gained renewed interest because of presumed fructan degradation and improved digestive tolerance [48–50]. However, a pilot study in patients with irritable bowel syndrome (IBS) did not confirm this [51].

Currently, the impact of fully characterized breads made with different wheat species and processing systems, and their effects on symptoms in NCWS has not been 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 2 parallel studies. Second, we investigated their effects on individual GI and extraintestinal symptoms. We hypothesized that consumption of YF and SF bread made from emmer would cause less symptoms than bread wheat and spelt.

Methods

Two parallel, randomized, double-blind, crossover, multicenter studies were conducted at Maastricht University and Wageningen University & Research, both in the Netherlands. Participants were recruited between September 11, 2020, and November 4, 2022, and measurements were completed on November 29, 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 & Research, and were 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 y who experience self-reported GI symptoms within 12 h after a single intake of bread, that is, 1–2 slices of bread (NCWS) were included. Medication had to be stable for $\geq$1 mo 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 >6 mo 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 $\geq$6 wk, an additional visit was scheduled for serological testing to rule out CD by total immunoglobulin A (IgA) and anti-tissue transglutaminase IgA. Furthermore, use of antibiotics, probiotics or prebiotics, participation in other studies 14 d before and during the study, excessive use of alcohol (>15 standard serving quantity per week) or any use of illicit drugs, and intentional weight loss during the study period were not allowed. Females 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," that is, to replace or avoid food products that they considered 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 $\geq$1 wk before the screening visit, overall GI symptoms had to be minimal, that is, $\leq$30 mm on a 100 mm visual analogue scale (VAS) [52]. The individual's symptom-free diet was maintained throughout the study period.

Medical history and Rome IV criteria for IBS [53] and functional dyspepsia (FD) [54] were assessed by the researcher during the screening visit. Smoking behavior (current, former, or nonsmoker) and alcohol intake were self-reported using predefined categories (none, <1 unit/wk, 1–5 units/wk, or 8–15 units/wk). Height and weight were self-reported or measured at the screening visit if unknown, and used to

calculate BMI. After inclusion into the study, but prior to starting the study period, participants completed the Generalized Anxiety Disorder assessment [55], Patient Health Questionnaire-9 [56], and the Patient Health Questionnaire-15 [57] to assess anxiety, depression, and somatic symptoms, respectively.

Study design

Two parallel, randomized, double-blind, crossover, multicenter studies were conducted (Figure 1). 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 5 slices (125–150 g in total) of these breads in a randomized order on 3 separate test days.

Randomization and blinding

The randomization list was generated by a colleague unconnected with the trial using a publicly available procedure (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Separate lists were made for studies A and B. Per study, the randomization list ensured an equal number of participants per treatment order (that is, randomized order of bread wheat, spelt, and emmer). The colleague provided the researcher with a randomization number, which corresponded with the labeled packages of the study breads.

Frozen packages of bread portions per test day (5 slices) were provided in sealed nontransparent plastic sachets so participants could not compare the appearance of the study breads. The sachets were labeled with a randomization number and a test day number according to the randomization list.

Participants were unaware of the different bread types under investigation, and the researchers were blinded to the randomization order. Data analysis was executed before unblinding of the researcher.

Study period

Participants received all 3 study breads (either YF or SF) at the end of the screening visit. As the full test period was completed at home, the order of consumption was indicated on the package (that is, test day 1, 2, or 3). Participants were instructed to consume the breads for breakfast and lunch, with the choice of consuming 2–3 slices per mealtime. The chosen quantity per mealtime was repeated on all subsequent test days. Each test day was preceded by a 3-d run-in period and separated by a wash-out period of ≥7 d (Figure 1). Participants received a reminder via a text message on the evening before each run-in period. For females, run-in periods and test days were not scheduled during the menses phase of their menstrual cycle, for which the wash-out period was prolonged if necessary.

On the evening of each test day and during the 3 run-in days, participants completed symptom diaries for GI and extraintestinal symptoms, and the Bristol Stool Scale [58] 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 individual's symptom-free diet, and, combined with photos of the study breads sent on the test day, to assess compliance to the intervention.

Because of limited shelf life of the study breads, study A was completed before starting study B. Hence, participants who completed study A could thereafter also participate in study B.

Study bread

All study breads were manufactured by the Dutch Bakery Center. Bread wheat (*Triticum aestivum* spp. *Aestivum*), spelt (*Triticum aestivum* ssp. *Spelta*), and emmer (*Tritordeum turgidum* var. *dicoccum*) were obtained from commercial growers. 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 the standard commercial bread baking process, with minor adjustments to the addition of water and yeast to obtain uniform-looking breads. For the SF breads, the commercial sourdough starter culture "Mailander Le Chef" (Böcker) 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. [59]. More details about baking procedures, and the analysis of the bread composition are included in the Supplementary Materials (Supplemental Tables 1–4 and Supplemental Figure 1), with a description of the comparison included in the Supplementary Results ("Comparing nutrient composition of the different bread types").

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. Secondary, the effects of these breads on individual GI symptoms (that is, abdominal discomfort, abdominal pain, belching, bloating, constipation, diarrhea, flatulence, fullness, nausea, urge to empty bowel) and extraintestinal symptoms (that is, confusion, headache, joint pains, loss of coordination, skin rash, tiredness) were investigated. All symptom scores were measured on a 100 mm VAS as part of the symptom diary.

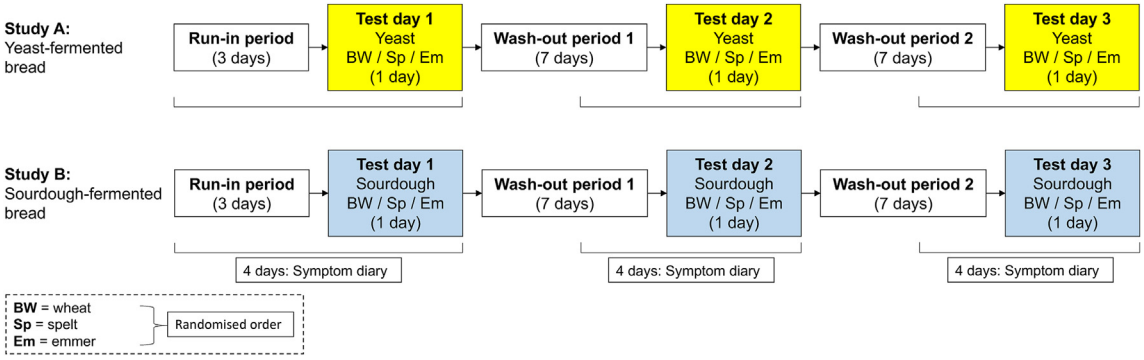

FIGURE 1. Study design.

Statistical analysis

Sample size was calculated using G*power version 3.1 (Heinrich Heine Universität). On the basis of a study by Biesiekierski et al. [21], a mean difference in VAS of 10.3 mm with SD of 12.8 mm 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 by the Medical Ethics Committee to include 2 extra participants per study if necessary.

Statistical analyses were conducted using IBM SPSS statistics version 26.0 (IBM Corp.) and figures were drawn using GraphPad Prism version 10.1.1 (GraphPad Software). Studies A and B were analyzed 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 IQR for nonnormal distributed continuous variables, and as frequencies with percentages for categorical variables.

To assess primary and secondary outcomes, delta VAS symptom scores (ΔVAS) were calculated per symptom for each bread as [score test day] – [average of 3-d run-in period], where the average of the 3-d run-in period was used as baseline. The ΔVAS per symptom was compared between breads using the nonparametric Friedman test, with the 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-d run-in period were compared with check for carry-over effects, and the ΔVAS of each test day to check for an order effect, both using the Friedman test with the post hoc Wilcoxon test.

Because of the large variation observed for each test day, in a post hoc analysis, responders and nonresponders were further explored. Responders were defined as participants with an increase of ≥15 mm on ΔVAS for overall GI symptoms and/or for predominant symptoms abdominal discomfort, abdominal pain, bloating, or flatulence [21,51,60]. The number of responders for each bread was compared by Cochran's Q test with the post hoc McNemar test.

Exploratively, the effects of dough processing using either YF or SF were assessed in the subgroup of participants that completed both studies 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

Study A was completed between September 11, 2020, and April 20, 2022. 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 self-reported not to result from bread ($n = 7$), 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 (Figure 2).

In study A, mean age was 42.8 ± 12.8 y, mean 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 <1 unit (that is, 1 standard serving quantity) (35%) or 1–5 units/wk (40%). Participants had been experiencing symptoms related to bread for 9.0 [IQR 3.5–28.0] y. Fifteen percent (3/20 participants) met de Rome IV criteria for IBS, and 5% (1/20) for FD. Full details are given in Table 1 [61] and Supplemental Table 5.

No carry-over effect or order-effect was found for any of the symptoms (for all symptoms $P > 0.05$) (Supplemental Figures 2 and 3).

Overall GI symptoms (Figure 3A) were comparable between YF breads made of bread wheat [median ΔVAS 5.7 mm (IQR 0–17.8 mm)],

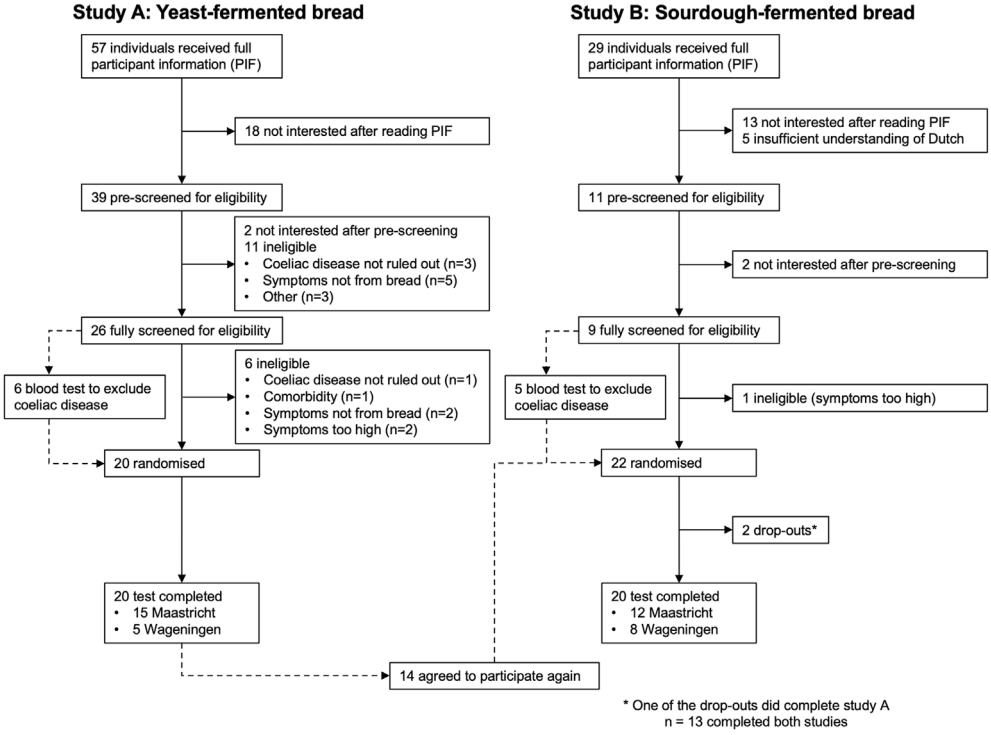

FIGURE 2. Flowchart of recruitment and inclusion.

TABLE 1
Baseline characteristics

	Study A ($n = 20$)[1]	Study B ($n = 20$)
Female	15 (75%)	18 (85%)
Age (y)	42.8 ± 12.8	41.9 ± 12.9
BMI[2] (kg/m^2)	25.6 ± 3.7	25.1 ± 4.8
Smoking		
Never	17 (85%)	16 (80%)
Current smoker	0 (0%)	0 (0%)
Quit smoking	3 (15%)	4 (20%)
Alcohol intake[3]		
None	4 (20%)	3 (15%)
<1 unit/wk	7 (35%)	7 (35%)
1–5 units/wk	8 (40%)	8 (40%)
6–7 units/wk	1 (5%)	1 (5%)
8–15 units/wk	0 (0%)	1 (5%)
Education level[4]		
Low	1 (5%)	1 (5%)
Middle	4 (20%)	4 (20%)
High	15 (75%)	15 (75%)
Start of bread-related symptoms (number of years ago)		
Gastrointestinal[5]	9.0 (3.5–28.0)	9.5 (5.0–23.5)
Extraintestinal[6]	18.0 (8.25–40.0)	11.0 (8.5–47.5)
Irritable bowel syndrome (Rome IV)	3 (15%)	3 (15%)
IBS-C	• 1 (5%)	• 1 (5%)
IBS-D	• 1 (5%)	• 0 (0%)
IBS-M	• 0 (0%)	• 0 (0%)
IBS-U	• 1 (5%)	• 2 (10%)
Functional dyspepsia (Rome IV)	1 (5%)	2 (10%)
Postprandial distress	• 0 (0%)	• 1 (5%)
Epigastric pain	• 0 (0%)	• 1 (5%)
Overlap syndrome	• 1 (5%)	• 0 (0%)
Anxiety (GAD-7)	0.0 (0.0–1.8)	1.0 (0.0–1.8)
Yes, anxiety (≥10)	• 0 (0%)	• 0 (0%)
Depression (PHQ-9)	1.0 (0.0–3.8)	1.0 (0.0–2.0)
Yes, depression (≥10)	• 1 (5%)	• 1 (5%)
Somatization (PHQ-15)	4.8 ± 3.4	4.9 ± 2.5
Minimal (<5)	• 9 (45%)	• 9 (45%)
Low (5–9)	• 9 (45%)	• 11 (55%)
Medium (10–14)	• 2 (10%)	• 0 (0%)
High (15+)	• 0 (0%)	• 0 (0%)

Continuous variables are displayed as mean ± SD for normally distributed data and as median (IQR) for non-normal distributed data. Categorical variables are displayed as number (percentage).
Abbreviations: FD, functional dyspepsia; GAD-7, Generalized Anxiety Disorder; IBS, irritable bowel syndrome; IBS-C, constipation predominant IBS; IBS-D, diarrhea predominant IBS; IBS-M, mixed stool pattern IBS; IBS-U, unspecified subtype IBS; PHQ-9, Patient Health Questionnaire-9; PHQ-15, Patient Health Questionnaire-15.
[1] Thirteen participants from study A also completed study B.
[2] BMI was calculated based on self-reported weight and height. If unknown, weight and height were measured during the screening visit.
[3] Alcohol use was classified in these predefined categories according to the average number of units (1 unit = 1 standard serving quantity) per week.
[4] Education level was categorized according to the Dutch education system [61].
[5] $n = 17$ for study A, because the other 3 participants could not recollect how long they had already experienced symptoms.
[6] $n = 8$ for study A and $n = 5$ for study B, because the other participants did not report extraintestinal symptoms after bread consumption.

spelt [median ΔVAS 0 mm (IQR −7.6 to 9.4 mm)], and emmer [median ΔVAS 1.3 mm (IQR 0–21.3 mm), $P = 0.267$]. 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 extraintestinal symptoms showed significant differences between YF breads (Figure 4).

Study B: SF breads

Study B was completed between May 3, 2022, and November 29, 2022. Fourteen participants from study A gave consent to also participate in study B. In addition, 29 new potential participants received the study information. Eleven completed the pre-screening and 9 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 2 participants dropped out after test day 1 [because of severe symptoms ($n = 1$), or found the study too time consuming ($n = 1$)].

Twenty participants completed study B (Figure 2). Of these, 18 were female (85%), mean age was 41.9 ± 12.9 y, 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/wk (40%). Participants had been experiencing symptoms related to bread for 9.5 [IQR 5.0–23.5] y. Fifteen percent (3/20 participants) met de Rome IV criteria for IBS and 10% (2/20) for FD. For full details, see Table 1 [61] and Supplemental Table 5.

No carry-over effect or order-effect was found for any of the symptoms (for all symptoms $P > 0.05$) (Supplemental Figures 4 and 5).

Overall GI symptoms (Figure 5A) were comparable between SF breads made of bread wheat [median ΔVAS 2.1 mm (IQR −3.1 to 31.5 mm)], spelt [median ΔVAS 8.5 mm (IQR 0–15.3 mm)], and emmer [median ΔVAS 0 mm (IQR −2.9 to 9.3 mm), $P = 0.144$]. 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 extraintestinal symptoms showed significant differences between SF breads (Figure 6).

Post hoc analyses

Responders compared with nonresponders

On a group level, no differences in symptom scores were found between YF breads nor between SF breads. Nevertheless, we noted a wide range in symptom scores, suggesting inter-individual variation in response. To further explore this, responders were defined as participants with an increase of ≥15 mm ΔVAS 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 (Supplemental Table 6) was comparable between YF breads made of bread wheat ($n = 6$), spelt ($n = 5$), and emmer ($n = 7$, $P = 0.761$). Seven participants (35%) responded to 1 type of bread, 4 participants (20%) to 2 types of bread, and 1 (5%) to all 3 breads (Supplemental Table 7). In total, 40% of participants were considered nonresponders.

For study B, the number of responders (Supplemental Table 8) was comparable between SF breads made of bread wheat ($n = 9$), spelt ($n = 7$), and emmer ($n = 8$, $P = 0.761$). Seven participants (35%) responded to 1 type of bread, 4 participants (20%) to 2 types of bread, and 3 (15%) to all 3 breads (Supplemental Table 9). In total, 30% of participants were considered nonresponders.

Yeast compared with 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 (Figure 2).

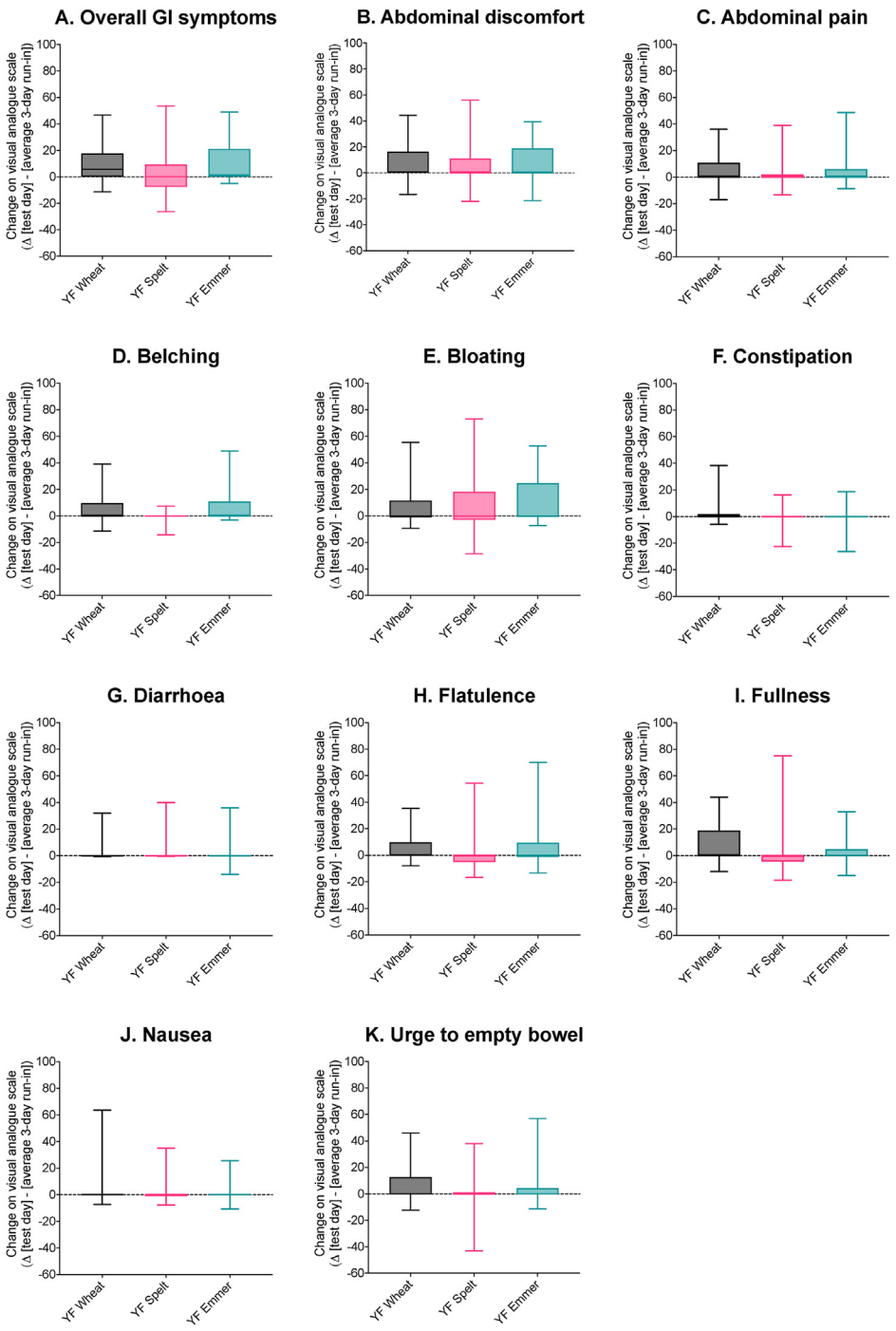

FIGURE 3. Gastrointestinal (GI) symptom scores, displayed as change on visual analogue scale (ΔVAS = [score test day] – [average of 3-d run-in period]) for yeast fermented (YF) breads made with bread wheat, spelt, or emmer (study A, $n = 20$). ΔVAS per symptom was compared between breads using the nonparametric Friedman test, with the post hoc Wilcoxon test.

Overall GI symptoms scores (Supplemental Figure 6A) were comparable between all YF and SF bread types ($P = 0.396$). None of the assessed individual GI symptoms (Supplemental Figure 6B–K) or extraintestinal symptoms (Supplemental Figure 7) showed significant differences between the 6 bread types. The number of responders (Supplemental Table 10) was comparable between all YF and SF breads ($P = 0.835$). None of the participants responded to the same combination of bread types across fermentation types (Supplemental Table 11).

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 h after bread consumption, whereas CD and WA were ruled out. When comparing the 3 wheat types, we found no differences in GI and extraintestinal symptoms between the YF or between the SF breads. On an individual level, however, we noted that more than half

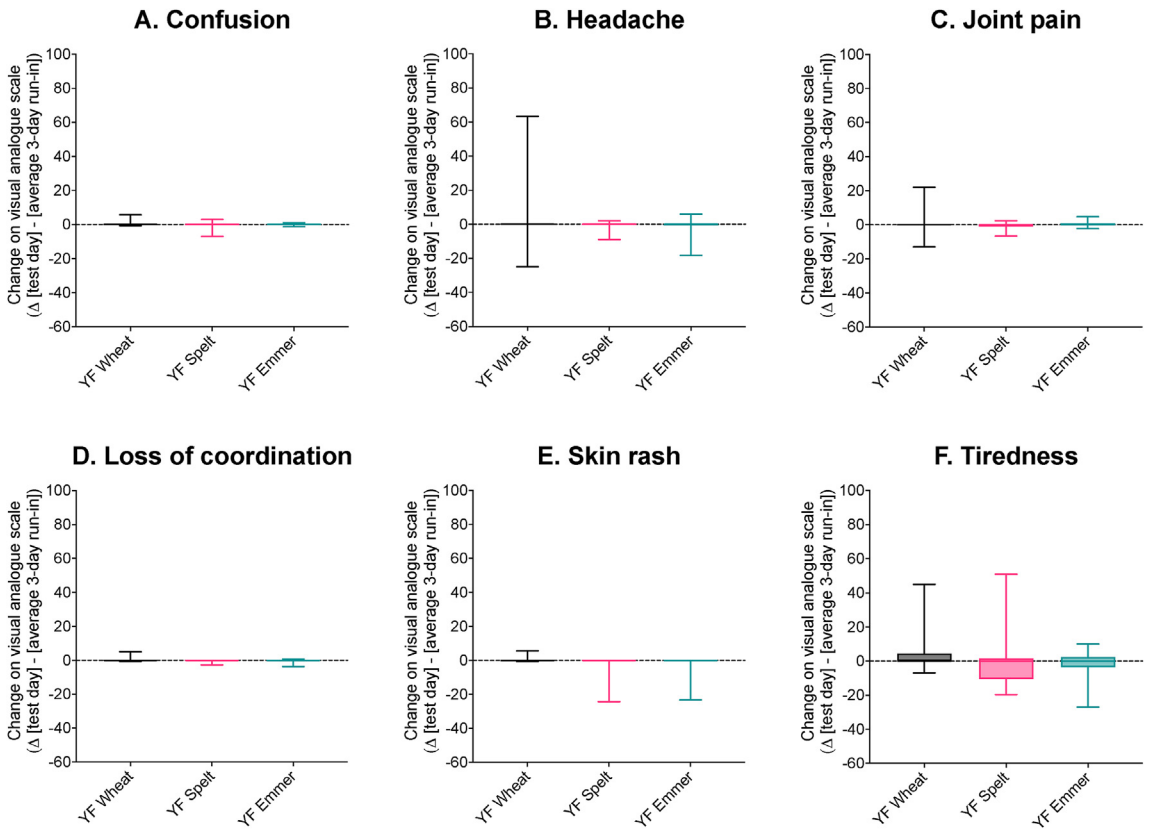

FIGURE 4. Extraintestinal symptom scores, displayed as change on visual analogue scale (ΔVAS = [score test day] – [average of 3-d run-in period]) for yeast fermented (YF) breads made with bread wheat, spelt, or emmer (study A, $n = 20$). ΔVAS per symptom was compared between breads using the nonparametric Friedman test, with the post hoc Wilcoxon test.

of the participants responded with GI symptoms to ≥1 of the breads. Because all bread types contained FODMAP, gluten, and ATIs, it was not possible to assign any of the reported symptoms to 1 of these components. Nevertheless, the number of responders did not differ between bread types.

Breads made from bread wheat, spelt, and emmer did not result in differences in GI symptoms in our study population. Although previous studies investigated the effects of gluten [20,22–26,62–66] and/or FODMAP [21,67–74] on symptoms in NCGS/NCWS, only a few studies compared breads made of different wheat species or using YF or SF. In line with our results, the only study using YF bread wheat and spelt also found no differences between bread types in NCWS individuals [40]. In contrast, a reduction of IBS symptoms was found from intake of ancient compared with modern durum wheat products [47], from tritordeum-based products compared with a habitual wheat-containing diet [75], and a tritordeum-based diet was just as effective as a low-FODMAP diet [72]. We included emmer as ancient grain in the current study. Although some differences were found in total fiber and fructans content [59], the absolute differences were rather small, and no clear benefit was found for the emmer bread. However, a comparison with our study population should be done with care, as the aforementioned studies included patients with IBS in whom CD was excluded, but not specifically characterized as NCWS [47,72,75].

Our study also showed no differences in extraintestinal symptoms between study breads. To our knowledge, this has been investigated in only 1 other study, showing a significant improvement of fatigue when eating ancient wheat products [47]. Possibly, the longer intervention (6 wk) was better suited to investigate extraintestinal symptoms, which usually have a longer time until onset [76].

The majority of previous studies on the effects of bread used different grains [77,78] or processing methods [70,79–82] to compare differences in specific compounds, usually FODMAP or gluten, as a potential trigger in NCWS. However, their joint presence in bread in varying amounts [38,44,83] hinders attributing effects of different breads to 1 specific compound. Additionally, growing conditions such as the location and soil type, environment, and agronomic practices also affect the composition of grain [84]. We therefore performed detailed analyses of our study breads [59], showing effects of wheat type and processing method. The clinical relevance of observed differences is unclear, but may contribute to the large variation between symptom responses of participants to individual breads, with no single bread causing the lowest symptoms.

Exploratively, we also compared YF and SF in a subset of participants, 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 direct comparison. Our findings are in line with a pilot study by Laatikainen et al. [51], but they did show that SF resulted in higher extraintestinal symptom scores, which they suggest may be explained by a nocebo response. The role of the nocebo effect in NCGS was recently confirmed by a randomized, double-blind, placebo-controlled, international multicenter study designed to assess the role of expectancy on adverse reactions after gluten intake [60]. As a nocebo response may induce an order effect in crossover studies, this was checked for the current study, but not found. Nevertheless, we cannot exclude any potential influence of a nocebo effect throughout the study.

There is no consensus on the definition and diagnostic criteria of NCWS as the trigger(s) remain unclear. The only diagnostic criteria so

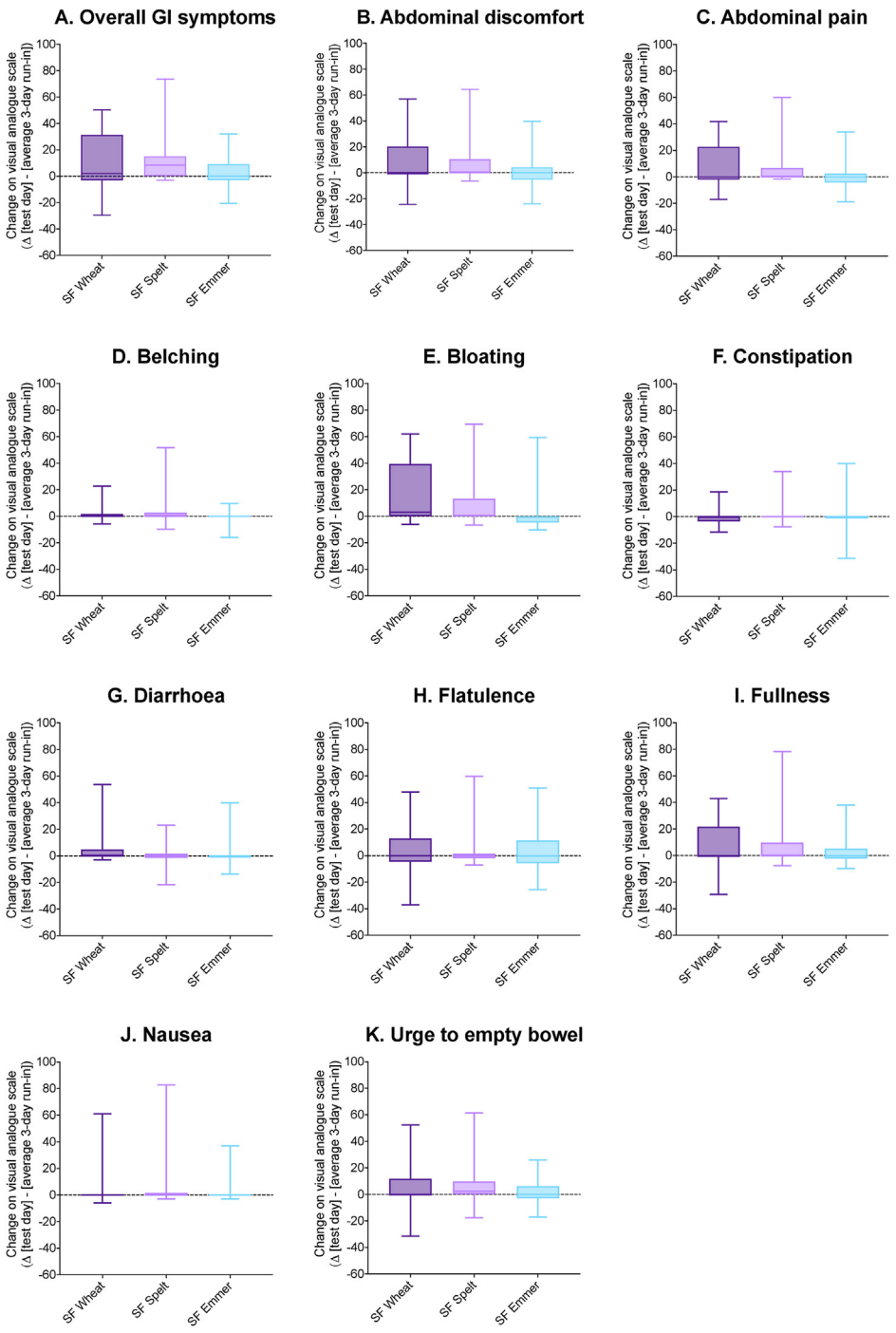

FIGURE 5. Gastrointestinal symptom scores, displayed as change on visual analogue scale (ΔVAS = [score test day] – [average of 3-d run-in period]) for sourdough fermented (SF) breads made with bread wheat, spelt or, emmer (study B, $n = 20$). ΔVAS per symptom was compared between breads using the nonparametric Friedman test, with the post hoc Wilcoxon test.

far are the Salerno experts' criteria [10], which focus on gluten and therefore may not always apply. We consider our definition of NCWS, that is, symptoms after the consumption of bread, clinically relevant in the Netherlands where bread is an important staple gluten-containing food product [85], but this may limit generalizability in other countries.

We feel that studies investigating wheat-based foods consumed "as part of a daily diet" are required to provide data that are useful for optimizing food processing, product development, and dietary recommendations. Participants consumed 5 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 have clinical relevance [85,86]. Because we wanted to compare breads that were as similar as possible to commercially available bread and mimic the real-life situation, levels of gluten, ATIs, or other components did not differ from commercially available bread. As only a few individuals responded to all different breads, this highlights the

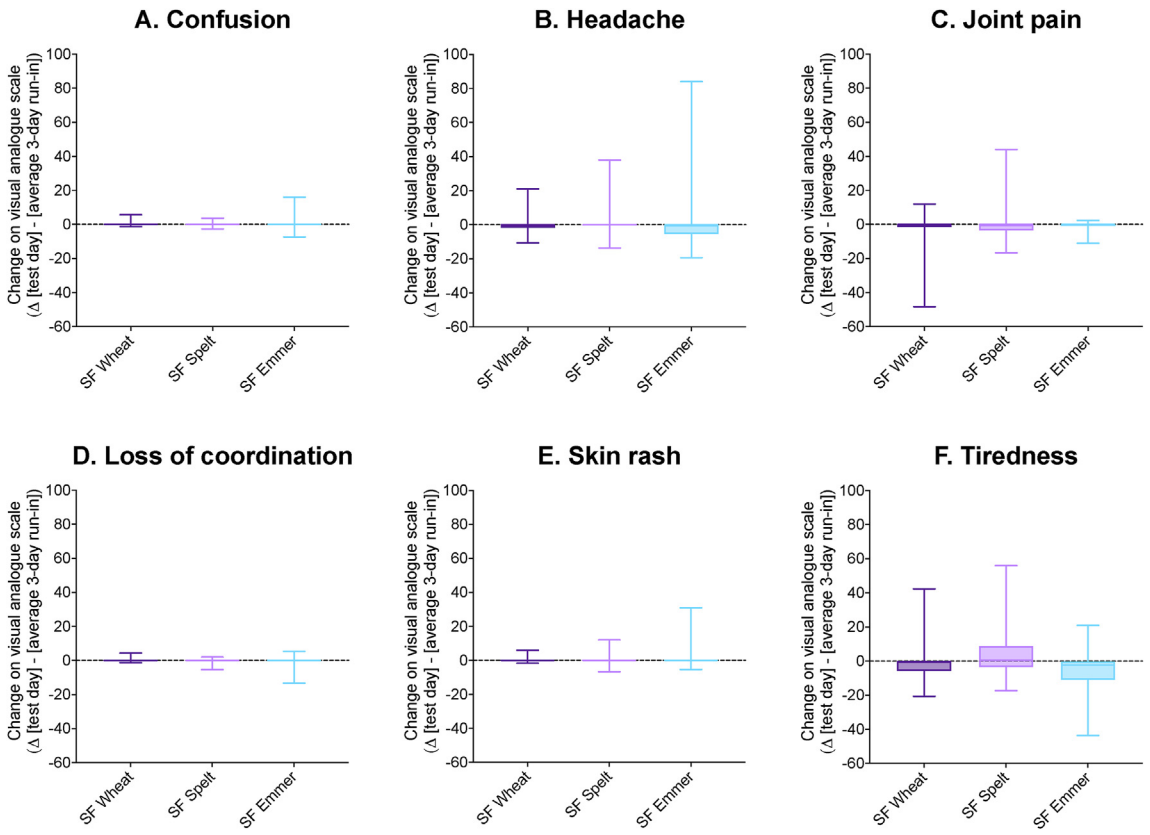

FIGURE 6. Extraintestinal symptom scores, displayed as change on visual analogue scale (ΔVAS = [score test day] – [average of 3-d run-in period]) for sourdough fermented (SF) breads made with bread wheat, spelt or emmer (study B, $n = 20$). ΔVAS per symptom was compared between breads using the nonparametric Friedman test, with the post hoc Wilcoxon test.

need for individualized dietary treatment. NCWS individuals in whom CD and WA have been excluded may benefit from trying different bread types.

We observed that there was large heterogeneity in our study population in symptom response and bread type(s) triggering symptoms, which may have contributed to no significant differences on a group level. However, a strength of the study was the crossover design comparing the effects within individuals, who themselves indicated to develop symptoms after consuming bread. The variation observed may also indicate 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 and as predominant symptoms are abdominal pain, bloating, and flatulence [19], the intestinal microbiota may be a relevant factor in symptom generation [87].

Contrary to previous studies, our intervention only consisted of 1 test day. Although we may have missed symptom responses after prolonged intake, previous studies show that most NCWS individuals report symptoms within 12 h [19]. This was also the group included in the current study. Another possible limitation of our study is the small sample size. Although this was considered sufficient based on the sample size calculation, the heterogeneity found in the symptom response may require a larger number to show differences between interventions. Furthermore, this limited the interpretation of the comparison between YF and SF breads.

With a crossover design, there is always the risk of a carry-over effect, especially with longer lasting symptoms [19]. However, symptom scores did not differ between run-in periods. Furthermore, although participants adhered to a symptom-free diet throughout the study, we found that some participants had higher symptom scores during run-in than on the test day. This may be because of the overlap with IBS and/or other factors, such as stress, that were not assessed in our study.

In conclusion, the majority of NCWS individuals experienced GI symptoms for ≥1 of the breads, but on the group level, no differences were found between different YF or SF breads. Nevertheless, these individual differences confirm the need for a personalized dietary treatment of NCWS.

Acknowledgments

Dutch Bakery Center (NBC) is a nonprofit organization subsidized by the Dutch Government (at the time of the project launch). NBC supported the project with their network in finding a qualified miller, purchase of required certified grains, storage and handling of grains, selection of appropriate sourdough cultures, and performance of required experimental baking trails to achieve acceptable test breads. Our special thanks goes to F. van Straaten, Z. Proos, P. Kuiper, and L. van Zonsbeek.

Author contributions

The authors' responsibilities were as follows – MCGdG, GvR, LJWJG, DK, FJPHB, DMAEJ: designed research; MCGdG, ET, BB: conducted research; BJMW, PJS, AHPA: provided essential materials, data, and resources; MCGdG, ET, BB, AL, AHPA: analyzed data; MCGdG, DK, FJPHB, DMAEJ: wrote the paper; MCGdG: had

primary responsibility for final content; ET, BB, GvR, BJMW, PJS, AHPA, LJWJG: reviewed and edited paper; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Funding

"Well on Wheat?" was financed by a grant from the Dutch Government "TKI – Top Knowledge Institute" and a wide range of additional partners* from the Agri-Food industry who donated unrestricted research grants. *These partners were: AB-Mauri Bakery Ingredients, Made, the Netherlands; Baking Industry Research Trust, Wellington, New Zealand; Borgesius Holding BV-Albert Heijn, Stadskanaal, the Netherlands; CSM Innovation Bakery Center, Bingen, Germany; CYMMIT, Texcoco, Mexico; DSM Food Specialties, Delft, the Netherlands; Fazer Bakeries Oy, Helsinki, Finland; Health Grain Forum, Vienna, Austria; ICC-Intl., Vienna, Austria; IWGA, Kansas 66210, USA; Lantmännen EK, Stockholm, Sweden; Mondelez, Saclay, France; Nederlands Bakkerij Centrum, Wageningen, the Netherlands; Nutrition et Sante, Revel, France; Puratos BV, Groot Bijgaarden, Belgium; Rademaker BV-Bakery equipment, Culemborg, the Netherlands; Sonneveld Group BV, Papendrecht, the Netherlands; Zeelandia-HJ Doeleman BV, Zierikzee, the Netherlands. The project was coordinated and executed by an academic research consortium team (ARCT). Research specialists from funding partners were entitled to ask questions and give suggestions to ARCT for consideration during biannual progress meetings. Decisions on study set-up, execution, and data interpretation of data were exclusively taken by ARCT. Scientific output communications were exclusively organized by ARCT without involvement of funding partners.

Data availability

Data described in the manuscript, code book, and analytical code will be made available upon request pending a methodologically sound proposal, and in line with EU regulations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.02.008.

References

[1] P.R. Shewry, S.J. Hey, The contribution of wheat to human diet and health, Food Energy Secur 4 (3) (2015) 178–202.
[2] P.L. Weegels, The future of bread in view of its contribution to nutrient intake as a starchy staple food, Plant Foods Hum. Nutr. 74 (1) (2019) 1–9.
[3] E.M. Barrett, M.J. Batterham, S. Ray, E.J. Beck, Whole grain, bran and cereal fibre consumption and CVD: a systematic review, Br. J. Nutr. 121 (8) (2019) 914–937.
[4] G.A. Gaesser, Whole grains, refined grains, and cancer risk: a systematic review of meta-analyses of observational studies, Nutrients 12 (12) (2020) 3756.
[5] M. Tieri, F. Ghelfi, M. Vitale, C. Vetrani, S. Marventano, A. Lafranconi, et al., Whole grain consumption and human health: an umbrella review of observational studies, Int. J. Food Sci. Nutr. 71 (6) (2020) 668–677.
[6] L.M. Sanders, Y. Zhu, M.L. Wilcox, K. Koecher, K.C. Maki, Effects of whole grain intake, compared with refined grain, on appetite and energy intake: a systematic review and meta-analysis, Adv. Nutr. 12 (4) (2021) 1177–1195.
[7] J.F. Ludvigsson, D.A. Leffler, J.C. Bai, F. Biagi, A. Fasano, P.H. Green, et al., The Oslo definitions for coeliac disease and related terms, Gut 62 (1) (2013) 43–52.
[8] A. Cianferoni, Wheat allergy: diagnosis and management, J. Asthma Allergy. 9 (2016) 13–25.
[9] P. Singh, A. Arora, T.A. Strand, D.A. Leffler, C. Catassi, P.H. Green, et al., Global prevalence of celiac disease: systematic review and meta-analysis, Clin. Gastroenterol. Hepatol. 16 (6) (2018) 823–836.e2.
[10] C. Catassi, L. Elli, B. Bonaz, G. Bouma, A. Carroccio, G. Castillejo, et al., Diagnosis of non-celiac gluten sensitivity (NCGS): the Salerno experts' criteria, Nutrients 7 (6) (2015) 4966–4977.
[11] F. Brouns, G. van Rooy, P. Shewry, S. Rustgi, D. Jonkers, Adverse reactions to wheat or wheat components, Compr. Rev. Food Sci. Food Saf. 18 (5) (2019) 1437–1452.
[12] C. Catassi, G. Catassi, L. Naspi, Nonceliac gluten sensitivity, Curr. Opin. Clin. Nutr. Metab Care. 26 (2023) 490–494.
[13] A. Carroccio, O. Giambalvo, F. Blasca, R. Iacobucci, A. D'Alcamo, P. Mansueto, Self-reported non-celiac wheat sensitivity in high school students: demographic and clinical characteristics, Nutrients 9 (7) (2017) 771.
[14] M.D.E. Potter, M.M. Walker, M.P. Jones, N.A. Koloski, S. Keely, N.J. Talley, Wheat intolerance and chronic gastrointestinal symptoms in an Australian population-based study: association between wheat sensitivity, celiac disease and functional gastrointestinal disorders, Am. J. Gastroenterol. 113 (7) (2018) 1036–1044.
[15] M. Potter, M.P. Jones, M.M. Walker, N.A. Koloski, S. Keely, G. Holtmann, et al., Incidence and prevalence of self-reported non-coeliac wheat sensitivity and gluten avoidance in Australia, Med. J. Aust. 212 (3) (2020) 126–131.
[16] P. Mansueto, A. D'Alcamo, A. Seidita, A. Carroccio, Food allergy in irritable bowel syndrome: the case of non-celiac wheat sensitivity, World J. Gastroenterol. 21 (23) (2015) 7089–7109.
[17] D. Schuppan, G. Pickert, M. Ashfaq-Khan, V. Zevallos, Non-celiac wheat sensitivity: differential diagnosis, triggers and implications, Best. Pract. Res. Clin. Gastroenterol. 29 (3) (2015) 469–476.
[18] R. De Giorgio, U. Volta, P.R. Gibson, Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? Gut 65 (1) (2016) 169–178.
[19] T. van Gils, P. Nijeboer, C.E. IJssennagger, D.S. Sanders, C.J. Mulder, G. Bouma, Prevalence and characterization of self-reported gluten sensitivity in The Netherlands, Nutrients 8 (11) (2016) 714.
[20] J.R. Biesiekierski, E.D. Newnham, P.M. Irving, J.S. Barrett, M. Haines, J.D. Doecke, et al., Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial, Am. J. Gastroenterol. 106 (3) (2011) 508–514, quiz 15.
[21] J.R. Biesiekierski, S.L. Peters, E.D. Newnham, O. Rosella, J.G. Muir, P.R. Gibson, No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates, Gastroenterology 145 (2) (2013) 320–328.e1–3.
[22] S.L. Peters, J.R. Biesiekierski, G.W. Yelland, J.G. Muir, P.R. Gibson, Randomised clinical trial: gluten may cause depression in subjects with non-coeliac gluten sensitivity – an exploratory clinical study, Aliment. Pharmacol. Ther. 39 (10) (2014) 1104–1112.
[23] A. Di Sabatino, U. Volta, C. Salvatore, P. Biancheri, G. Caio, R. De Giorgio, et al., Small amounts of gluten in subjects with suspected nonceliac gluten sensitivity: a randomized, double-blind, placebo-controlled, cross-over trial, Clin. Gastroenterol. Hepatol. 13 (9) (2015) 1604–1612.e3.
[24] L. Elli, C. Tomba, F. Branchi, L. Roncoroni, V. Lombardo, M.T. Bardella, et al., Evidence for the presence of non-celiac gluten sensitivity in patients with functional gastrointestinal symptoms: results from a multicenter randomized double-blind placebo-controlled gluten challenge, Nutrients 8 (2) (2016) 84.
[25] H.F. Dale, J.G. Hatlebakk, N. Hovdenak, S.O. Ystad, G.A. Lied, The effect of a controlled gluten challenge in a group of patients with suspected non-coeliac gluten sensitivity: a randomized, double-blind placebo-controlled challenge, Neurogastroenterol. Motil. (2018).
[26] S.M. Moleski, A. Shah, P. Durney, M. Matthews, G. Kaushal, C. Smith, et al., Symptoms of gluten ingestion in patients with non-celiac gluten sensitivity: a randomized clinical trial, Nutrition 81 (2021) 110944.
[27] J.P. Algera, M.K. Magnusson, L. Öhman, S. Störsrud, M. Simrén, H. Törnblom, Randomised controlled trial: effects of gluten-free diet on symptoms and the gut microenvironment in irritable bowel syndrome, Aliment. Pharmacol. Ther. 56 (9) (2022) 1318–1327.
[28] C. Crawley, N. Savino, C. Halby, S.D. Sander, A.N. Andersen, M. Arumugam, et al., The effect of gluten in adolescents and young adults with gastrointestinal symptoms: a blinded randomised cross-over trial, Aliment. Pharmacol. Ther. 55 (9) (2022) 1116–1127.
[29] S. Geisslitz, P. Shewry, F. Brouns, A.H.P. America, G.P.I. Caio, M. Daly, et al., Wheat ATIs: characteristics and role in human disease, Front Nutr 8 (2021) 667370.
[30] Y. Junker, S. Zeissig, S.J. Kim, D. Barisani, H. Wieser, D.A. Leffler, et al., Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4, J. Exp. Med. 209 (13) (2012) 2395–2408.

[31] M. Cuccioloni, M. Mozzicafreddo, L. Bonfili, V. Cecarini, M. Giangrossi, M. Falconi, et al., Interfering with the high-affinity interaction between wheat amylase trypsin inhibitor CM3 and toll-like receptor 4: in silico and biosensor-based studies, Sci. Rep. 7 (1) (2017) 13169.
[32] A. Caminero, J.L. McCarville, V.F. Zevallos, M. Pigrau, X.B. Yu, J. Jury, et al., Lactobacilli degrade wheat amylase trypsin inhibitors to reduce intestinal dysfunction induced by immunogenic wheat proteins, Gastroenterology 156 (8) (2019) 2266–2280.
[33] G. Pickert, S. Wirtz, J. Matzner, M. Ashfaq-Khan, R. Heck, S. Rosigkeit, et al., Wheat consumption aggravates colitis in mice via amylase trypsin inhibitor-mediated dysbiosis, Gastroenterology 159 (2020) 257–272.e17.
[34] X. Huang, D. Schuppan, L.E. Rojas Tovar, V.F. Zevallos, J. Loponen, M. Gänzle, Sourdough fermentation degrades wheat alpha-amylase/trypsin inhibitor (ATI) and reduces pro-inflammatory activity, Foods 9 (7) (2020) 943.
[35] G. Iacomino, V. Rotondi Aufiero, L. Di Stasio, S. Picascia, N. Iannaccone, N. Giardullo, et al., Triticum monococcum amylase trypsin inhibitors possess a reduced potential to elicit innate immune response in celiac patients compared to Triticum aestivum, Food Res. Int. 145 (2021) 110386.
[36] P.R. Gibson, S.J. Shepherd, Food choice as a key management strategy for functional gastrointestinal symptoms, Am. J. Gastroenterol. 107 (5) (2012) 657–666, quiz 67.
[37] W.S. Nanayakkara, P.M. Skidmore, L. O'Brien, T.J. Wilkinson, R.B. Gearry, Efficacy of the low FODMAP diet for treating irritable bowel syndrome: the evidence to date, Clin. Exp. Gastroenterol. 9 (2016) 131–142.
[38] J.U. Ziegler, D. Steiner, C.F.H. Longin, T. Würschum, R.M. Schweiggert, R. Carle, Wheat and the irritable bowel syndrome – FODMAP levels of modern and ancient species and their retention during bread making, J. Funct. Foods. 25 (2016) 257–266.
[39] M. Schmidt, E. Sciurba, Determination of FODMAP contents of common wheat and rye breads and the effects of processing on the final contents, Eur. Food Res. Technol. 247 (2) (2021) 395–410.
[40] J. Zimmermann, F.H. Longin, A. Schweinlin, M. Basrai, S.C. Bischoff, No difference in tolerance between wheat and spelt bread in patients with suspected non-celiac wheat sensitivity, Nutrients 14 (14) (2022) 2800.
[41] J. Dvorak, K.R. Deal, M.C. Luo, F.M. You, K. von Borstel, H. Dehghani, The origin of spelt and free-threshing hexaploid wheat, J. Hered. 103 (3) (2012) 426–441.
[42] L.J.W.J. Gilissen, M.J.M. Smulders, Chapter 6 - Gluten quantity and quality in wheat and in wheat-derived products, in: M. Rossi (Ed.), Biotechnological Strategies for the Treatment of Gluten Intolerance, Academic Press, 2021, pp. 97–129.
[43] F. Brouns, S. Geisslitz, C. Guzman, T.M. Ikeda, A. Arzani, G. Latella, et al., Do ancient wheats contain less gluten than modern bread wheat, in favour of better health? Nutr. Bull. 47 (2) (2022) 157–167.
[44] S. Geisslitz, C.F.H. Longin, K.A. Scherf, P. Koehler, Comparative study on gluten protein composition of ancient (Einkorn, Emmer and Spelt) and modern wheat species (Durum and Common Wheat), Foods 8 (9) (2019) 409.
[45] T. Šuligoj, A. Gregorini, M. Colomba, H.J. Ellis, P.J. Ciclitira, Evaluation of the safety of ancient strains of wheat in coeliac disease reveals heterogeneous small intestinal T cell responses suggestive of coeliac toxicity, Clin. Nutr. 32 (6) (2013) 1043–1049.
[46] T. Asledottir, R. Rehman, G. Mamone, G. Picariello, T.G. Devold, G.E. Vegarud, et al., Ancestral wheat types release fewer celiac disease related T cell epitopes than common wheat upon ex vivo human gastrointestinal digestion, Foods 9 (9) (2020) 1173.
[47] F. Sofi, A. Whittaker, A.M. Gori, F. Cesari, E. Surrenti, R. Abbate, et al., Effect of Triticum turgidum subsp. turanicum wheat on irritable bowel syndrome: a double-blinded randomised dietary intervention trial, Br. J. Nutr. 111 (11) (2014) 1992–1999.
[48] J. Loponen, M.G. Gänzle, Use of sourdough in low FODMAP baking, Foods 7 (7) (2018) 96.
[49] L. Ribet, R. Dessalles, C. Lesens, N. Brusselaers, M. Durand-Dubief, Nutritional benefits of sourdoughs: a systematic review, Adv. Nutr. 14 (1) (2023) 22–29.
[50] V. D'Amico, M. Gänzle, L. Call, B. Zwirzitz, H. Grausgruber, S. D'Amico, et al., Does sourdough bread provide clinically relevant health benefits? Front. Nutr. 10 (2023).
[51] R. Laatikainen, J. Koskenpato, S.M. Hongisto, J. Loponen, T. Poussa, X. Huang, et al., Pilot study: comparison of sourdough wheat bread and yeast-fermented wheat bread in individuals with wheat sensitivity and irritable bowel syndrome, Nutrients 9 (11) (2017).
[52] D.A. Delgado, B.S. Lambert, N. Boutris, P.C. McCulloch, A.B. Robbins, M.R. Moreno, et al., Validation of digital visual analog scale pain scoring with a traditional paper-based visual analog scale in adults, J. Am. Acad. Orthop. Surg. Glob. Res. Rev. 2 (3) (2018) e088.
[53] F. Mearin, B.E. Lacy, L. Chang, W.D. Chey, A.J. Lembo, M. Simren, et al., Bowel disorders, Gastroenterology, 2016.
[54] V. Stanghellini, F.K. Chan, W.L. Hasler, J.R. Malagelada, H. Suzuki, J. Tack, et al., Gastroduodenal disorders, Gastroenterology 150 (6) (2016) 1380–1392.
[55] R.L. Spitzer, K. Kroenke, J.B. Williams, B. Lowe, A brief measure for assessing generalized anxiety disorder: the GAD-7, Arch. Intern. Med. 166 (10) (2006) 1092–1097.
[56] L. Stafford, M. Berk, H.J. Jackson, Validity of the Hospital Anxiety and Depression Scale and Patient Health Questionnaire-9 to screen for depression in patients with coronary artery disease, Gen. Hosp. Psychiatry. 29 (5) (2007) 417–424.
[57] K. Kroenke, R.L. Spitzer, J.B. Williams, The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms, Psychosom. Med. 64 (2) (2002) 258–266.
[58] L.J. O'Donnell, J. Virjee, K.W. Heaton, Detection of pseudodiarrhoea by simple clinical assessment of intestinal transit rate, BMJ 300 (6722) (1990) 439–440.
[59] P.R. Shewry, A.H.P. America, A. Lovegrove, A.J. Wood, A. Plummer, J. Evans, et al., Comparative compositions of metabolites and dietary fibre components in doughs and breads produced from bread wheat, emmer and spelt and using yeast and sourdough processes, Food Chem 374 (2022) 131710.
[60] M.C.G. de Graaf, C.L. Lawton, F. Croden, A. Smolinska, B. Winkens, M.A.M. Hesselink, et al., The effect of expectancy versus actual gluten intake on gastrointestinal and extra-intestinal symptoms in non-coeliac gluten sensitivity: a randomised, double-blind, placebo-controlled, international, multicentre study, Lancet Gastroenterol. Hepatol. 9 (2) (2024) P110–P123.
[61] Centraal Bureau voor de Statistiek [Internet], Opleidingsniveau (2023) [August 2023]. Available from: https://www.cbs.nl/nl-nl/nieuws/2019/33/verschil-levensverwachting-hoog-en-laagopgeleid-groeit/opleidingsniveau.
[62] A.K. Cartee, R.S. Choung, K.S. King, S. Wang, J.L. Dzuris, R.P. Anderson, et al., Plasma IL-2 and symptoms response after acute gluten exposure in subjects with celiac disease or nonceliac gluten sensitivity, Am. J. Gastroenterol. 117 (2) (2022) 319–326.
[63] A. Picarelli, R. Borghini, M. Di Tola, M. Marino, C. Urciuoli, C. Isonne, et al., Intestinal, systemic, and oral gluten-related alterations in patients with nonceliac gluten sensitivity, J. Clin. Gastroenterol. 50 (10) (2016) 849–858.
[64] V. Ponzo, I. Ferrocino, I. Goitre, M. Pellegrini, M. Bruno, M. Astegiano, et al., Non-celiac gluten/wheat sensitivity: clinical characteristics and microbiota and mycobiota composition by response to the gluten challenge test, Nutrients 13 (4) (2021) 1260.
[65] L. Roncoroni, K.A. Bascuñán, M. Vecchi, L. Doneda, M.T. Bardella, V. Lombardo, et al., Exposure to different amounts of dietary gluten in patients with non-celiac gluten sensitivity (NCGS): an exploratory study, Nutrients 11 (1) (2019) 136.
[66] B. Zanini, R. Baschè, A. Ferraresi, C. Ricci, F. Lanzarotto, M. Marullo, et al., Randomised clinical study: gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-coeliac gluten sensitivity, Aliment. Pharmacol. Ther. 42 (8) (2015) 968–976.
[67] M. Ajamian, G. Rosella, E.D. Newnham, J.R. Biesiekierski, J.G. Muir, P.R. Gibson, Effect of gluten ingestion and FODMAP restriction on intestinal epithelial integrity in patients with irritable bowel syndrome and self-reported non-coeliac gluten sensitivity, Mol. Nutr. Food Res. (2021) e1901275.
[68] M. Barone, E. Gemello, M.T. Viggiani, F. Cristofori, C. Renna, A. Iannone, et al., Evaluation of non-celiac gluten sensitivity in patients with previous diagnosis of irritable bowel syndrome: a randomized double-blind placebo-controlled crossover trial, Nutrients 12 (3) (2020) 705.
[69] W. Dieterich, D. Schuppan, M. Schink, R. Schwappacher, S. Wirtz, A. Agaimy, et al., Influence of low FODMAP and gluten-free diets on disease activity and intestinal microbiota in patients with non-celiac gluten sensitivity, Clin. Nutr. 38 (2) (2019) 697–707.
[70] R. Laatikainen, J. Koskenpato, S.M. Hongisto, J. Loponen, T. Poussa, M. Hillila, et al., Randomised clinical trial: low-FODMAP rye bread vs. regular rye bread to relieve the symptoms of irritable bowel syndrome, Aliment. Pharmacol. Ther. 44 (5) (2016) 460–470.
[71] E. Nordin, C. Brunius, R. Landberg, P.M. Hellström, Fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs), but not gluten, elicit modest symptoms of irritable bowel syndrome: a double-blind, placebo-controlled, randomized three-way crossover trial, Am. J. Clin. Nutr. 115 (2) (2022) 344–352.
[72] F. Russo, G. Riezzo, A. Orlando, M. Linsalata, B. D'Attoma, L. Prospero, et al., A comparison of the low-FODMAPs diet and a tritordeum-based diet on the gastrointestinal symptom profile of patients suffering from irritable bowel syndrome-diarrhea variant (IBS-D): a randomized controlled trial, Nutrients 14 (8) (2022) 1544.
[73] G.I. Skodje, V.K. Sarna, I.H. Minelle, K.L. Rolfsen, J.G. Muir, P.R. Gibson, et al., Fructan, rather than gluten, induces symptoms in patients with self-reported non-celiac gluten sensitivity, Gastroenterology 154 (3) (2018) 529–539.e2.

[74] G.I. Skodje, I.H. Minelle, K.L. Rolfsen, M. Iacovou, K.E.A. Lundin, M.B. Veierød, et al., Dietary and symptom assessment in adults with self-reported non-coeliac gluten sensitivity, Clin. Nutr. ESPEN. 31 (2019) 88–94.
[75] F. Russo, G. Riezzo, M. Linsalata, A. Orlando, V. Tutino, L. Prospero, et al., Managing symptom profile of IBS-D patients with tritordeum-based foods: results from a pilot study, Front. Nutr. 9 (2022) 797192.
[76] A. Carroccio, M. Soresi, M. Chiavetta, F. La Blasca, S. Compagnoni, A. Giuliano, et al., Frequency and clinical aspects of neurological and psychiatric symptoms in patients with non-celiac wheat sensitivity, Nutrients 13 (6) (2021) 1971.
[77] M. Dinu, D. Macchia, G. Pagliai, A.M. Gori, F. Cesari, R. Marcucci, et al., Symptomatic efficacy of buckwheat products in Non-Celiac Gluten Sensitivity (NCGS), Asia Pac, J. Clin. Nutr. 26 (4) (2017) 630–636.
[78] S. Sánchez-León, C. Haro, M. Villatoro, L. Vaquero, I. Comino, A.B. González-Amigo, et al., Tritordeum breads are well tolerated with preference over gluten-free breads in non-celiac wheat-sensitive patients and its consumption induce changes in gut bacteria, J. Sci. Food Agric. 101 (8) (2021) 3508–3517.
[79] R. Laatikainen, J. Jalanka, J. Loponen, S.M. Hongisto, M. Hillilä, J. Koskenpato, et al., Randomised clinical trial: effect of low-FODMAP rye bread versus regular rye bread on the intestinal microbiota of irritable bowel syndrome patients: association with individual symptom variation, BMC Nutr 5 (2019) 12.
[80] M. Calasso, R. Francavilla, F. Cristofori, M. De Angelis, M. Gobbetti, New protocol for production of reduced-gluten wheat bread and pasta and clinical effect in patients with irritable bowel syndrome: a randomised, double-blind, cross-over study, Nutrients 10 (12) (2018) 1873.
[81] C. Haro, M. Villatoro, L. Vaquero, J. Pastor, M.J. Giménez, C.V. Ozuna, et al., The dietary intervention of transgenic low-gliadin wheat bread in patients with non-celiac gluten sensitivity (NCGS) showed no differences with gluten free diet (GFD) but provides better gut microbiota profile, Nutrients 10 (12) (2018) 1964.
[82] D. Rees, G. Holtrop, G. Chope, K.M. Moar, M. Cruickshank, N. Hoggard, A randomised, double-blind, cross-over trial to evaluate bread, in which gluten has been pre-digested by prolyl endoprotease treatment, in subjects self-reporting benefits of adopting a gluten-free or low-gluten diet, Br. J. Nutr. 119 (5) (2018) 496–506.
[83] S. Geisslitz, C. Ludwig, K.A. Scherf, P. Koehler, Targeted LC-MS/MS reveals similar contents of alpha-amylase/trypsin-inhibitors as putative triggers of nonceliac gluten sensitivity in all wheat species except einkorn, J. Agric. Food Chem. 66 (46) (2018) 12395–12403.
[84] A. Lovegrove, J. Dunn, T.K. Pellny, J. Hood, A.J. Burridge, A.H.P. America, et al., Comparative compositions of grain of bread wheat, emmer and spelt grown with different levels of nitrogen fertilisation, Foods 12 (4) (2023) 843.
[85] C.T.M. van Rossum, E.J.M. Buurma-Rethans, C.S. Dinnissen, M.H. Beukers, H.A.M. Brants, A.L.M. Dekkers, et al., The diet of the Dutch – results of the Dutch National Food Consumption Survey 2019-2021 on food consumption and evaluation with dietary guidelines, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, 2023.
[86] L. Brink, A. Postma-Smeets, A. Stafleu, D. Wolvers, Richtlijnen Schijf van Vijf. Den Haag, Stichting Voedingscentrum Nederland, the Netherlands, 2020.
[87] T.S. King, M. Elia, J.O. Hunter, Abnormal colonic fermentation in irritable bowel syndrome, Lancet 352 (9135) (1998) 1187–1189.