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Wheat and the irritable bowel syndrome – FODMAP levels of modern and ancient species and their retention during bread making

Jochen U. Ziegler [a], Deborah Steiner [a], C. Friedrich H. Longin [b], Tobias Würschum [b], Ralf M. Schweiggert [a,*], Reinhold Carle [a,c]

[a] Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstraße 25, D-70599 Stuttgart, Germany

[b] State Plant Breeding Institute, University of Hohenheim, Fruwirthstraße 21, D-70599, Stuttgart, Germany

[c] Biological Science Department, Faculty of Science, King Abdulaziz University, P.O. Box 80257, Jeddah 21589, Saudi Arabia

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ABSTRACT

Dietary intake of fermentable oligo-, di- and monosaccharides, and polyols (FODMAP) has previously been shown to aggravate the symptoms of the irritable bowel syndrome (IBS), furthermore being associated with wheat sensitivity and a bread wheat-specific intolerance. FODMAP in whole grain flours and breads made of different varieties of bread wheat, spelt, durum, emmer, and einkorn were determined by high-performance anion exchange chromatography with pulsed amperometric detection. Fructans and raffinose were the only FODMAP detected in wheat flour. Total FODMAP contents ranged from 1.24 ± 0.38 to 2.01 ± 0.42 g/100 g DM in emmer and einkorn flours, respectively. During bread making, prolonging dough proofing times (>4 hours) allowed to effectively diminish FODMAP levels of the final product by up to 90%. Therefore, the applied processing method was substantially more important than the selection of the used variety in order to obtain low-FODMAP wheat bakery products, suitable for consumption by IBS patients.

1. Introduction

The irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder with a prevalence of up to 12% in the European population (Hungin, Whorwell, Tack, & Mearin, 2003; Lovell & Ford, 2012), more common in females than males (Lovell & Ford, 2012). Typical IBS symptoms are abdominal pain, bloating, and altered bowel habits combined with intermittent diarrhoea (IBS-D) or constipation (IBS-C) (Spiller et al., 2007). Therefore, IBS considerably affects the patients' quality of life and generates significant health care costs (Spiller et al., 2007). Dietary habits are believed to play a major role in its pathogenesis (El-Salhy & Gundersen, 2015; Gibson, Varney, Malakar, & Muir, 2015) and, in agreement, a diet low in specific short-chain carbohydrates has been shown to markedly alleviate IBS

* Corresponding author. Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstraße 25, D-70599 Stuttgart, Germany. Tel: +49 (0) 711 459 22995; fax: +49 (0) 711 459 24110.
E-mail address: ralf.schweiggert@uni-hohenheim.de (R.M. Schweiggert).

Abbreviations: DM, dry matter; DP, degree of polymerization; FFN, 1^F-fructofuranosylnystose; FODMAP, fermentable oligo-, di- and monosaccharides, and polyols; FW, fresh weight; HPAEC, high-performance anion exchange chromatography; IBS, irritable bowel syndrome; NCWS, non-celiac wheat sensitivity; PAD, pulsed amperometric detection

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symptoms in numerous cases (Gibson & Shepherd, 2010; Rao, Yu, & Fedewa, 2015). The presumably responsible carbohydrates are known by their acronym FODMAP, meaning fermentable oligo-, di- and monosaccharides, and polyols. The availability of low-FODMAP products may thus help to conserve or enhance health of the respective IBS patients. Due to high daily consumption of wheat in many countries worldwide, broad screenings to discover high- and low-FODMAP varieties or species as well as the development of technological strategies to reduce FODMAP levels are of particular interest for wheat and derived products, potentially allowing the production of functional, i.e. well-digestible, wheat-based foods with low FODMAP levels for IBS patients. FODMAP comprise the monosaccharide fructose, the disaccharide lactose, oligosaccharides named fructans and galactans, and sugar polyols such as sorbitol and mannitol (Gibson & Shepherd, 2010). As FODMAP are often poorly absorbed in the small intestine, they reach the colon where they may cause osmotic stress drawing water into the gut lumen. In addition, they are rapidly fermented by the colonic microbiota, releasing further osmotically active metabolites and substantial amounts of gas (Gibson & Shepherd, 2005). The resulting luminal distension has been proposed to be a potential cause of many IBS symptoms (Hayes, Fraher, & Quigley, 2014), although solid evidence from well-powered randomized and controlled clinical trials is still scarce.

The intestinal malabsorption of FODMAP is believed to be mainly related to genetic factors (Gibson & Shepherd, 2005), particularly the lack or insufficient expression of hydrolysing enzymes (oligosaccharides, lactose) and limited absorption capacity (fructose, polyols) due to ineffective or even deficient transporter proteins. In particular, fructose absorption capacity has been shown to substantially vary among individuals (Gibson, Newnham, Barrett, Shepherd, & Muir, 2007; Rumessen, 1992). Since fructose absorption is significantly enhanced in the presence of glucose due to the glucose-dependent fructose co-transporter GLUT2 (Gibson et al., 2007), only the portion of fructose ingested in excess to glucose (so-called 'excess fructose' (Gibson et al., 2007)) may reach the colon, and was therefore included in the FODMAP concept (Gibson et al., 2007; Gibson & Shepherd, 2005). While dietary uptake of excess fructose mostly results from high-fructose beverages and fruit consumption (Rumessen, 1992), major sources of fructans and galactans include legumes, Jerusalem artichoke, onion, globe artichoke, pear, and wheat (Muir et al., 2009; van Loo, Coussement, Leenheer, de Hoebregs, & Smits, 1995). The latter has been suggested to account for a major part of daily consumed FODMAP, mostly comprising fructans (Gibson & Shepherd, 2010; van Loo et al., 1995). However, fructans also possess prebiotic properties, and can provide health promoting effects for non-IBS individuals (Verspreet, Dornez, van den Ende, Delcour, & Courtin, 2015a).

Besides their relevance to IBS patients, FODMAP from wheat have also been suggested to aggravate or possibly even cause non-celiac wheat sensitivity (NCWS) (Biesiekierski et al., 2013), which is a group of various ill-defined wheat intolerances different from celiac disease and wheat allergy (Kucek, Veenstra, Amnuaycheewa, & Sorrells, 2015). Numerous individuals have reported a series of health implications after consuming bread wheat, whereas they claimed spelt products to be easily digestible (Stallknecht, Gilbertson, & Ranney, 1996). Biesiekierski et al. (2011) suggested that lower FODMAP contents in spelt products might be the reason for these observations. Gibson and Shepherd (2010) recommended consuming spelt products instead of bread wheat, although a broad database comprehensively supporting the suggested low-FODMAP character of ancient wheat species like spelt is unavailable, and thus, most urgently needed. Several studies on the effect of genetic, environmental, horticultural, and processing-related factors on fructans in wheat are available (Brandolini, Hidalgo, Plizzari, & Erba, 2011; Huynh et al., 2008; Knez, Abbott, & Stangoulis, 2014; van den Ende, 2013; Verspreet et al., 2013a, 2015a). However, further studies on broad wheat collections grown under different environments and processed under different production parameters are needed to clarify to which extent the aforementioned factors impact the FODMAP content of the final wheat product.

Therefore, the aim of the present study was to compare the FODMAP levels in flours of different modern and ancient wheat species, originating from the most important cultivated *Triticum* species bread wheat (*Triticum aestivum* L.), spelt (*T. spelta* L.), durum (*T. durum* Desf.), emmer (*T. dicoccum* Schrank), and einkorn (*T. monococcum* L.) grown at different locations. Breads prepared from bread wheat and spelt flour were investigated, since the degradation of fructans during bread making has been previously reported (Andersson, Fransson, Tietjen, & Åman, 2009; Fretzdorff & Welge, 2003a; Gélinas, McKinnon, & Gagnon, 2015; Knez et al., 2014; Nilsson, Öste, & Jägerstad, 1987; Verspreet et al., 2013b). The targeted results should improve estimates of the FODMAP intake from wheat consumption. This is critical because of the growing number of individuals who report tolerating the consumption of spelt but not of bread wheat products. Consequently, this study should show which wheat species and processing methods would be applicable for producing low-FODMAP wheat products that are harmless to IBS patients. This might help manufacturers of cereal products to develop guidelines for the production of breads for a special 'IBS-diet'. Furthermore, insights into the causal relationship of FODMAP and bread wheat–specific wheat sensitivity will be discussed.

2. Materials and methods

2.1. Solvents and reagents

Methanol, sodium hydroxide, sodium chloride, and maltose were purchased from VWR International (Leuven, Belgium). Sodium acetate, sucrose, fructose, and glucose monohydrate were obtained from Merck (Darmstadt, Germany). 1-Kestotriose (1-kestose), 1,1-kestotetraose (1-nystose), and 1,1,1-kestopentaose (1^F-fructofuranosylnystose, FFN) were from Wako Chemicals (Neuss, Germany), stachyose hydrate (from *Stachys tuberifera* Naudin), mannitol and lactose monohydrate from Sigma-Aldrich (Taufkirchen, Germany), raffinose pentahydrate from Alfa Aesar (Karlsruhe, Germany), and sorbitol from Avantor Performance Materials (Deventer, the Netherlands). The purity of all authentic reference standards was ≥ 99%, except for 1,1,1-kestopentaose (>80%). Purified water was prepared using a Sartorius arium 611 Ultrapure Water System (Sartorius, Göttingen, Germany), and used throughout the study.

2.2. Sample material and preparation

Five varieties each of bread wheat, spelt, and durum, as well as two varieties each of emmer and einkorn were grown at four sites in southern Germany (Hohenheim, Oberer Lindenhof, Seligenstadt, and Eckartsweier). A list of the used varieties is displayed in Table 1. Additional information about the sample set and growing locations are described elsewhere (Longin et al., 2016; Ziegler, Schweiggert, Würschum, Longin, & Carle, 2016). All wheat samples were produced by the State Plant Breeding Institute (University of Hohenheim, Stuttgart, Germany). After harvest in 2013, spelt, emmer, and einkorn samples were dehulled and cleaned using a laboratory seed cleaner (Samatec-Roeber, Bad Oeynhausen, Germany). Whole grains were ground to a particle size ≤ 0.5 mm with a laboratory mill ZM1 (Retsch, Haan, Germany). Dry matter content of the obtained whole grain flour was determined with an infrared moisture analyser MA 40 (Sartorius, Göttingen, Germany). Baked products were lyophilized (Lyovac GT2, Finn-Aqua Santasalo-Sohlberg, Munich, Germany), and ground with mortar and pestle to obtain a fine powder. All samples were stored at -20 °C until FODMAP analyses.

2.3. Bread making

The bread wheat variety 'Tobak' and the spelt variety 'Zollernspelz' (grown in Hohenheim) were used for baking experiments. 120 g whole grain flour, 2.2 g sodium chloride, 5 g commercial fresh baker's yeast from a local market, and 72 mL water were mixed and kneaded with a spiral hook for 5 min at 100 rpm (Model K5SS, Kitchenaid, St. Joseph, MI, USA). The resulting dough was kept in a proofing chamber for 0.5, 2, and 4 h, respectively, at 30 °C and approximately 75% humidity to avoid dehydration. After proofing, bread rolls of 50 g each were formed and leavened for an additional 30 min. Subsequently, the rolls were baked at 195 °C for 20 min applying four vapour injections in a Monsun City 680 C oven (Debag, Bautzen, Germany). The baked bread rolls were frozen at -20 °C and lyophilized prior to FODMAP analyses. Baking experiments were performed in duplicate.

2.4. FODMAP extraction

An aliquot of 0.4 g whole grain flour or lyophilized bread powder was thoroughly mixed with 1 mL methanol in order to inactivate amylase activities and prevent excessive starch hydrolysis during the extraction process. Subsequently, 20 mL water was added, and the mixture was homogenized at room temperature for 2 × 15 s using a probe sonicator (Sonopuls HD 3100, Bandelin electronic, Berlin, Germany) equipped with a microtip MS73. After centrifugation for 5 min at 1520 × *g* and 20 °C (Heraeus Labofuge 400R, Thermo Fisher Scientific, Osterode, Germany), the liquid phase was separated, and the solid residue was re-extracted with 20 mL water. The combined extracts were made up to a volume of 200 mL, and filtered through a 0.45 µm polyamide syringe filter (Chromafil AO-45/15, Macherey-

Table 1 – Saccharide concentration in whole grain flours of different varieties of bread wheat, spelt, durum, emmer, and einkorn.

Species	Variety	Concentration (g/100 g DM)[a]			
		Glucose (2)	Fructose (3)	Raffinose (5)	Total fructans (sum of DP3–15)
Bread wheat	Event	0.064 ± 0.011	0.052 ± 0.009	0.230 ± 0.025	1.383 ± 0.244
	Genius	0.080 ± 0.006	0.049 ± 0.004	0.196 ± 0.016	1.568 ± 0.187
	JB Asano	0.070 ± 0.003	0.053 ± 0.002	0.206 ± 0.032	1.575 ± 0.228
	Julius	0.082 ± 0.009	0.064 ± 0.007	0.207 ± 0.015	1.438 ± 0.180
	Tobak	0.116 ± 0.008	0.092 ± 0.009	0.229 ± 0.047	1.966 ± 0.194
	Mean	*0.082 ± 0.018*[ab]	*0.062 ± 0.016*[a]	*0.214 ± 0.014*[a]	*1.568 ± 0.204*[a]
Spelt	Badenkrone	0.077 ± 0.023	0.058 ± 0.015	0.259 ± 0.067	1.555 ± 0.195
	Divimar	0.080 ± 0.012	0.058 ± 0.008	0.273 ± 0.081	1.447 ± 0.140
	Franckenkorn	0.059 ± 0.023	0.045 ± 0.017	0.382 ± 0.134	0.968 ± 0.209
	Oberkulmer Rotkorn	0.063 ± 0.013	0.054 ± 0.017	0.287 ± 0.089	1.159 ± 0.157
	Zollernspelz	0.054 ± 0.008	0.045 ± 0.009	0.343 ± 0.072	1.158 ± 0.125
	Mean	*0.067 ± 0.010*[a]	*0.052 ± 0.006*[a]	*0.309 ± 0.046*[a]	*1.257 ± 0.213*[ab]
Durum	Auradur	0.118 ± 0.031	0.078 ± 0.020	0.250 ± 0.112	1.221 ± 0.025
	Karur	0.144 ± 0.032	0.079 ± 0.009	0.194 ± 0.096	1.273 ± 0.112
	Logidur	0.076 ± 0.012	0.055 ± 0.013	0.206 ± 0.079	0.940 ± 0.144
	Lunadur	0.136 ± 0.018	0.102 ± 0.016	0.194 ± 0.076	1.199 ± 0.121
	Wintergold	0.091 ± 0.025	0.061 ± 0.009	0.254 ± 0.065	1.212 ± 0.062
	Mean	*0.113 ± 0.023*[b]	*0.075 ± 0.016*[a]	*0.220 ± 0.027*[a]	*1.169 ± 0.117*[b]
Emmer	Osiris	0.102 ± 0.036	0.079 ± 0.025	0.301 ± 0.090	1.080 ± 0.294
	Ramses	0.073 ± 0.013	0.063 ± 0.014	0.283 ± 0.044	0.819 ± 0.190
	Mean	*0.088 ± 0.015*[ab]	*0.071 ± 0.008*[a]	*0.292 ± 0.009*[a]	*0.950 ± 0.130*[b]
Einkorn	Terzino	0.063 ± 0.024	0.057 ± 0.023	0.301 ± 0.066	1.674 ± 0.381
	Tifi	0.081 ± 0.012	0.077 ± 0.007	0.241 ± 0.105	1.804 ± 0.240
	Mean	*0.072 ± 0.009*[ab]	*0.067 ± 0.010*[a]	*0.271 ± 0.030*[a]	*1.739 ± 0.065*[a]

[a] Mean ± standard deviation (n = 4 locations).
Values with different superscript letters in a column are significantly different from each other ($p < 0.05$).

Nagel, Düren, Germany) into HPLC vials. Extractions were carried out in duplicate.

2.5. Quantitation of FODMAP by HPAEC-PAD

Saccharide analyses were performed by high-performance anion exchange chromatography applying pulsed amperometric detection (HPAEC-PAD) according to the method described by Schütz, Muks, Carle, and Schieber (2006) with slight modifications. Briefly, a Dionex ICS 3000 system was equipped with a Dionex CarboPac PA200 (3 × 250 mm) column protected by a CarboPac PA200 (3 × 50 mm) guard column, both operated at 25 °C. The mobile phase consisted of purified water (A), 225 mM NaOH (B), and 500 mM sodium acetate (C). The gradient programme applied is shown in Table 2. Eluents were degassed using helium, and maintained under helium atmosphere. Total runtime was 130 min at a flow rate of 0.25 mL/min. The injection volume was 10 µL. Calibrations were performed using authentic reference standards of sorbitol, mannitol, glucose, fructose, sucrose, raffinose, 1-kestotriose, maltose, 1,1-kestotetraose, and 1,1,1-kestopentaose at six concentration levels between 0.5 and 10 mg/L. All calibrations showed excellent linearity with correlation coefficients (r^2) > 0.99. Fructans with a degree of polymerization (DP) of 3, 4, and 5 were quantitated as 1-kestotriose, 1,1–kestotetraose, and 1,1,1-kestopentaose, respectively. Fructans of higher DP (6–11) were quantitated by the molar calibration curve of 1,1,1-kestopentaose applying a respective molar weight correction. Confirming fructan assignment, inulinase (from Megazyme HK Fructan kit, Megazyme, Bray, Ireland) was applied for the selective enzymatic hydrolysis of fructans. Limits of detection (LOD) and quantitation (LOQ) were determined by HPAEC-PAD signal-to-noise ratios of 3 and 10, respectively (International Conference on Harmonisation, 1994). Values ranging between the LOD and the LOQ were denoted as 'traces' (tr.), whereas eventual signals below the LOD were denoted as 'not detectable' (n.d.). Total fructans refers to the sum of all quantifiable fructans spanning from DP3 to DP11. FODMAP concentrations in both flours and breads were calculated as g FODMAP per 100 g flour dry matter (DM).

Table 2 – Gradient program used for the separation of saccharides from wheat.

Time (min)	Eluent A (%)[a]	Eluent B (%)[b]	Eluent C (%)[c]	Status
0	70.0	27.5	2.5	acquisition
30	42.5	27.5	30.0	
50	26.5	27.5	46.0	
95	0.0	27.5	72.5	
100	0.0	0.0	100.0	
115	0.0	66.0	34.0	
120	70.0	27.5	2.5	clean-up
130	70.0	27.5	2.5	re-equilibration

[a] Purified water.
[b] 225 mM NaOH.
[c] 500 mM sodium acetate.

2.6. Enzymatic quantitation of fructans

To validate the applied fructan extraction and HPAEC-PAD quantitation method, a Megazyme Fructan HK enzymatic assay kit (Megazyme, Bray, Ireland) was used for the determination of total fructan contents in all wheat samples grown in Hohenheim. The enzymatic assay was based on established standard methods (AOAC method 999.03 and AACC method 32.32.01).

2.7. Statistical analyses

The analysed wheat species comprised different numbers of varieties as described above. To evaluate such unbalanced sample sets, a non-parametric Kruskal–Wallis rank sum test and subsequent pairwise comparisons using the Nemenyi Post-Hoc test with Chi-squared approximation for independent samples were applied to detect significant differences between wheat species. Analysis of variance (ANOVA) and Tukey's HSD test were computed as significance tests among varieties within the wheat species. Calculation of means, standard deviations, heritability, Wilcoxon signed-rank correlation test, and the above mentioned significance tests were carried out using the software R version 3.2.4 (R Foundation for Statistical Computing, 2016, available at www.r-project.org) with the package 'car' for ANOVA and Tukey's HSD test, and the package 'PMCMR' (Pohlert, 2014) for non-parametric significance tests. The calculation of heritability was carried out as described previously (Piepho & Möhring, 2007) using the ASReml 3.0 software (Gilmour, Gogel, Cullis, & Thompson, 2009).

3. Results and discussion

3.1. Identification of FODMAP in wheat

Fig. 1a illustrates a representative HPAEC-PAD chromatogram of an aqueous extract obtained from a whole grain flour of the bread wheat variety 'Tobak'. Our study focused on whole grain flours, since substantial amounts of FODMAP, particularly fructans, are present in higher concentrations in the bran (Haskå, Nyman, & Andersson, 2008).

Glucose (2), sucrose (4), and maltose (7) were the non-FODMAP components observed at highest signal intensities. Regarding FODMAP for which authentic standards were available, the presence of trace amounts of mannitol (1) was found, while sorbitol, lactose, and stachyose were not detected in any of the investigated wheat samples. These findings were in agreement with previous reports (Stone & Morell, 2009). Moreover, fructose (3), raffinose (5), 1–kestotriose (6), 1,1-kestotetraose (8), and 1,1,1-kestopentaose (9) were identified by comparison of their retention times to those of authentic standards. Since further authentic reference standards of branched and higher polymerized fructans were commercially unavailable, the tentative identification of the respective peaks (DP 3–14) was carried out as follows. After enzymatic hydrolysis with inulinase, peaks corresponding to DP 3–14 quantitatively disappeared, while fructose simultaneously increased in equivalent amounts (data not shown), confirming their assignment to fructans (Fig. 1). The tentative assignment of each degree of polymerization (DP) was

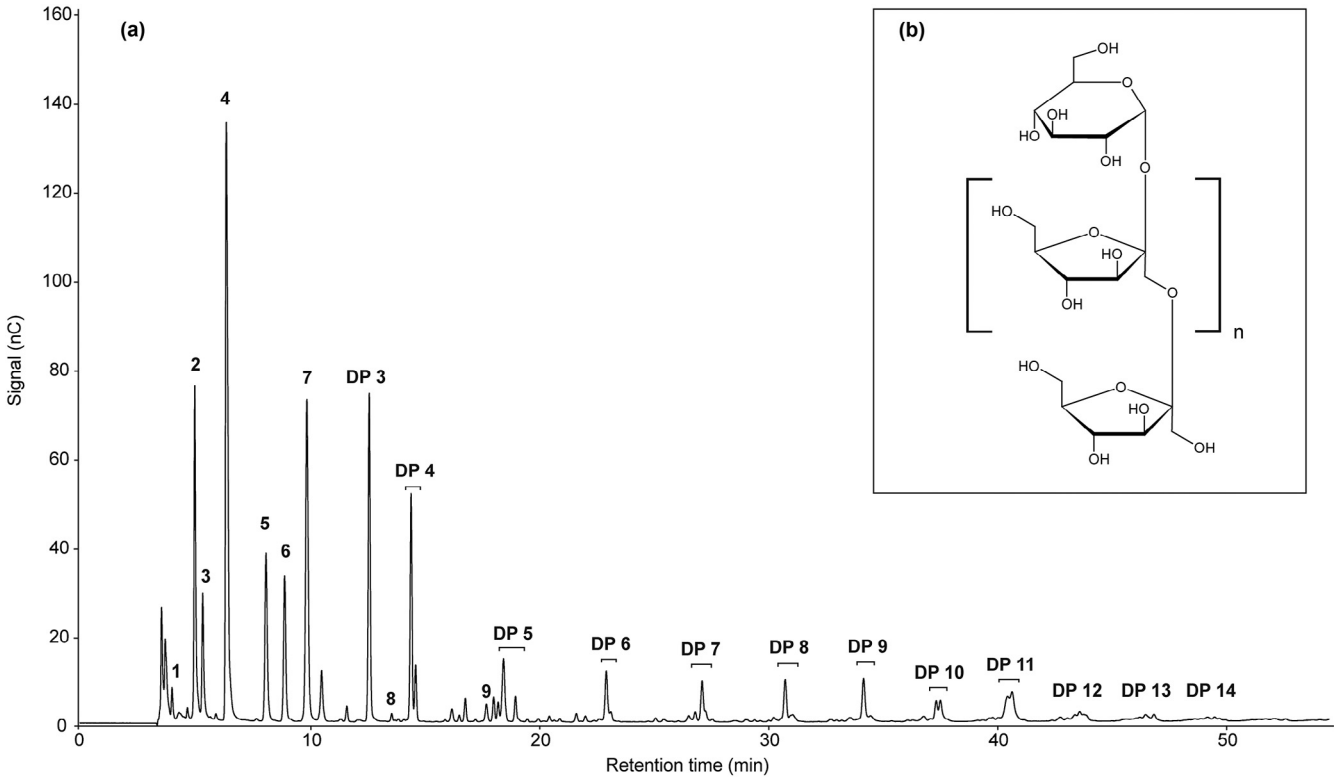

Fig. 1 – (a) HPAEC-PAD chromatogram of the bread wheat variety 'Tobak' (1) mannitol, (2) glucose, (3) fructose, (4) sucrose, (5) raffinose, (6) 1-kestose, (7) maltose, (8) 1,1-kestotetraose (1-nystose), (9) 1,1,1-kestopentaose (FFN), (DP3–DP14) tentatively identified fructans with a degree of polymerization (DP) of 3–14. (b) Exemplary structure of an inulin-type fructan.

based on previously described HPAEC elution orders (Shiomi, Onodera, & Sakai, 1997; Verspreet et al., 2015b), based on synthesized standards or mass spectrometric analyses. In these studies, branched fructans of an identical DP of *n* were reported to consistently elute after their linear analogue (DP of *n*), but prior to the unbranched compound with a DP of *n+1*. Therefore, the peaks eluting in between compounds 6 (kestotriose) and 8 (1,1-kestotetraose) were assigned to fructans of DP 3, except for those peaks that did not disappear upon inulinase treatment (e.g., peak 7, maltose). By analogy, the peaks eluting in between 1,1-kestotetraose (8) and 1,1,1-kestopentaose (9) were assigned to DP 4. The assignment of DP 5–14 was based on the homologous behaviour of their retention times, i.e., on their equidistant consecutive increase in retention time by ca. 3.8–4.9 min per fructosyl unit. Such retention behaviour has previously been reported on HPAEC-based separations (Schütz et al., 2006). In the wheat samples investigated, fructan signals with assigned DP ranging from 3 to 11 were quantifiable (>LOQ), while those of fructans with DP of 12 to 14 were present in trace amounts. These results were in agreement with previous reports (Haskå et al., 2008; Verspreet et al., 2015a). The validity of our HPAEC-PAD-based identification and the later described quantitation was corroborated by comparing the HPAEC-PAD results with those obtained *via* a commercial enzymatic test kit. After analysing all wheat samples grown at the Hohenheim location, differences between the results of the enzymatic determination and those of the HPAEC-PAD quantitation using molecular weight correction factors for single fructans were non-significant ($p > 0.05$, see Supplemental Figure S1).

3.2. *Quantitation of FODMAP in diverse wheat species and varieties*

Sucrose was the predominant saccharide in whole grain flours of all wheat samples, ranging from 0.655 g/100 g DM in the bread wheat variety 'Julius' to 1.115 g/100 g DM in the durum variety 'Lunadur' (Supplemental Tables S1 and S3). Levels of glucose (0.067–0.113 g/100 g DM) were consistently higher than those of fructose (0.052–0.075 g/100 g DM) in all wheat species and varieties investigated (Table 1). Although concentrations found were low, these findings are of potential importance, since fructose contents exceeding concomitant glucose contents are viewed as FODMAP due to the potential malabsorption of such 'excess fructose' (Gibson et al., 2007; Gibson & Shepherd, 2005).

The galactosyl-oligosaccharide raffinose was found to range from 0.194 g/100 g DM (durum variety 'Karur') to 0.382 g/100 g DM (spelt variety 'Zollernspelz', Table 1). Among the species, spelt (0.309 ± 0.046 g/100 g DM) tended to have higher contents than bread wheat (0.214 ± 0.014 g/100 g DM) and durum (0.220 ± 0.027 g/100 g DM), however, significant differences in raffinose contents were not observed (Table 1). These data are in agreement with previously reported raffinose levels in bread wheat, spelt, and durum (Fretzdorff & Welge, 2003b; Huynh et al., 2008).

All five wheat species under investigation contained substantial amounts of total fructans, ranging from 0.819 g/100 g DM in the emmer variety 'Ramses' to 1.966 g/100 g DM in the bread wheat variety 'Tobak'. Mean levels in einkorn (1.739 ± 0.065 g/100 g DM) and bread wheat (1.568 ± 0.204 g/100 g DM) were

significantly higher than in durum (1.169 ± 0.117 g/100 g DM) and emmer (0.950 ± 0.130 g/100 g DM, Table 1). Previous studies reported fructan concentrations in bread wheat and durum from 0.7 to 2.9 g/100 g DM (Andersson et al., 2013; Fretzdorff & Welge, 2003b; Huynh et al., 2008; Verspreet et al., 2012), consistent with these findings. Reports on fructan levels in ancient wheat species are scarce. A single emmer variety was previously described to contain 1.5 g/100 g DM fructans (Gélinas et al., 2015). Fretzdorff and Welge (2003b) described average total fructan contents in spelt to be 1.1 g/100 g DM, while Verspreet et al. (2012) determined 0.6 g/100 g in flour of one spelt variety. Furthermore, Brandolini et al. (2011) reported einkorn to have higher fructan contents than bread wheat (1.90 vs. 1.29 g/100 g DM), however, only one bread wheat variety was investigated. Due to the significant and substantial variations between the varieties and growing locations in our study (Supplemental Tables S1–S4), conclusions regarding differences among wheat species should be based on sample sets with more than one variety per species, grown at a minimum of two different locations.

Total FODMAP contents, considering total fructans, raffinose, and excess fructose, in the wheat species investigated are illustrated in Fig. 2. Gibson and Shepherd (2010) recommended the consumption of spelt products over that of bread wheat products, assuming lower FODMAP contents in spelt. In our study, differences between FODMAP concentrations in whole grain flours of bread wheat and spelt were non-significant ($p = 0.50$), being additionally characterized by a high variability among varieties within one species (Table 1). Based on our data, dietary recommendations in favour or against the consumption of selected wheat species regarding their FODMAP contents would be untenable. Although emmer and durum yielded significantly lower values than bread wheat and einkorn, individual varieties of the aforementioned species may still contain considerable amounts of FODMAP, such as the emmer variety 'Osiris' grown at Eckartsweier (1.840 g/100 g DM, Supplemental Table S4). Furthermore, FODMAP levels in the flours of three spelt varieties, i.e., Franckenkorn (0.681–1.213 g/100 g DM), Oberkulmer Rotkorn (0.93–1.26 g/100 g DM), and Zollernspelz (1.02–1.33 g/100 g DM), were slightly lower than those of the investigated five bread wheat varieties (1.38–1.97 g/100 g DM). Nevertheless, these differences were small as compared to those achievable by particular bread making measures as described below. This is of great importance, because bread wheat and spelt flours may often be processed differently due to their differing techno-functional characteristics (Schober, Clarke, & Kuhn, 2002).

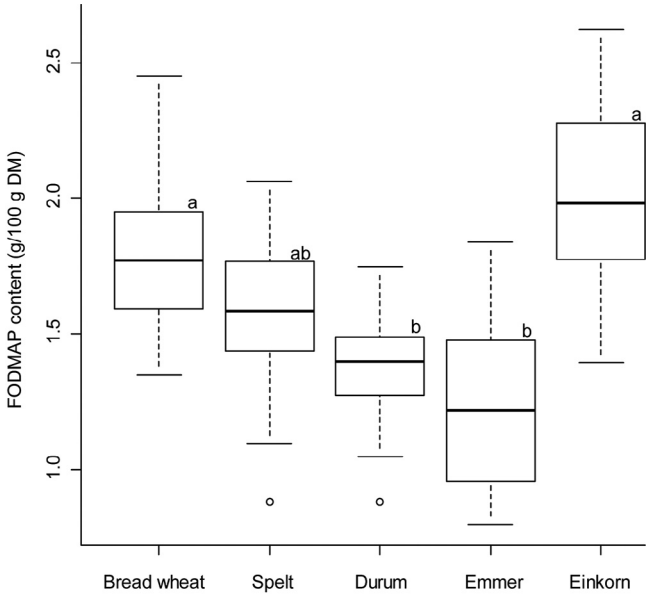

Fig. 2 – Total FODMAP (fermentable oligo-, di-, and monosaccharides, and polyols) contents in whole grain flours. n = 20 for bread wheat, spelt, and durum, and n = 8 for emmer and einkorn.

Besides different processing strategies, targeted breeding for low-fructan and, thus, low-FODMAP wheat varieties may be another tool for the manufacture of products being harmless to IBS patients. In contrast, Shimbata et al. (2011) sought to produce high-fructan wheat varieties in order to yield prebiotic products for healthy individuals. Our results strongly suggest a distinct genetic influence on fructan concentrations in wheats due to the very high heritability value ($h^2 = 0.87$, Supplemental Table S5) as determined for all varieties of the five wheat species under investigation. In agreement, previous studies reported fructan accumulation to be predominantly under genetic control (Brandolini et al., 2011; Huynh et al., 2008). By analogy to fructans, a high heritability of $h^2 = 0.69$ was observed for raffinose content in the investigated wheat species. Despite the high heritability values, it should be noted that the growing location influenced the total fructan and raffinose levels (Supplemental Tables S1-S4) in agreement with a previous report (Brandolini et al., 2011). The influence of the growing location on the FODMAP levels among the individual wheat species or among the overall means of the five species was investigated by performing a Wilcoxon signed-rank correlation test. Samples from the location Eckartsweier (EKW) showed significantly higher raffinose contents. However, this effect was not seen on the other locations. Environmental influences of the location on total fructan contents were not observed (data not shown).

Hence, concentrations of the major FODMAP in wheat flour, i.e., fructans and raffinose, may be altered by future plant breeding. However, since fructans and raffinose are reserve carbohydrates, membrane stabilizers, and stress tolerance mediators exerting further functions in disease prevention in plants (van den Ende, 2013), such genetic modifications would most likely affect crop productivity. Therefore, food technological modulation of FODMAP levels during wheat processing may represent a more promising approach as described in the following.

3.3. *Influence of processing on FODMAP content in baked wheat products*

The majority of wheat grains are processed prior to human consumption, mainly to bakery products. Bread wheat is the major crop used for bread making in Germany, although spelt use is increasing. Whole grain flours of the widely grown bread wheat variety 'Tobak' and the most widely grown spelt variety 'Zollernspelz' were processed into yeast-leavened bread rolls to monitor their FODMAP levels, implementing different total proofing times of 1, 2.5, or 4.5 h as described above (Fig. 3). Fur-

thermore, these varieties were selected due to their different FODMAP content. The bread wheat variety 'Tobak' contained 2.45 g/100 g DM, representing a worst case scenario of a high-FODMAP flour. The spelt variety 'Zollernspelz' was characterized by comparably low FODMAP levels of 1.53 g/100 g DM.

In whole grain flours of both 'Zollernspelz' and 'Tobak', glucose levels were consistently higher than fructose levels, i.e., excess fructose was absent, thus not contributing to FODMAP. However, bread making had a substantial influence on the glucose to fructose ratio, irrespective of the used variety. After a total proofing time of 1 h, fructose concentrations significantly increased from 0.087 to 1.905 g/100 g DM (bread wheat variety 'Tobak') during processing, due to the almost complete hydrolysis of sucrose (data not shown) and partial enzymatic degradation of fructans. By analogy, glucose concentrations increased after 1 h of proofing; however, to a significantly smaller extent (0.11 to 0.35 g/100g DM). As a result, fructose levels exceeded those of glucose, thus being considered 'excess fructose' representing a contribution to FODMAP levels.

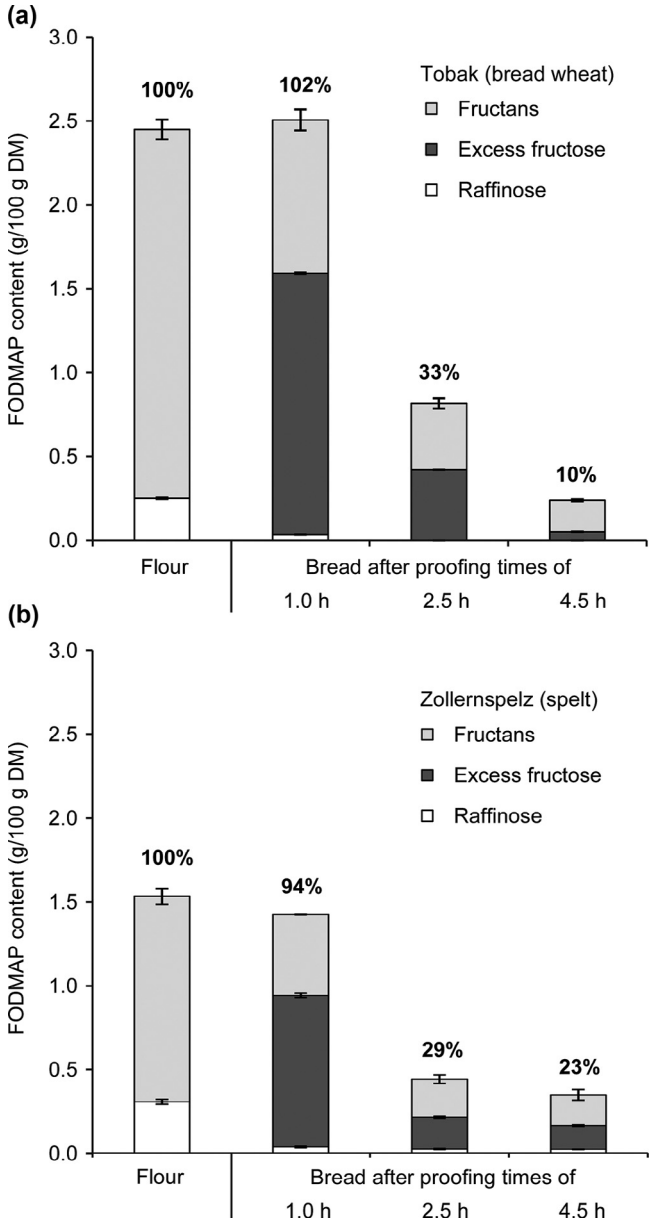

Fig. 3 – Total FODMAP (fermentable oligo-, di-, and monosaccharides, and polyols) contents in whole grain flours and breads of the bread wheat variety 'Tobak' (a) and the spelt variety 'Zollernspelz' (b) after dough proofing for 1, 2.5, and 4.5 h, respectively.

Fructose and glucose levels during bread making were similar in 'Tobak' and the spelt variety 'Zollernspelz' (Fig. 3). In agreement, Verspreet et al. (2013b) observed an initial increase of fructose content during yeasted dough fermentation followed by an almost complete fructose degradation within 2 h of fermentation. However, in our study, the increase in fructose was accompanied by a simultaneous decrease of fructan and raffinose concentrations (see below); therefore, FODMAP levels of whole grain flour and 1 h-leavened breads were not statistically different, irrespective of the wheat varieties used (Fig. 3). After proofing for 2.5 h, both excess fructose and fructan concentrations decreased to 0.42 and 0.40 g/100 g DM (bread wheat 'Tobak'), respectively. Although being less pronounced, further reductions were achieved after 4.5 h resulting in 0.05 g/100 g DM excess fructose and 0.19 g/100 g DM total fructans in the bread wheat variety 'Tobak'. Proportionally, similar decreases of both saccharide classes in breads from spelt were observed (Fig. 3). In previous studies, the activity of yeast during proofing has been suggested to be responsible for the degradation of fructan levels by 40–80% of its initial content in both whole grain and refined flours (Gélinas et al., 2015; Knez et al., 2014; Nilsson et al., 1987; Verspreet et al., 2013b), whereas the baking step has been shown to exert only marginal effects (Andersson et al., 2009; Fretzdorff & Welge, 2003a; Gélinas et al., 2015; Knez et al., 2014). Endogenous invertase and inulinase activities in wheat grains have earlier been demonstrated to play a minor role, since enzymatic fructan degradation has been described to be non-significant in bread leavened without yeast (Andersson et al., 2009; Knez et al., 2014; Verspreet et al., 2013b).

Similarly to total fructans, we observed a degradation of raffinose with only 13.4% and 12.1% of the initial flour concentrations being retained after 1 h in bread wheat and spelt, respectively. After 4.5 h, raffinose was undetectable in bread wheat-based breads, whereas minor amounts (7.2%) were retained in spelt breads (Fig. 3). As a result of decreasing fructose, fructan, and raffinose levels, total FODMAP levels were diminished to 29–33% of their initial contents after 2.5 h, and to 10–23% after 4.5 h of proofing (Fig. 3).

The above-described processing-related reduction of excess fructose and FODMAP levels are expected to have major nutritional relevance for IBS patients. The occurrence of excess fructose is strongly suggested to increase the risk of fructose malabsorption and related health implications (Gibson et al., 2007; Gibson & Shepherd, 2005). In contrast, breads leavened for only one hour contained excess fructose (1.56 and 0.90 g/100g DM in bread wheat and spelt bread, respectively), although in lower concentrations than other food sources like pear (up to 5 g/100 g fresh weight (FW)) and peach (4 g/100 g FW, (Muir et al., 2009)). Gibson & Shepherd (2010) suggested tolerable excess fructose of foods and beverages to be less than 0.5 g/100 g FW in order to prevent the risk of inducing IBS symptoms (Gibson & Shepherd, 2010). Considering their moisture

content, our bread wheat-based breads baked after 1 h proofing time exhibited excess fructose levels of 1.01 g/100 g FW, thus by far exceeding the aforementioned threshold value. In contrast, bread wheat-based breads produced after 4.5 h of proofing time only contained 0.03 g/100 g FW. Consequently, the frequent consumption of short-leavened breads may potentially aggravate the symptoms of fructose malabsorption, whereas long-leavened breads may be better digestible.

Because of lowered excess fructose and fructan levels, longer proofing times can diminish FODMAP levels (Fig. 3) irrespective of the wheat variety used. Total FODMAP contents in fresh breads leavened for 1h were 1.63 g/100 g FW (bread wheat) and 0.93 g/100 g FW (spelt), while they were 0.16 g/100 g FW (bread wheat) and 0.23 g/100g FW (spelt) after 4.5 h of proofing. Consequently, for the production of low-FODMAP breads palatable by IBS and fructose-intolerant patients, the application of yeasted dough and extended proofing times appears a promising approach. However, further studies are required to investigate the influence of such processing methods on the loaf quality of resulting products.

Acceptable intake levels for total FODMAP consumption are not clearly defined (Gibson & Shepherd, 2010), and therefore, a recommendation of maximum tolerable amounts of short or long-leavened bread depends on individual patients' conditions. A further reduction of fructans during bread making has previously been achieved by using sourdough (Andersson et al., 2009; Escrivá & Martínez-Anaya, 2000; Fretzdorff & Welge, 2003a), since lactic bacteria are suggested to foster fructan degradation by creating acidic conditions favouring yeast invertase activity (Kucek et al., 2015; Nilsson et al., 1987).

4. Concluding remarks

Since a diet low in FODMAP can alleviate IBS symptoms (Rao et al., 2015), we sought to discover particular wheat species with low FODMAP concentrations. In particular, ancient wheat species including spelt were thought to be generally more suitable for IBS patients than modern bread wheat varieties. Unexpectedly, differences in the FODMAP content of the bread wheat and spelt species under investigation were not observed, although flours of three spelt varieties contained slightly lower FODMAP levels than most bread wheat varieties. Most importantly, the ancient wheat species einkorn even showed the highest FODMAP contents. In order to produce low-FODMAP products for IBS management, however, these differences appear to be of minor importance due to the achievable reductions by implementing specific bread making steps. Our study demonstrates that low-FODMAP bakery products can be produced when applying prolonged proofing times (>4 h) on yeast-based dough, irrespective of the variety used. Such prolonged procedures are mainly carried out by smallholders adhering to traditional methods including long dough fermentation times. While prolonged dough proofing was thought to solely improve sensory appearance of the resulting products, our study clearly shows that it also reduced the FODMAP levels of the bakery product. Since spelt doughs are often leavened for longer times than bread wheat doughs due to more traditional recipes and in order to improve their rather poor technological performance (Schober et al., 2002), observations of individuals better tolerating spelt than bread wheat products might be rather related to different processing than to species-related causes. Although prolonging proofing times reduced FODMAP contents, their complete removal from bakery products may not be desirable in all cases, since they are prebiotic, and offer health promoting effects for individuals without IBS, when consumed in moderate amounts (Verspreet et al., 2015a). While low-FODMAP wheat products might be digestible for IBS patients, their suitability for individuals suffering from an apparent NCWS remains unclear. The relevance of FODMAP for NCWS is currently under debate (Catassi et al., 2013; Kucek et al., 2015; Verspreet et al., 2015a), and further research on differentiation of NCWS from IBS should be encouraged.

Conflict of interest

The authors have declared no conflict of interest.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2016.05.019.

REFERENCES

Andersson, A. A., Andersson, R., Piironen, V., Lampi, A.-M., Nyström, L., Boros, D., Fraś, A., Gebruers, K., Courtin, C. M., Delcour, J. A., Rakszegi, M., Bedo, Z., Ward, J. L., Shewry, P. R., & Åman, P. (2013). Contents of dietary fibre components and their relation to associated bioactive components in whole grain wheat samples from the HEALTHGRAIN diversity screen. *Food Chemistry*, *136*, 1243–1248.

Andersson, R., Fransson, G., Tietjen, M., & Åman, P. (2009). Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry*, 57, 2004–2008.

Biesiekierski, J. R., Peters, S. L., Newnham, E. D., Rosella, O., Muir, J. G., & Gibson, P. R. (2013). 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, 320–328.

Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J., Gibson, P. R., & Muir, J. G. (2011). Quantification of fructans, galacto-oligosaccharides and other short-chain

carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics*, 24, 154–176.
Brandolini, A., Hidalgo, A., Plizzari, L., & Erba, D. (2011). Impact of genetic and environmental factors on einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*) polysaccharides. *Journal of Cereal Science*, 53, 65–72.
Catassi, C., Bai, J. C., Bonaz, B., Bouma, G., Calabrò, A., Carroccio, A., Castillejo, G., Ciacci, C., Cristofori, F., Dolinsek, J., Francavilla, R., Elli, L., Green, P., Holtmeier, W., Koehler, P., Koletzko, S., Meinhold, C., Sanders, D., Schumann, M., Schuppan, D., Ullrich, R., Vécsei, A., Volta, U., Zevallos, V., Sapone, A., & Fasano, A. (2013). Non-celiac gluten sensitivity: The new frontier of gluten related disorders. *Nutrients*, 5, 3839–3853.
El-Salhy, M., & Gundersen, D. (2015). Diet in irritable bowel syndrome. *Nutrition Journal*, 14, 17.
Escrivá, C., & Martínez-Anaya, M. A. (2000). Influence of enzymes on the evolution of fructosans in sourdough wheat processes. *European Food Research and Technology*, 210, 286–292.
Fretzdorff, B., & Welge, N. (2003a). Abbau von getreideeigenen Fructanen während der Herstellung von Roggen-Vollkornbrot. *Getreide, Mehl und Brot*, 57, 147–151.
Fretzdorff, B., & Welge, N. (2003b). Fructan- und Raffinosegehalte im Vollkorn einiger Getreidearten und Pseudo-Cerealien. *Getreide, Mehl und Brot*, 57, 3–8.
Gélinas, P., McKinnon, C., & Gagnon, F. (2015). Fructans, water-soluble fibre and fermentable sugars in bread and pasta made with ancient and modern wheat. *International Journal of Food Science & Technology*, doi:10.1111/ijfs.13022.
Gibson, P. R., Newnham, E., Barrett, J. S., Shepherd, S. J., & Muir, J. G. (2007). Review article: Fructose malabsorption and the bigger picture. *Alimentary Pharmacology & Therapeutics*, 25, 349–363.
Gibson, P. R., & Shepherd, S. J. (2005). Personal view: Food for thought – western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Alimentary Pharmacology & Therapeutics*, 21, 1399–1409.
Gibson, P. R., & Shepherd, S. J. (2010). Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. *Journal of Gastroenterology and Hepatology*, 25, 252–258.
Gibson, P. R., Varney, J., Malakar, S., & Muir, J. G. (2015). Food components and irritable bowel syndrome. *Gastroenterology*, 148, 1158–1174, e4.
Gilmour, A., Gogel, B., Cullis, B. R., & Thompson, R. (2009). *ASReml User Guide release 3.0*. Hemel Hempstead: VSN International Ltd.
Haskå, L., Nyman, M., & Andersson, R. (2008). Distribution and characterisation of fructan in wheat milling fractions. *Journal of Cereal Science*, 48, 768–774.
Hayes, P. A., Fraher, M. H., & Quigley, E. M. M. (2014). Irritable bowel syndrome: The role of food in pathogenesis and management. *Gastroenterology & Hepatology*, 10, 164–174.
Hungin, A., Whorwell, P., Tack, J., & Mearin, F. (2003). The prevalence, patterns and impact of irritable bowel syndrome: An international survey of 40000 subjects. *Alimentary Pharmacology & Therapeutics*, 17, 643–650.
Huynh, B.-L., Palmer, L., Mather, D. E., Wallwork, H., Graham, R. D., Welch, R. M., & Stangoulis, J. C. (2008). Genotypic variation in wheat grain fructan content revealed by a simplified HPLC method. *Journal of Cereal Science*, 48, 369–378.
International Conference on Harmonisation (1994). Validation of analytical procedures: Test and Methodology Q2(R1). <http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf> Accessed 20.08.13.
Knez, M., Abbott, C., & Stangoulis, J. C. R. (2014). Changes in the content of fructans and arabinoxylans during baking processes of leavened and unleavened breads. *European Food Research and Technology*, 239, 803–811.
Kucek, L. K., Veenstra, L. D., Amnuaycheewa, P., & Sorrells, M. E. (2015). A grounded guide to gluten: How modern genotypes and processing impact wheat sensitivity. *Comprehensive Reviews in Food Science and Food Safety*, 14, 285–302.
Longin, C. F. H., Ziegler, J. U., Schweiggert, R. M., Koehler, P., Carle, R., & Würschum, T. (2016). Comparative study of hulled (einkorn, emmer and spelt) and naked wheats (durum and bread wheat): Agronomic performance and quality traits. *Crop Science*, 56, 302–311.
Lovell, R. M., & Ford, A. C. (2012). Global prevalence of and risk factors for irritable bowel syndrome: A meta-analysis. *Clinical Gastroenterology and Hepatology*, 10, 712–721, e4.
Muir, J. G., Rose, R., Rosella, O., Liels, K., Barrett, J. S., Shepherd, S. J., & Gibson, P. R. (2009). Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry*, 57, 554–565.
Nilsson, U., Öste, R., & Jägerstad, M. (1987). Cereal fructans: Hydrolysis by yeast invertase, *in vitro* and during fermentation. *Journal of Cereal Science*, 6, 53–60.
Piepho, H.-P., & Möhring, J. (2007). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*, 177, 1881–1888.
Pohlert, T. (2014). The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package. <http://cran.r-project.org/package=PMCMR>.
Rao, S. S. C., Yu, S., & Fedewa, A. (2015). Systematic review: Dietary fibre and FODMAP-restricted diet in the management of constipation and irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*, 41, 1256–1270.
Rumessen, J. J. (1992). Fructose and related food carbohydrates: Sources, intake, absorption, and clinical implications. *Scandinavian Journal of Gastroenterology*, 27, 819–828.
Schober, T., Clarke, C., & Kuhn, M. (2002). Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. *Cereal Chemistry*, 79, 408–417.
Schütz, K., Muks, E., Carle, R., & Schieber, A. (2006). Separation and quantification of inulin in selected artichoke (*Cynara scolymus* L.) cultivars and dandelion (*Taraxacum officinale* WEB. ex WIGG.) roots by high-performance anion exchange chromatography with pulsed amperometric detection. *Biomedical Chromatography*, 20, 1295–1303.
Shimbata, T., Inokuma, T., Sunohara, A., Vrinten, P., Saito, M., Takiya, T., & Nakamura, T. (2011). High levels of sugars and fructan in mature seed of sweet wheat lacking GBSSI and SSIIa enzymes. *Journal of Agricultural and Food Chemistry*, 59, 4794–4800.
Shiomi, N., Onodera, S., & Sakai, H. (1997). Fructo-oligosaccharide content and fructosyltransferase activity during growth of onion bulbs. *New Phytologist*, 136, 105–113.
Spiller, R., Aziz, Q., Creed, F., Emmanuel, A., Houghton, L., Hungin, P., Jones, R., Kumar, D., Rubin, G., Trudgill, N., & Whorwell, P. (2007). Guidelines on the irritable bowel syndrome: Mechanisms and practical management. *Gut*, 56, 1770–1798.
Stallknecht, G., Gilbertson, K., & Ranney, J. (1996). Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale. In J. Janick (Ed.), *Proceedings of the Third National Symposium: New crops, new opportunities, new technologies, Indianapolis, Indiana, October 22–25, 1996* (pp. 156–170). Alexandria, VA: ASHS Press.
Stone, B., & Morell, M. (2009). Carbohydrates. In K. Khan & P. R. Shewry (Eds.), *Wheat: chemistry and technology* (pp. 299–362). St. Paul, MN: AACC International.
van den Ende, W. (2013). Multifunctional fructans and raffinose family oligosaccharides. *Frontiers in Plant Science*, 4, 247.

van Loo, J., Coussement, P., Leenheer, L., de Hoebregs, H., & Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science and Nutrition*, *35*, 525–552.

Verspreet, J., Cimini, S., Vergauwen, R., Dornez, E., Locato, V., Le Roy, K., De Gara, L., Van den Ende, W., Delcour, J. A., & Courtin, C. M. (2013a). Fructan metabolism in developing wheat (*Triticum aestivum* L.) kernels. *Plant and Cell Physiology*, *54*, 2047–2057.

Verspreet, J., Dornez, E., van den Ende, W., Delcour, J. A., & Courtin, C. M. (2015a). Cereal grain fructans: Structure, variability and potential health effects. *Trends in Food Science and Technology*, *43*, 32–42.

Verspreet, J., Hemdane, S., Dornez, E., Cuyvers, S., Delcour, J. A., & Courtin, C. M. (2013b). Maximizing the concentrations of wheat grain fructans in bread by exploring strategies to prevent their yeast (*Saccharomyces cerevisiae*)-mediated degradation. *Journal of Agricultural and Food Chemistry*, *61*, 1397–1404.

Verspreet, J., Holmgaard Hansen, A., Dornez, E., Delcour, J. A., van den Ende, W., Harrison, S. J., & Courtin, C. M. (2015b). LC-MS analysis reveals the presence of graminan- and neo-type fructans in wheat grains. *Journal of Cereal Science*, *61*, 133–138.

Verspreet, J., Pollet, A., Cuyvers, S., Vergauwen, R., van den Ende, W., Delcour, J. A., & Courtin, C. M. (2012). A simple and accurate method for determining wheat grain fructan content and average degree of polymerization. *Journal of Agricultural and Food Chemistry*, *60*, 2102–2107.

Ziegler, J. U., Schweiggert, R. M., Würschum, T., Longin, C. F. H., & Carle, R. (2016). Lipophilic antioxidants in wheat (*Triticum* spp.): A target for breeding new varieties for future functional cereal products. *Journal of Functional Foods*, *20*, 594–605.