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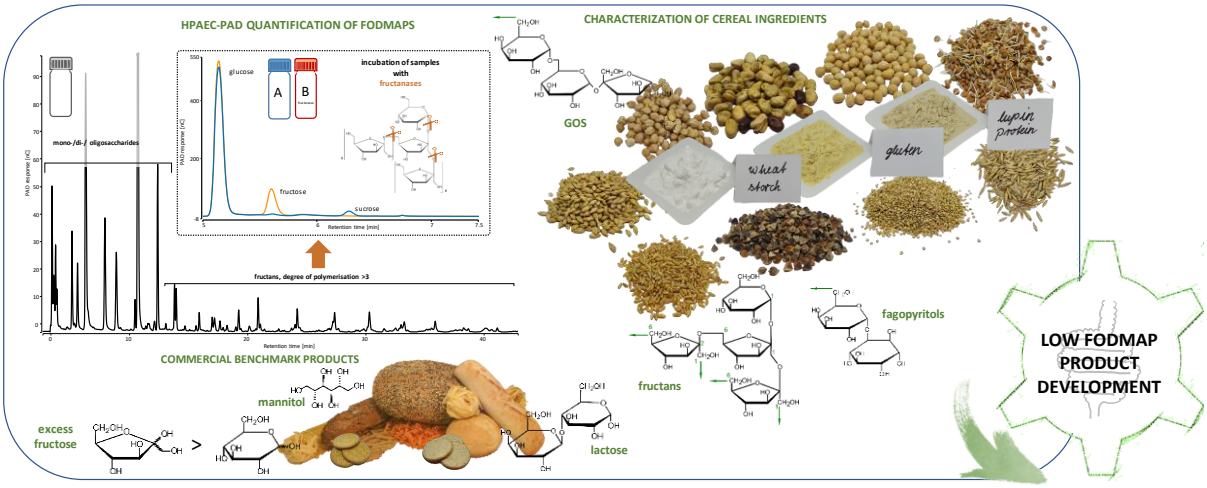
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CRediT author statement

Lilit Ispiryan: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing- Original draft preparation. **Emanuele Zannini:** Conceptualization, Methodology, Funding acquisition, Writing- Reviewing & Editing. **Elke K. Arendt:** Conceptualization, Methodology Supervision, Funding acquisition, Writing- Reviewing & Editing

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Characterization of the FODMAP-profile in Cereal-product Ingredients

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Keywords: FODMAPs, IBS, cereals, ingredients, HPAEC-PAD, fructans, galactooligosaccharides, product-development, fagopyritols

Abbreviations: FODMAP, fermentable oligo-, di, monosaccharides and polyols; HPAEC-PAD, high performance anion exchange chromatography coupled with pulsed amperometric detection; FOS, fructooligosaccharides; GOS, galactooligosaccharides; DP_{av}, average degree of polymerization; RFO, raffinose family oligosaccharides; IBS, irritable bowel syndrome; DM, dry matter; PC, protein concentrate; PI, protein isolate

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ABSTRACT:

Cereal-based products, such as bread, are staple foods in the western diet. Due to the nature of their basic ingredients and the diversity of recipes, the amount of fermentable short-chain carbohydrates (FODMAPs) in those products may be high. This study characterized the FODMAP-profiles of a broad range of cereal-product ingredients, serving as a basis for low FODMAP product development. Different cereals, pseudo-cereals, gluten-free flours, pulses, pulse protein ingredients, commercial sprouts, and other cereal-product ingredients were analyzed, using anion-exchange chromatography with electrochemical detection. Wheat and related cereals were high in fructans. Pulses, such as peas contained high galactooligosaccharides (GOS) amounts. Whereas GOS levels in pulse protein ingredients varied, depending on their production. Gluten-free flours, for instance, rice-flour, showed low FODMAP-profiles. Amongst those, buckwheat, which does not contain any of the FODMAPs investigated, contained high amounts of other soluble non-digestible carbohydrates, namely fagopyritols; these may have a similar effect on a sensitive gut as GOS. Finally, ingredients contained mainly high levels of fructans and GOS. Yet, the analysis of commonly consumed commercial cereal products, including bread, pasta, crackers and biscuits, highlighted the relevance of lactose, fructose in excess of glucose and polyols. These products serve as benchmarks for further product development.

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1. Introduction

Small dietary carbohydrates, which are not digested in the human intestine, and fermented by bacteria in the colon, are entitled with the well-recognized acronym FODMAPs (fermentable oligo-, di-, and monosaccharides and polyols). These carbohydrates can have beneficial or adverse effects on the human health. For individuals with functional gastrointestinal disorders, such as irritable bowel syndrome (IBS) the ingestion may be problematic leading to different symptoms, for instance bloating and abdominal pain or an altered bowel habit. IBS can be a very severe condition and thus, highly implicate on the patient's quality of life. Research over the past two decades has shown that dietary therapy with a reduced intake of FODMAPs (the low FODMAP diet) is successful in the treatment of IBS. Due to this fact the low FODMAP diet has been largely in focus lately. This often led to misinterpretation, incorrect application of the diet and thus, criticism in terms of lack of nutrients and long-term effects on the health. As emphasized in the recent review by Halmos and Gibson (2019) the correct application of a personalized, individual low FODMAP diet for each patient is crucial for a successful treatment of IBS (Halmos and Gibson, 2019).

The often quoted, and exclusively investigated, list of FODMAPs comprises the most abundant dietary non-digestible, osmotically active and readily fermentable carbohydrates with galactooligosaccharides (GOS), fructans and fructooligosaccharides (FOS), lactose, fructose in excess of glucose and polyols. Due to the nature of the basic cereal-product ingredients and the diversity of product-recipes, these products can contain high levels of FODMAPs.

GOS (also named raffinose family oligosaccharides, RFO, or α-galactosides) are known to be storage carbohydrates with protective plant-physiological functions in seeds of pulses (legumes). These oligosaccharides are α (1 → 6) linked galactosyl-derivates from sucrose with the most common homologues raffinose, stachyose, and verbascose. Due to the absence of the enzyme α-galactosidase, GOS are not digested in the human gut and fermented by the microflora in the colon. This leads to gastrointestinal discomfort and to symptoms, such as bloating and abdominal pain, in IBS-patients as well as healthy individuals. The metabolism

of GOS in plants also involves cyclitols, such as inositol or pinitol and their galactosides such as galactinol and ciceritol (Martínez-Villaluenga et al., 2008). Their contribution to the flatulence-causing effect is being investigated. Similarly, non-digestible fructans are storage carbohydrates in different plants, including cereals and serve the plant with energy during drought and other extreme conditions (Verspreet et al., 2015). Cereal fructans are predominantly composed of branched β (2 → 1) and β (2 → 6) linked fructose chains with a terminal glucose (graminan-type). Stems and leaves of the oat plant also accumulate neo-levan type fructans with β (2 → 1) and/or β (2 → 6) linked fructose chains, with the glucose residue linked internally (Livingston et al., 1993). The disaccharide lactose, which is the main FODMAP in dairy products, may also be found in cereal-based products depending on their formulation, as later highlighted in this study. Fructose may occur in high excess to glucose in some fermented cereal-products (Ziegler et al., 2016). Likewise polyols (sugar-alcohols), such as mannitol, the reduced form of fructose, may be produced during fermentation in cereal-products (Sahin et al., 2019)

Due to the lack of definition and regulations of FODMAPs in the EU legislation, very few products with a low FODMAP labelling are available on the European market. Only gluten-free products, which predominantly are made from ingredients naturally low in FODMAPs serve as alternative for people following the low FODMAP diet. Also most products, labelled meanwhile by different organizations in addition to the official certification by the Monash University, are mainly gluten-free products (Monash University, 2019). However, often these products are lacking sensory appeal and nutritional value. Thus, the development of palatable functional low FODMAP products with a high nutritional value is an emerging area of research. This is a fundamental study on the characterization of the FODMAP-profiles of a broad range of cereal-product ingredients. The gained knowledge serves as a basis for the development of products with a lowered FODMAP content using different (bio-) technological approaches. Furthermore, this study aimed to highlight relevant FODMAPs in cereal-products, other than fructans and GOS deriving from the ingredients. Therefore, commonly consumed products of different categories, which also serve as benchmarks for further product development, have

been analyzed. Different studies have been conducted, characterizing the FODMAP content in a wide range of food, as it is consumed (Biesiekierski et al., 2011; Muir et al., 2009). However, these studies serve as dietetic guide for patients following the low FODMAP diet. A dry matter-based characterization of the FODMAP profiles of raw ingredients as a tool for product-development remains scarce. The HPAEC-PAD method for the quantification of FODMAPs, applied in this study allowed a detailed characterization of the ingredients and their respective products (Ispiryan et al., 2019).

2. Experimental

2.1. Ingredients and food products.

All ingredients for analysis were commercially sourced, except for the fababean flour and the protein isolates from lupin and fababean, which were provided by Fraunhofer Institute (IVV), Germany. The suppliers of all ingredients are compiled in Table S1 (supporting information). Food products, available on the Irish market, represent examples of commonly consumed cereal-products of different categories, including bread, pasta, biscuits and crackers, and their gluten-free alternatives, respectively (Table 1).

2.2. Sample preparation and FODMAP quantification.

Commercial flours of the different ingredients were used for analysis as supplied. Whole grains, seeds and the raw pasta were milled with a Bühler laboratory disc mill (Braunschweig, Germany) or disrupted using a QIAGEN Tissue Lyser II (Hilden, Germany), to a particle size of ≤ 0.5 mm (Ispiryan et al., 2019). The breads, biscuits, crackers as well as cooked pasta (cooked according to instructions on packaging), were freeze-dried and ground to a fine powder. Three packets of each product were purchased, and equal amounts of each packet pooled, disrupted into small pieces and approx. 10-15 g freeze-dried for 3 d.

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The quantification of mono-, di-, galactooligosaccharides, fructans, and polyols was conducted via high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), performed on a Dionex™ ICS-5000+ system (Sunnyvale, CA, USA) as described by Ispiryan et al. (2019). All carbohydrates, except for the fructans have been quantified using authentic reference standards, as specified in the previous study (Ispiryan et al., 2019). Raffinose and stachyose have been determined as the sum of both sugars using raffinose pentahydrate from Sigma-Aldrich (Darmstadt, Germany) as a reference standard, performed on the Thermo Scientific™Dionex™CarboPac™PA200 column. A qualitative separation and analysis of raffinose and stachyose was achieved on the Thermo Scientific™Dionex™CarboPac™PA1 column. The total fructan content and the average degree of polymerization have been determined after enzymatic hydrolysis with two enzyme mixtures A and B, where only B contained fructan degrading inulinases. The calculation was based on the quantification of the monomers glucose and fructose released from the fructan molecules (Ispiryan et al., 2019). The significance of the fructose released from sucrose and the fructose released from the hydrolysis with the enzyme mixture B has been determined for samples in which the levels of sucrose exceeded the theoretically calculated fructan levels. Samples in which no significant difference was determined and all levels below 0.1 g/ 100 g are referred to as n.d. (not detected) in further discussions. A flow chart summarizing the extraction procedure and the fructan determination according to Ispiryan et al. (2019) is illustrated in Figure S1 (supporting information).

All extractions were carried out in duplicate. The results of the ingredients are presented in g analyte per 100 g sample on a dry weight basis (g/ 100 g DM), whereas the results of the products are additionally presented on the fresh weight basis (“as is”). The weight before and after freeze-drying was recorded and used for the calculation of the FODMAP contents on the “as is” basis. The dry matter of the ingredients and products after freeze-drying was determined according to AACC 44-15.02.

2.3. Reference analysis of fructans in oat.

The fructan assay kit K-FRUC (Megazyme, Bray, Ireland), last updated in October 2018, has been applied for the determination of fructans in oat flour and oat bran, as reference to the determination via HPAEC-PAD. The optimized assay contained in addition to exo- and endoinulinases also levanases; latter are specifically applied to cleave levan type fructans as well as highly branched fructans. Not in the kit supplied, α-galactosidase (E-AGLANP, Megazyme, Ireland, Bray) has been used to take into account interfering GOS. Samples extracts were incubated with the additional enzyme prior to the degradation with the first enzyme mixture, according to controls and precautions of the assay procedure.

2.4. Ash determination.

The ash contents of the different wheat flours, the wheat starch and the semolina were determined according to AACC 08-01.01, 08-17.01 and 08-12.01, respectively.

2.5. Statistical analysis.

The statistical analysis has been performed with SPSS Statistic 24 (IBM Corp., Armonk, NY, USA). Within the fructan analysis via HPAEC-PAD, a significant difference of the sucrose content in the sample and the fructose released from sucrose and potentially additional fructans after incubation with inulinase was determined by means of an independent t-test ($p = 0.05$); cf. Ispiryan et al. (2019) for details on the fructan analysis. One-way ANOVA followed by Tuckey's test ($p = 0.05$) have been applied to determine statistical significance between the fructan levels of the different wheat flours and wheat isolates.

3. Results and Discussion

3.1. FODMAP levels in the ingredients.

The FODMAP levels are reported in five groups of the ingredients. The categorization is based on the plant-origin of the ingredients and their composition, the typical use and the type of the ingredients.

3.1.1. Group I – fructan containing cereals.

Wheat, spelt, rye and barley are commonly used ingredients in cereal products. Amongst them wheat is the basic ingredient in a number of staple foods being part of the western diet, such as bread and pasta. Depending on the product type also milling fractions or isolates from different components from the wheat grains are applied in formulations. Their HPAEC-PAD profiles and the FODMAP levels are presented in Figure 1 and Table 2. The abundant FODMAPs in wheat as well as in the other cereals, were fructans ranging from 0.85 - 1.88 g/ 100 g DM (Figure 2). The analysis of the different wheat flours, revealed similar values as reported by Haskå et al. (2008). Cereal fructans, such as those found in wheat, spelt, rye or barley are of the branched, graminan-type and contain β (2 → 1) as well as β (2 → 6) linked fructose monomers to the terminal sucrose. They are mainly located in the outer layers of the wheat grain (Haskå et al., 2008). Thus, the bran contained the highest amounts of fructans (3.40 g/ 100 g DM), whereas the lowest level was detected in the baker's flour with 1.19 g/ 100 g DM, followed by the whole meal (1.88 g/ 100 g DM). The difference in these two flours is resulting from the lower extraction-rate of the baker's flour, which thus contains fewer outer parts from the grain. The ash content of a flour is characteristic for the extraction-rate of the flour-product and correlates also with the fructan levels in the flour. Both components of the wheat grain, the minerals as well as the fructans, are mainly located in the outer layers of the grains (Figure S2, supporting information). Thus, the fructan content of commercial flours has a strong dependence on the extraction-rate of the flour-product.

Biscuit flour, baker's flour and semolina are produced from different varieties of wheat, namely soft, hard and durum wheat, respectively. The differentiation of soft wheat and hard wheat for non-breadmaking and breadmaking wheat is according to the North American terminology. Wheat, which is easier to crush (soft wheat) is used for biscuits, while wheat which is harder to crush (hard wheat) is used for breadmaking (Delcour and Hoseney, 2010). The fructan contents of baker's flour from hard wheat and durum wheat semolina were similar (1.19 and 1.20 g/ 100 g DM), whereas biscuit flour produced from soft wheat had slightly higher fructan

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158 levels (1.48 g/ 100 g DM). Spelt, which also represents a wheat species, contained lower
159 amounts of fructans (0.85 g/ 100 g DM) than the flours from soft wheat, hard wheat and the
160 durum wheat semolina. Ziegler et al. (2016) determined the fructan contents in a number of
161 different varieties of bread wheat, spelt as well as durum wheat. In contrast to the results in
162 this study, no significant difference in the fructan-contents in spelt and bread wheat
163 (corresponding to hard wheat in Northern American terminology) was determined, while
164 fructans in durum were slightly lower. However, the fructan levels of the different varieties of
165 each species varied significantly. Thus, as Ziegler et al. (2016) state, a general categorization
166 of the different species of wheat to contain higher or lower levels of fructans is not possible.
167 Furthermore, as mentioned above in the context of baker's flour and whole meal, commercial
168 flour-products have different extraction rates. The spelt flour analyzed in this study was 'Type
169 630'. The type number corresponds to the extraction rate of a flour. It reflects the ash-content
170 (%) multiplied by 1000 (Belitz et al., 2009). Hence, a flour of 'Type 630' contains less parts of
171 the outer layer of the grain, than flours from the whole grain which have a Type-number of
172 > 1000 (Belitz et al., 2009).
173 The analysis of the isolates from wheat, revealed that about one third of the wheat fructans
174 were detected in the gluten-isolate (0.60 g/ 100 g DM), unlike the wheat starch which did not
175 contain any soluble carbohydrates. Thus, the contribution of the gluten-ingredient to the total
176 FODMAP content should be considered for applications in low FODMAP products.
177 Nemeth et al. (2014) reported that the content of fructans in barley is dependent on breeding
178 and cultivar of the plant, with levels ranging from 0.9 - 4.2 g/ 100 g DM. The barley grains of
179 the variety Beatrix (brewer's barley), analyzed in this study, contained 1.38 g fructans in
180 100 g DM. The fructan levels in rye (3.61 g/ 100 g DM), were comparable to those found in
181 other studies and significantly higher than the levels in wheat, spelt and barley (Karppinen et
182 al., 2003). The average degrees of polymerization in the cereals investigated were 4 – 9 with
183 the shortest chain-lengths in spelt and barley and the longest chain-length in rye. Previous
184 studies reported similar degrees of polymerization for these cereals (Nemeth et al., 2014;
185 Verspreet et al., 2012).

Furthermore, GOS were found in the ingredients of Group I. The main representative of those oligosaccharides is the trisaccharide raffinose. Stachyose occurs only in low levels or in traces (Henry and Saini, 1989). The next higher saccharide in this series of oligosaccharides, verbascose, was not found in any of these ingredients. The GOS levels ranged from 0.06 g/ 100 g DM in the baker's flour to 0.56 g/ 100 g DM in barley. No other FODMAPs were found in considerable amounts in any of the Group I ingredients. The disaccharide lactose was not detected in any of the ingredients of Group I - V (Figure 2) and will not be further discussed for the following groups. Only very low amounts of fructose were determined, which did not exceed the levels of glucose in any of the samples. The sugar alcohols sorbitol and mannitol were detected in very low levels only (0.01 – 0.04 g/ 100 g DM). This would not be clinically relevant in food products, according to the cutoff level of 0.4 g total polyols per serving, defined by Varney et al. (2017).

3.1.2. Group II – low FODMAP and gluten-free cereals and pseudo-cereals.

The ingredients of this group, the cereals oat, millet and rice, the pseudo-cereals quinoa and buckwheat as well as the fractions and isolates oat bran, corn starch and potato starch are basic ingredients of most gluten-free products.

Apart from being gluten-free, all these ingredients are also very low in FODMAPs (cf. HPAEC-PAD profiles in Figure 3 and values in Table 2). Only low to moderate levels of GOS were detected, with the lowest levels in quinoa and the highest levels in oat bran (0.09 and 0.33 g/ 100 g DM, respectively).

None of these ingredients contained fructans at levels above 0.1 g/ 100 g DM. However, fructan levels in oat found in literature were contradictory to the findings in this study. Dodevska et al. (2013) as well as Biesiekierski et al. (2011) determined 0.35 % and 0.32 % fructans in oat flakes (as eaten, moisture not excluded from total weight), respectively. Given that oat contains ~ 0.3 % GOS, additional 0.3 % fructans would add up to 0.6 %; this would exceed the cutoff level for oligosaccharides (0.3 g/ serving; Varney et al., 2017). Both studies used the HK-FRUC Megazyme assay without correction of the GOS-interference with α-galactosidase

(Biesiekierski et al., 2011; Dodevska et al., 2013). Since inulinases used in the assay to cleave fructans also cleave the terminal fructose from GOS, fructan-results from the assay, obtained without the addition of α-galactosidase are overestimated. However, high levels of fructans were reported in other parts of the oat plant, such as stems and leaves (Livingston et al., 1993). These fructans have in contrast to other cereal fructans a different structure. As described in section 3.1.1, most cereals contain graminan-type fructans where fructose units are β (2 → 1) and β (2 → 6) linked to the terminal sucrose. The fructans found in oat hay are called neolevan-type fructans; they are branched or linear and consist of β (2 → 1) and/or β (2 → 6) linked fructose chains with an internal glucose residue. Fructan molecules consisting of exclusively β (2 → 6) bound fructose chains were also reported in parts of the oat plant (Livingston et al., 1993). The enzymes supplied in the HK-FRUC assay as well as in the inulinases applied for the fructan determination via HPAEC-PAD in this study, are specifically cleaving inulin-type and graminan-type fructans but underestimate levan-type fructans (McCleary et al., 2019). The neolevan-type fructans with exclusively β (2 → 6) bound fructose are similar to levan-type fructans. An updated version (October 2018) of the K-FRUC Megazyme assay contains in addition to the inulinases also levanases in the fructan cleaving enzyme mixture. Thus, in order to exclude an underestimation of potential fructans in oat the optimized assay has been applied. However, the analysis of the whole oat flour and the oat bran with that assay, alike the HPAEC-PAD determination, revealed that no fructans were detectable.

No other carbohydrates which are considered as FODMAPs were found in these ingredients. Yet, the FODMAPs determined in this study, as well as in other FODMAP-literature, are not the only non-digestible and readily fermentable dietary sugars. Other carbohydrates such as passively absorbed monosaccharides other than fructose, the disaccharide lactulose or oligosaccharides other than fructans and GOS may also be considered as FODMAPs (Halmos and Gibson, 2019).

Based on the findings of this study the classification of buckwheat may change from a low FODMAP to a high FODMAP ingredient. To date, there is no other scientific study, where the

241 FODMAP profile of buckwheat was determined. As shown in Table 2, buckwheat did not
242 contain any of the FODMAPs commonly analyzed. Also, according to the mobile-app from the
243 Monash University, buckwheat is listed as a low FODMAP grain (Monash University, 2019).
244 However, oligosaccharides called fagopyritols represent the majority of soluble carbohydrates
245 in buckwheat. These compounds are α-galactosides from D-*chiro*-inositiol and occur in levels
246 up to ~5 %, with fagopyritol B1 as the most abundant representative (Horbowicz et al., 1998).
247 An estimation of the peak in the HPAEC-PAD profile suspected to be fagopyritol B1 (Figure 3)
248 as sucrose revealed an approximate concentration of ~1 g/ 100 g DM; a reference standard
249 was not available. These oligosaccharides have similar structural properties to GOS (Figure
250 3) and require the enzyme α-galactosidase for the hydrolysis and absorption into the intestinal
251 mucosa; it is well known that the human gut lacks this enzyme. Fagopyritols are, thus, non-
252 digestible, fermentable carbohydrates. On the one hand, there have been studies indicating a
253 beneficial, blood glucose lowering effect from inositol and fagopyritols for diabetes patients
254 (Kawa et al., 2003). On the other hand, there is evidence, that these carbohydrates may have
255 a similar impact on a sensitive gut, such as in IBS patients, as GOS found in pulses (Horbowicz
256 et al., 1998). In addition to fagopyritols and GOS, buckwheat and pulses contain so called
257 cyclitols and their α-galactosides. These are precursor and intermediate compounds of the
258 biosynthesis of fagopyritols and GOS (Horbowicz et al., 1998; Martínez-Villaluenga et al.,
259 2008). Also these small carbohydrates may cause an altered bowel habit, similar to polyols.
260 *In vitro* and *in vivo* studies are required to support this hypothesis.

261 *3.1.3. Group III – seeds and flours from pulses.*

262 Pulses have traditionally been consumed along with cereals. Nowadays cereal products,
263 including bakery products as well as beverages based on, or with the addition of pulses have
264 gained major importance. This trend appeared due to their composition, particularly the high
265 protein-content. Pulse ingredients are applied in bakery products, providing beneficial techno-
266 functional properties and leading to products with a high nutritional value. However, pulses
267 generally reveal in their native composition a high FODMAP content. Thereby GOS are the

main saccharides in pulses, described as FODMAPs, with up to 10 % (Martínez-Villaluenga et al., 2008).

In accordance with literature, stachyose was the predominant GOS detected in all ingredients of this group. Raffinose appeared in lower levels, whereas the levels of verbascose ranged from 0.05 g in chickpea-flour up to 3.45 g of 100 g DM in fababean-flour (Figure 2, Table 2). These two ingredients also represented the two extremes of the total GOS levels, with 2.11 g in the chickpea-flour and 4.87 g of 100 g DM in the fababean-flour; the amounts analyzed in all pulses were within the ranges described in other studies (Kannan et al., 2018; Martínez-Villaluenga et al., 2008).

No other FODMAPs were determined in substantial amounts. No fructans were found in any of the pulses. However, besides the fact that very little information on the quantification of fructans in raw pulses is available, some studies described moderate to very high fructan levels in cooked pulses. Dodevska et al. (2013) determined 0.8 - 1.5 g/ 100 g DM fructans in beans and lentils, respectively. As discussed in section 3.1.2. and in a previous study this overestimation is due to the interference of GOS in the analysis using the enzyme assay without the appropriate correction (Biesiekierski et al., 2011; Dodevska et al., 2013; Ispiryan et al., 2019). In accordance with the findings in this study, Huynh et al. (2008), did not determine any fructans in fababeans and pea, applying a similar method of fructan determination via HPAEC-PAD after enzymatic hydrolysis and with the inclusion of α-galactosidase. McCleary et al. (2019) conducted different trials, presenting the GOS-interference in the Fructan Assay Kits. They presented an apparent fructan content of 2.85 - 3.05 % in mung beans (contain 3.05 % GOS). The analysis with the inclusion of α-galactosidase revealed values below the limit of quantification of the assay (0.1 - 0.11 %) (McCleary et al., 2019).

Furthermore, as discussed for Group II, the precursor and intermediate derivates of GOS, cyclitols and their α-galactosides, are also found in pulses, and may have a similar effect on the GI-tract as GOS, thus contributing to even higher FODMAP levels.

295 *3.1.4. Group IV – pulse protein ingredients.*

296 Since pulses are a very good sources of protein, they are often used as raw material to
297 produce protein-isolates (PI; ~ 90 % protein) or protein concentrates (PC; 40 - 75 % protein)
298 as ingredients for the food industry; these are used for instance for protein fortification to
299 increase the nutritional value (e.g. in gluten-free products), and as techno-functional
300 ingredients to improve the rheology properties of the end-products (Singhal et al., 2016).
301 The FODMAP levels, in particular GOS, in the different PI and PC varied highly. The PI from
302 fababean (85 % protein content) and lupin (94 % protein content), which were developed as
303 part of an EU (Protein2Food project, grant no. 635727) project in Fraunhofer Institute (IVV),
304 contained only traces of GOS. In contrast to that the commercial protein ingredients from carob
305 (48 % protein content) and lupin (38 - 42 % protein content) contained very high amounts of
306 GOS, 5.51 g and 10.74 g of 100 g DM, respectively. Carob (locust bean), also belongs to
307 pulses and is rich in GOS, as shown in Table 2. However, this is the first study reporting the
308 contents of GOS in carob. The occurrence of raffinose, stachyose and verbascose in carob
309 seeds was identified qualitatively (Amuti and Pollard, 1977). In lupin seeds, similarly high
310 values of 9.1 ± 2.6 g/ 100 g DM were determined in another study (Andersen et al., 2005). The
311 lupin PC had the highest levels of GOS in all pulses (Figure 2). Due to the high amount of
312 GOS in both PC, presumably the preparation of these commercial protein ingredients did not
313 involve a separation of the soluble carbohydrates. In contrast to this, the PI from fababean
314 and lupin, were obtained by isoelectric point precipitation. Within that preparation process,
315 soluble carbohydrates, including GOS were removed. The commercial PI from pea (85 %
316 protein content) also contained moderate levels of GOS (1.16 g/ 100 g DM); the production
317 process is not known.
318 The results in Table 2 reveal that different approaches to produce pulse protein ingredients,
319 have a major impact on the FODMAP contents and should thus be investigated prior to low
320 FODMAP applications.

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The application of the PC from carob and lupin or the PI from pea, may result in high FODMAP-products, depending on the proportion of the ingredient in the formulation. In contrast to this for instance the PI from fababean and lupin may directly be used in any type of products.

3.1.5. Group V – commercial sprouts.

Sprouted grains and seeds are applied in bakery products. High enzyme activities favour techno-functional properties and a higher bioavailability of nutrients increases the nutritional value. Horstmann et al. (2019) for instance, applied different sprouts in gluten-free bread systems. These ingredients displayed functional attributes relating to higher dough quality as well as improved nutritional value of the bread.

The flours from the commercial sprouts from buckwheat and quinoa did not contain substantial amounts of any carbohydrate, currently considered as FODMAPs (Figure 2, Table 2). The semi quantitatively determined amount of fagopyritol B1 in the buckwheat sprouts was lower in comparison to the buckwheat flour (0.29 vs. 0.89 g/ 100 g DM).

In pulses, such as in lupin, lentil or pea, germination or sprouting have been often reported to be effective for the removal of GOS; depending on the germination conditions the levels of α-galactosides can be diminished to different extents. This effect has been identified to occur due to increased activities of the native enzyme from the pulses, α-galactosidase, which cleaves the α (1 → 6) linkages in GOS molecules (Kannan et al., 2018; Martínez-Villaluenga et al., 2008).

The GOS levels in the commercial lentil and lupin sprouts (2.03 g and 3.44 g in 100 g DM, respectively) were markedly lower than in the lentil flour and the commercial lupin PC (3.98 g and 10.74 g in 100 g DM, respectively). Also the flour from sprouted peas contained less GOS (3.69 g/ 100 g DM) than the flour from raw green and yellow peas (4.48 g and 4.75 g/ 100 g DM, respectively). Nevertheless, the lower amounts were still relatively high, considering the clinical cutoff level of 0.3 g oligosaccharides per serve (Varney et al., 2017). However, only small levels (5 %) are added to the bread formulations (Horstmann et al., 2019). Thus, even though the commercial sprouts from pulses still contained relatively high levels of

GOS, resulting bakery products may still be considered as low in FODMAPs, depending on other components of the recipes.

3.2. FODMAP levels in commercial cereal-products and their gluten-free alternatives.

The FODMAP profiles of commonly consumed, representative cereal-products from the Irish market from different categories, including bread, pasta, biscuits and crackers and their gluten-free alternatives were determined and serve as benchmarks for further low FODMAP product development

The FODMAP levels of all products are presented in Table 3. Furthermore, Table 3 shows whether, the products meet the low FODMAP criteria established by Varney et al. (2017) The white wheat loaf, the Irish soda breads as well as the sourdough bread were all based on wheat as the basic ingredient (cf. Table 1). As demonstrated in section 3.1.1. the main FODMAP in wheat are fructans (1.19 - 1.88 g/ 100 g DM). However, the FODMAP profiles of the breads differed greatly. The white wheat loaf contained only low levels, 0.14 g/ 100 g (0.22 g/ 100 g DM), of fructans. From the list of ingredients, it can be seen that the bread was produced from a yeast-fermented dough. Different studies have shown that yeast-fermentation is capable to degrade the fructans in the wheat flour (Struyf et al., 2018; Ziegler et al., 2016). However, the hydrolysis of the fructans by the yeast-invertase (in the case of baker's yeast) leads to the release of fructose and thus fructose in excess of glucose. The white bread analyzed in this study contained 0.19 g/ 100 g (0.30 g/ 100 g DM) fructose in excess of glucose. A number of studies reported the degradation of fructans in bread by different yeast under different conditions. For instance Ziegler et al. (2016) have shown, the amount of fructose remaining in the bread after the fermentation with baker's yeast is dependent on the fermentation time. In contrast to this, soda breads are produced without any fermentation; chemical leavening with soda leads to the production of gas during the baking process and hence, the higher bread volume. Thus, high amounts of fructans, 1.07 g (1.81 g) and 1.32 g/ 100 g (2.30 g/ 100 g DM), in the brown soda bread and the whole wheat soda bread, respectively, were determined. The fructan content in the whole wheat soda bread was

higher due to the additional wheatgerm, and, presumably, slightly different proportions of whole meal flour and wheat flour in the recipes. Furthermore, both breads contained lactose (0.50 g and 0.79 g/ 100 g), the main sugar found in milk; both formulations included ~40 % buttermilk. Also in the sourdough bread only moderate levels of fructans (0.41 g [0.69 g] / 100 g [DM]), were found, due to the fermentation with lactic acid bacteria (LAB) and yeast in sourdoughs (Loponen and Gänzle, 2018). In comparison to the yeast-fermented white loaf, the sourdough bread contained more fructans. However, the extent of fructan degradation is dependent on different fermentation conditions and most importantly the application of different species and strains. Low levels of mannitol were also detected (0.19 g [0.33 g] /100 g [DM]). Sourdough bread, is fermented with yeast and a range of LAB. This bread, called “San Franciscan Sourdough” was fermented amongst other LAB with *Lactobacillus sanfranciscensis*, which has an obligately heterofermentative metabolism (Gobbetti and Corsetti, 1997). Certain heterofermentative LAB possess the enzyme mannitol-dehydrogenase, which reduces the fructose released from the fructan-hydrolysis to mannitol. Depending on the fermentation conditions and the substrates available, some LAB are capable to produce very high levels of mannitol (Sahin et al., 2019). Lastly, the gluten-free bread, did not contain any ingredient which is naturally high in FODMAPs (cf. Table 1 and Figure 2), except for the pea protein, which may contain higher GOS levels, depending on the production of that ingredient (cf. section 3.1.4.). However, the pea protein is only a small portion of the formulation. Thus the bread had overall a very low FODMAP-profile.

Two different wheat-based crackers were analyzed; the plain crackers had an overall low FODMAP-content with 0.20 g/ 100 g (0.21 g/ 100 g DM) fructans and 0.05 g/ 100 g (0.06 g/ 100 g DM) fructose in excess of glucose. According to the ingredients list (Table 1), also this product was produced from a yeast-fermented dough. The wheat-based garlic crackers on the contrary, had high amounts of fructans, deriving from the wheat flour and the garlic powder in the formulation; latter is a rich source of fructans (Muir et al., 2009). The garlic crackers contained additionally high levels of fructose in excess of glucose 0.34 g/ 100 g (0.36 g/ 100 g DM), since also the dough of these crackers was fermented with yeast. Similar

to the gluten-free bread, also the gluten-free oat-based crackers did not contain substantial amounts of any FODMAPs. Low levels of fructose in excess of glucose were found (0.11 g [0.12 g] /100 g [DM]), deriving from the honey in the formulation, and low levels of GOS (0.20 g [0.21 g] /100 g [DM]), which naturally occur in oats, were detected

The wheat-based biscuits contained, besides high fructan levels (1.34 g [1.41 g] /100 g [DM]), also high amounts of lactose (0.96 g [1.00 g] /100 g [DM]), originated from milk as part of the formulation. The gluten-free oat-based biscuits, did not contain any high FODMAP ingredients and thus only low levels of GOS (0.13 g [0.14 g] /100 g [DM]) were detected.

The pasta was analyzed before and after cooking. The durum wheat pasta contained 1.34 g/ 100 g (1.50 g/ 100 g DM) fructans before, and 0.32 g/ 100 g (0.92 g/ 100 g DM) after cooking; 40 % of the wheat-fructans were lost in the cooking water. This corresponds to findings from Gélinas et al. (2016). The gluten-free pasta, which was made from the low FODMAP ingredients corn flour and rice flour (Figure 2), did consequently not contain any FODMAPs.

3.3. Conclusion

This work is the first comprehensive study providing a dry matter-based characterization of the FODMAP-profiles of a wide range of cereal-product ingredients. Existing FODMAP-literature predominantly serves as nutritional guidance for individuals adhering to the low FODMAP diet. This study, on the contrary, serves as a tool for the development of functional food products with a lowered FODMAP content. The extensive knowledge on compositional information of the raw ingredients enables a targeted application of (bio-) technological approaches to lower FODMAP levels.

In accordance with other studies wheat, rye, spelt and barley were confirmed as rich sources of fructans, while pulses had high GOS levels (Biesiekierski et al., 2011; Haskå et al., 2008; Martínez-Villaluenga et al., 2008; Nemeth et al., 2014). Protein ingredients from pulses had varying levels of GOS, depending on their production process. Commercial sprouts from pulses contained moderately high amounts of GOS, despite the GOS-degrading effect of the

sprouting process (Kannan et al., 2018; Martínez-Villaluenga et al., 2008). The gluten-free cereals oat, millet and rice as well as the pseudo-cereals quinoa and buckwheat did not contain substantial amounts of any of the FODMAPs investigated. However, buckwheat, which is currently listed as low FODMAP grain, was outstanding due to the major fraction of soluble carbohydrates, fagopyritols. Those sugars are non-digestible, fermentable and structurally similar to GOS and may have a similar effect on a sensitive gut. Further studies are needed to identify the contribution of fagopyritols to the flatulence-causing effect in IBS-patients. In addition to the FODMAPs found in the ingredients, the relevance of fructose in excess of glucose, polyols and lactose was highlighted by means of the analysis of representative, commonly consumed cereal-products of different categories. This study is the foundation for the development of high-quality cereal products with a lowered FODMAP content and improved flavour and nutritional properties.

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REFERENCES

Amuti, K.S., Pollard, C.J., 1977. Soluble carbohydrates of dry and developing seeds. Phytochemistry 16, 529–532.

Andersen, K.E., Bjergegaard, C., Møller, P., Sørensen, J.C., Sørensen, H., 2005. Compositional variations for α-galactosides in different species of Leguminosae, Brassicaceae, and barley: A chemotaxonomic study based on chemometrics and high-performance capillary electrophoresis. J. Agric. Food Chem. 53, 5809–5817.

Belitz, H.-D., Grosch, W., Schieberle, P., 2009. Food Chemisty, 4th ed. Springer-Verlag Berlin Heidelberg 2009.

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-oligosacharides and other short-chain carbohydrates in processed grains and cereals. J. Hum. Nutr. Diet. 24, 154–176.

Delcour, J.A., Hoseney, R., 2010. Principles of Cereal Science and Technology, 3rd ed. AACC International, Inc.

Dodevska, M.S., Djordjevic, B.I., Sobajic, S.S., Miletic, I.D., Djordjevic, P.B., Dimitrijevic-Sreckovic, V.S., 2013. Characterisation of dietary fibre components in cereals and legumes used in Serbian diet. Food Chem. 141, 1624–1629.

Gélinas, P., Mckinnon, C., Gagnon, F., 2016. Fructans, water-soluble fibre and fermentable sugars in bread and pasta made with ancient and modern wheat. Int. J. Food Sci. Technol. 51, 555–564.

Gobbetti, M., Corsetti, A., 1997. Lactobacillus sanfrancisco a key sourdough lactic acid bacterium: A review. Food Microbiol. 14, 175–187.

Halmos, E.P., Gibson, P.R., 2019. Controversies and reality of the FODMAP diet for patients with irritable bowel syndrome. J. Gastroenterol. Hepatol. 34, 1134–1142.

Haskå, L., Nyman, M., Andersson, R., 2008. Distribution and characterisation of fructan in wheat milling fractions. J. Cereal Sci. 48, 768–774.

Henry, R.J., Saini, H., 1989. Characterization of Cereal Sugars and Oligosaccharides. Cereal Chem. 66, 362–365.

Horbowicz, M., Brenac, P., Obendorf, R.L., 1998. Fagopyritol B1, O-α-D-galactopyranosyl-(1→2)-D-chiro-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. Planta 205, 1–11.

Horstmann, S.W., Atzler, J.J., Heitmann, M., Zannini, E., Lynch, K.M., Arendt, E.K., 2019. A comparative study of gluten-free sprouts in the gluten-free bread-making process. Eur. Food Res. Technol. 245, 617–629.

Huynh, B.L., Palmer, L., Mather, D.E., Wallwork, H., Graham, R.D., Welch, R.M., Stangoulis, J.C.R., 2008. Genotypic variation in wheat grain fructan content revealed by a simplified HPLC method. J. Cereal Sci. 48, 369–378.

Ispiryan, L., Heitmann, M., Hoehnel, A., Zannini, E., Arendt, E.K., 2019. Optimization and Validation of a HPAEC-PAD Method for the Quantification of FODMAPs in Cereals and Cereal-based Products. J. Agric. Food Chem. 67, 4384–4392.

Kannan, U., Sharma, R., Gangola, M.P., Chibbar, R.N., 2018. Improving grain quality in pulses:

strategies to reduce raffinose family oligosaccharides in seeds. Ekin J. Crop Breed. Genet. 4, 70–88.
Karppinen, S., Myllymäki, O., Forssell, P., Poutanen, K., 2003. Fructan content of rye and rye products. Cereal Chem. 80, 168–171.
Kawa, J.M., Taylor, C.G., Przybylski, R., 2003. Buckwheat Concentrate Reduces Serum Glucose in Streptozotocin-Diabetic Rats. J. Agric. Food Chem. 51, 7287–7291.
Livingston, D.P., Chatteron, N.J., Harrison, P.A., 1993. Structure and quantity of fructan oligomers in oat (*Avena* spp.). New Phytol. 123, 725–734.
Loponen, J., Gänzle, M., 2018. Use of Sourdough in Low FODMAP Baking. Foods 7, 96.
Martínez-Villaluenga, C., Frias, J., Vidal-Valverde, C., 2008. Alpha-galactosides: Antinutritional factors or functional ingredients? Crit. Rev. Food Sci. Nutr. 48, 301–316.
McCleary, B. V., Charmier, L.M.J., Mckie, V.A., Mcloughlin, C., Rogowski, A., 2019. Determination of fructan (inulin, fos, levan, and branched fructan) in animal food (animal feed, pet food, and ingredients): Single-laboratory validation, first Action 2018.07. J. AOAC Int. 102, 883–892.
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). J. Agric. Food Chem. 57, 554–565.
Nemeth, C., Andersson, A.A.M., Andersson, R., Mangelsen, E., Sun, C., Åman, P., 2014. Relationship of Grain Fructan Content to Degree of Polymerisation in Different Barleys. Food Nutr. Sci. 05, 581–589.
Sahin, A.W., Rice, T., Zannini, E., Axel, C., Coffey, A., Lynch, K.M., Arendt, E.K., 2019. *Leuconostoc citreum* TR116: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns. Int. J. Food Microbiol. 302, 80–89.
Struyf, N., Vandewiele, H., Herrera-Malaver, B., Verspreet, J., Verstrepen, K.J., Courtin, C.M., 2018. *Kluyveromyces marxianus* yeast enables the production of low FODMAP whole wheat breads. Food Microbiol. 76, 135–145.
Varney, J., Barrett, J., Scarlata, K., Catsos, P., Gibson, P.R., Muir, J.G., 2017. FODMAPs: food composition, defining cutoff values and international application. J. Gastroenterol. Hepatol. 32, 53–61.
Verspreet, J., Dornez, E., van den Ende, W., Delcour, J.A., Courtin, C.M., 2015. Cereal grain fructans: Structure, variability and potential health effects. Trends Food Sci. Technol. 43, 32–42.
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. J. Agric. Food Chem. 60, 2102–2107.
Ziegler, J.U., Steiner, D., Longin, C.F.H., Würschum, T., Schweiggert, R.M., Carle, R., 2016. Wheat and the irritable bowel syndrome - FODMAP levels of modern and ancient species and their retention during bread making. J. Funct. Foods 25, 257–266.

WEB REFERENCES

Edwards, N., 2017. Genesis R&D Food Documentation. Reference Amounts Customarily Consumed (RACC) Bakery Products. Accessed at: https://esharesearch.atlassian.net/wiki/spaces/GENFOOD/pages/133070950/RACC+Bakery+Products.
Monash University, 2019. The Monash University Low FODMAP App. Accessed at: http://www.med.monash.edu/ cecs/gastro/fodmap/.
Singhal, A., Karaca, A.C., Tyler, R., Nickerson, M., 2016. Pulse Proteins: From Processing to Structure-Function Relationships. In: Goyal A.K. (ed.), Grains Legumes, IntechOpen, DOI:10.5772/64020. Accessed at: https://www.intechopen.com/books/grain-legumes/pulse-proteins-from-processing-to-structure-function-relationships, pp. 55–78.

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Tables

Table 1. Selected commercial cereal products available on the Irish market

Product	Ingredients on packaging	Nutritional value/100g
	Bread	
White wheat loaf	Wheat Flour (Wheat, Calcium Carbonate, Iron, Thiamin, Niacin), Water, Yeast, Salt, Vegetable Oil (Rapeseed), Soya Flour, Emulsifier: E472e, Flour Treatment Agent: Ascorbic Acid (Vitamin C), Vegetable Fat (Palm).	Energy (kcal) 219, Energy (kJ) 920, Protein (g) 8.7, Carbohydrate (g) 43, Sugars (g) 2.42, Fat total (g) 1.4, Saturated (g) 0.4, Dietary fibre (mg) 2.8, Sodium (g) 1,1
Brown Soda bread	Wheatmeal, Buttermilk (38%), Wheatflour, Sugar, Rapeseed Oil, Raising Agent: Sodium Hydrogen Carbonate, Salt.	Energy (kcal) 239, Energy (kJ) 1010, Protein (g) 8.5, Carbohydrate (g) 43, Sugars (g) 4.2, Fat total (g) 2.45, Saturated (g) 0.32, Dietary fibre (g) 5.65, Sodium (g) 1.7
Wholewheat Soda bread	Fresh Buttermilk (36%), Wholemeal Wheat Flour (36%), Wheat Flour, Raising Agents: Sodium Hydrogen Carbonate, Diphosphates, Salt, Wheatgerm.	Energy (kcal) 219, Energy (kJ) 927, Protein (g) 7.9, Carbohydrate (g) 41.3, Sugars (g) 1.75, Fat total (g) 1.0, Saturated (g) Trace, Dietary fibre (g) 6.5, Sodium (g) 1.4
Wheat sourdough bread (San Franciscan style sourdough)	Wheat flour, water, salt, yeast	Energy (kcal) 245, Energy (kJ) 1025, Protein (g) 9.2, Carbohydrate (g) 48.8, Sugars (g) 1.3, Fat total (g) 1, Saturated (g) 0.2, Dietary fibre (mg) 3, Sodium (g) 1.4
Gluten free white loaf	Water, Potato Flour, Corn Starch, Tapioca Starch, White Rice Flour, Buckwheat Flour, Thickening Agent (Xanthan Gum, Cellulose, Agar), Rice Bran, Pea Protein, Yeast, Sourdough (Fermented Quinoa, Rice and Maize Flour), Psyllium Husk, Salt, Rapeseed Oil, Flour Treatment Agent (Ascorbic Acid), Acidifier (Glucono-Delta-Lactone), Acids (Citric Acid, Malic Acid, Tartaric Acid).	Energy (kcal) 200, Energy (kJ) 838, Protein (g) 7.7, Carbohydrate (g) 35.7, Sugars (g) 0.6, Fat total (g) 1,1, Saturated (g) 0.3, Dietary fibre (mg) 8.3, Sodium (g) 1.05
	Crackers	
Wheat-based garlic crackers	Wheat Flour (Wheat Flour, Niacin, Iron, Thiamin, Riboflavin, Folic Acid), Sunflower Oil, Garlic Powder (3.5%), Palm Oil, Salt, Sugar, Rice Flour, Inactive Yeast (Wheat, Barley), Cane Sugar Syrup, Flavouring, Yeast.	Energy (kcal) 488, Energy (kJ) 2045, Protein (g) 8.1, Carbohydrate (g) 6.4, Sugars (g) 2.4, Fat, total (g) 21.5, Saturated (g) 3.4, Dietary fibre (g) 4.4, Sodium (g) 1.0
Wheat-based plain crackers	Flour (Wheat Flour, Calcium, Iron, Niacin, Thiamin), Vegetable Oil (Palm), Salt, Raising Agent (Sodium Bicarbonate), Yeast.	Energy (kcal) 440, Energy (kJ) 1851, Protein (g) 10, Carbohydrate (g) 67.7, Sugars (g) 1.4, Fat total (g) 13.5, Saturated (g) 6.2, Dietary fibre (g) 3.8, Sodium (g) 1.3

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Gluten free oat-crackers	Wholegrain Oats (86%), Sustainable Palm Fruit Oil, Maize Starch, Sea Salt, Raising Agent: Ammonium Bicarbonate, Honey.	Energy (kcal) 460, Energy (kJ) 1922, Protein (g) 10.6, Carbohydrate (g) 58.9, Sugars (g) 1.8, Fat total (g) 16.8, Saturated (g) 6.6, Dietary fibre (g) 7.6, Sodium (g) 1.8
	Biscuits	
Wheat-based biscuits	Flour (54%) (Wheat Flour, Calcium, Iron, Niacin, Thiamin), Vegetable Oil (Palm), Wholemeal Wheat Flour (16%), Sugar, Partially Inverted Sugar Syrup, Raising Agents (Sodium Bicharbonate, Malic Acid, Ammonium Bicarbonate), Salt, Dried Skimmed Milk	Energy (kcal) 473, Energy (kJ) 1973, Protein (g) 7.3, Carbohydrate (g) 68.7, Sugars (g) 16.7, Fat total (g) 20.7, Saturated (g) 2, Dietary fibre (g) 3.3, Sodium (g) 1.3
Gluten free biscuits	Gluten Free Oat Flour (Oat Flour), Vegetable Margarine, Muscovado Sugar, Cornflour, Partially Inverted Sugar Syrup, Raising Agent (Sodium Bicarbonate), Flavouring, Vegetable Margarine contains: Palm Oil, Rapeseed Oil, Water, Salt, Emulsifier (Mono- and Di-Glycerides of Fatty Acids), Muscovado Sugar contains: Sugar, Molasses, Colour (Plain Caramel).	Energy (kcal) 476, Energy (kJ) 1998, Protein (g) 6.4, Carbohydrate (g) 66.9, Sugars (g) 24.8, Fat total (g) 19.4, Saturated (g) 7.6, Dietary fibre (g) 4.3, Sodium (g) 0.4
	Pasta	
Wheat spaghetti	Durum Wheat Semolina	Energy (kcal) 176, Energy (kJ) 748, Protein (g) 5.8, Carbohydrate (g) 35.7, Sugars (g) 1.1, Fat total (g) 0.7, Saturated (g) 0.2, Dietary fibre (g) 2.2, Sodium (g) 0.1
Gluten free spaghetti	Corn Flour 79.8%, Rice Flour 19.7%, Emulsifier: Mono and Diglycerides of Fatty Acids.	Energy (kcal) 356, Energy (kJ) 1510, Protein (g) 6.5, Carbohydrate (g) 79, Sugars (g) 0.5, Fat total (g) 1.5, Saturated (g) 0.5, Dietary fibre (g) 1.2, Sodium (g) 0.02

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Table 2. FODMAP contents of cereal-product ingredients

Ingredient	FODMAP contents ± standard deviation [g/100g DM] [a]											
	Mono-/Disaccharides [b, c]			Polyols [b]				Oligosaccharides				
	Glucose	Fructose	EF [d]	Xylitol (cyclitol) [e]	Sorbitol (cyclitol) [f]	Mannitol	∑	Raffinose/ Stachyose [b]	Verbascose (FP-B1) [b,g]	∑	Total fructan [h]	DP_{av}
Group I												
Whole meal	0.18 ± 0.02	0.07 ± 0.01	-	n.d.	0.04 ± 0.00	0.01 ± 0.00	0.05	0.14 ± 0.00	n.d.	0.14	1.88 ± 0.09	6.7
Bakers flour	0.06 ± 0.00	0.06 ± 0.00	-	n.d.	0.01 ± 0.00	n.d.	0.01	0.06 ± 0.00	n.d.	0.06	1.19 ± 0.00	5.6
Biscuit flour	0.07 ± 0.00	0.05 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.00	n.d.	0.09	1.48 ± 0.03	5.4
Semolina	0.13 ± 0.02	0.04 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.31 ± 0.00	n.d.	0.31	1.20 ± 0.02	4.3
Vital gluten	0.14 ± 0.00	0.18 ± 0.01	-	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00	n.d.	0.03	0.60 ± 0.00	4.4
Wheat starch	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Wheat bran	0.41 ± 0.03	0.15 ± 0.01	-	n.d.	0.02 ± 0.00	0.04 ± 0.00	0.06	0.41 ± 0.01	n.d.	0.41	3.40 ± 0.15	5.0
Spelt	0.07 ± 0.00	0.05 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.00	n.d.	0.13	0.85 ± 0.01	4.1
Rye	0.64 ± 0.02	0.08 ± 0.01	-	n.d.	n.d.	0.01 ± 0.00	0.01	0.13 ± 0.00	n.d.	0.13	3.61 ± 0.08	8.9
Barley (whole grains)	0.56 ± 0.02	0.05 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.56 ± 0.02	n.d.	0.56	1.38 ± 0.09	3.7
Group II												
Whole Oat flour	0.04 ± 0.00	0.03 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.29 ± 0.00	0.02 ± 0.00	0.31	n.d.	-
Oat bran	0.02 ± 0.00	0.03 ± 0.00	0.01	n.d.	n.d.	n.d.	n.d.	0.29 ± 0.00	0.04 ± 0.00	0.33	n.d.	-
Quinoa	0.26 ± 0.00	0.13 ± 0.00	-	n.d.	0.28 ± 0.01 [f]	n.d.	0.28	0.09 ± 0.00	n.d.	0.09	n.d.	-
Millet	0.06 ± 0.00	0.03 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.15 ± 0.00	n.d.	0.15	n.d.	-
Buckwheat flour	0.09 ± 0.00	0.05 ± 0.00	-	0.07 ± 0.00 [e]	0.17 ± 0.00 [f]	n.d.	0.24	0.01 ± 0.00	0.89 ± 0.00 [g]	0.01	n.d.	-
Brown rice	0.09 ± 0.01	0.02 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.01	n.d.	0.13	n.d.	-
Corn starch	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Potato starch	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Group III												
Lentil (whole seeds)	0.24 ± 0.01	0.04 ± 0.00	-	n.d.	0.95 ± 0.03 [f]	n.d.	0.95	2.54 ± 0.02	1.44 ± 0.01	3.98	n.d.	-

Chickpea flour	0.05 ± 0.00	n.d.	-	0.01 ± 0.00 [e]	n.d.	n.d.	0.01	2.05 ± 0.06	0.05 ± 0.00	2.11	n.d.	-
Soy (whole seeds)	0.11 ± 0.00	0.02 ± 0.00	-	0.04 ± 0.00 [e]	0.06 ± 0.01 [f]	n.d.	0.10	3.37 ± 0.04	0.19 ± 0.00	3.55	n.d.	-
Green pea (whole seeds)	0.14 ± 0.00	0.01 ± 0.00	-	n.d.	0.01 ± 0.00 [f]	n.d.	0.01	1.87 ± 0.05	2.61 ± 0.09	4.48	n.d.	-
Yellow pea (whole seeds)	0.13 ± 0.00	0.01 ± 0.00	-	n.d.	0.02 ± 0.00 [f]	n.d.	0.02	2.12 ± 0.05	2.63 ± 0.06	4.75	n.d.	-
Fababean (prot.rich flour)	0.13 ± 0.00	0.09 ± 0.00	-	n.d.	0.03 ± 0.00 [f]	n.d.	0.03	1.42 ± 0.01	3.45 ± 0.01	4.87	n.d.	-
Group IV												
Fababean prot. ** (85%)	0.01 ± 0.00	0.02 ± 0.00	0.01	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00	0.06 ± 0.00	0.08	n.d.	-
Carob prot. * (≥ 48%)	0.02 ± 0.00	0.05 ± 0.00	0.03	n.d.	n.d.	n.d.	n.d.	4.15 ± 0.02	1.36 ± 0.00	5.51	n.d.	-
Pea prot. * (≥ 83%)	0.02 ± 0.00	n.d.	-	n.d.	n.d.	n.d.	n.d.	0.57 ± 0.01	0.59 ± 0.00	1.16	n.d.	-
Lupin prot. ** (94%)	0.01 ± 0.00	0.01 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Lupin prot. * (≥ 38%)	0.05 ± 0.00	0.06 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	9.55 ± 0.02	1.19 ± 0.04	10.74	n.d.	-
Group V												
Quinoa sprouts	0.57 ± 0.05	n.d.	-	n.d.	n.d.	n.d.	n.d.	0.20 ± 0.01	n.d.	0.20	n.d.	-
Pea sprouts	0.19 ± 0.07	0.02 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	2.59 ± 0.04	1.1 ± 0.01	3.69	n.d.	-
Lupin sprouts	0.09 ± 0.01	0.01 ± 0.00	-	0.15 ± 0.00 [e]	n.d.	n.d.	0.15	3.44 ± 0.05	n.d.	3.44	n.d.	-
Buckwheat sprouts	0.59 ± 0.04	0.08 ± 0.00	-	0.10 ± 0.00 [e]	0.01 ± 0.00 [f]	n.d.	0.11	n.d.	0.29 ± 0.01[g]	n.d.	n.d.	-
Lentil sprouts	0.06 ± 0.00	0.17 ± 0.00	0.11	n.d.	n.d.	n.d.	n.d.	1.35 ± 0.02	0.68 ± 0.01	2.03	n.d.	-

[a] extractions carried out in duplicates and measured via HPAEC-PAD, results referred to dry matter (DM). [b] n.d., not detected or levels below 0.005 g / 100 g DM. [c] no lactose detected in any of the ingredients. [d] EF, excess fructose = fructose – glucose. [e,f] unidentified cyclitols suspected to be for instance chiro-inositol, myo-inositol or pinitol, estimated as xylitol or sorbitol, respectively. [g] FP-B1, fagopyritol B1, estimated as sucrose. [h] n.d., not detected in means of no significant difference in sucrose values and fructose values determined from difference of assay A and B in fructan determination, or levels below 0.1 g / 100 g DM. (*) commercial protein ingredients, (**) protein ingredients delivered from research projects

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Table 3. FODMAP contents of commercial cereal-products from Irish market

Products	FODMAP contents ± standard deviation [g/100g as is] [a]								
	Mono-/Disaccharides [b]				Polyols	Oligosaccharides			
	Glucose	Fructose	EF [c]	Lactose	∑Polyols (xylitol, sorbitol, mannitol)	∑GOS (raffinose, stachyose, verbascose)	Total fructan [d]	Serve [g] [e]	Meets low FODMAP criteria [f]
Bread									
White wheat loaf	0.11 ± 0.00	0.30 ± 0.01	0.19	n.d.	n.d.	0.01	0.14 ± 0.01		Yes
Brown Soda bread	0.22 ± 0.02	0.20 ± 0.01	-	0.50 ± 0.03	n.d.	0.05	1.07 ± 0.01		No
Wholewheat Soda bread	0.23 ± 0.01	0.07 ± 0.00	-	0.79 ± 0.01	n.d.	0.07	1.32 ± 0.06	50	No
Wheat sourdough bread	0.10 ± 0.00	0.06 ± 0.00	-	n.d.	0.21	n.d.	0.41 ± 0.00		Yes
Gluten free white loaf	0.10 ± 0.00	0.04 ± 0.00	-	n.d.	0.03	n.d.	n.d.		Yes
Crackers									
Wheat-based plain crackers	0.08 ± 0.00	0.13 ± 0.01	0.05	n.d.	n.d.	0.01	0.2 ± 0.01		Yes
Wheat-based garlic crackers	0.71 ± 0.01	1.05 ± 0.04	0.34	n.d.	n.d.	0.01	2.44 ± 0.05	30	No
Gluten free oat-crackers	0.36 ± 0.00	0.47 ± 0.00	0.11	n.d.	n.d.	0.20	n.d.		Yes
Biscuits									
Wheat-based biscuits	0.38 ± 0.02	0.31 ± 0.01	-	0.96 ± 0.08	n.d.	0.04	1.36 ± 0.09	30	No
Gluten free biscuits	0.45 ± 0.01	0.45 ± 0.01	-	n.d.	0.01	0.13	n.d.		Yes
Pasta									
Wheat spaghetti uncooked	0.18 ± 0.00	0.10 ± 0.00	-	n.d.	0.01	0.08	1.34 ± 0.11	55	No
Wheat spaghetti cooked	0.07 ± 0.01	0.02 ± 0.00	-	n.d.	n.d.	0.01	0.32 ± 0.03	140	No
Gluten-free spaghetti uncooked	0.20 ± 0.00	0.19 ± 0.01	-	n.d.	0.03	n.d.	n.d.	55	Yes
Gluten-free spaghetti cooked	0.03 ± 0.00	0.03 ± 0.00	-	n.d.	n.d.	n.d.	n.d.	140	Yes

[a] extractions carried out in duplicates and measured via HPAEC-PAD, results referred to fresh weight (as is). [b] n.d., not detected or levels below 0.005 g / 100 g DM. [c] EF, excess fructose = fructose – glucose. [d] n.d., not detected in means of no significant difference in sucrose values and fructose values determined from difference of assay A and B in fructan determination, or levels below 0.1 g/100 g DM. [e] serving sizes based on suggestions according Edwards, 2017. [f] cutoff levels per serve for each FODMAP according to Varney et al. (2017): 0.3 g oligosaccharides, 0.4 g polyols, 0.15 g excess fructose, 1 g lactose

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Figures

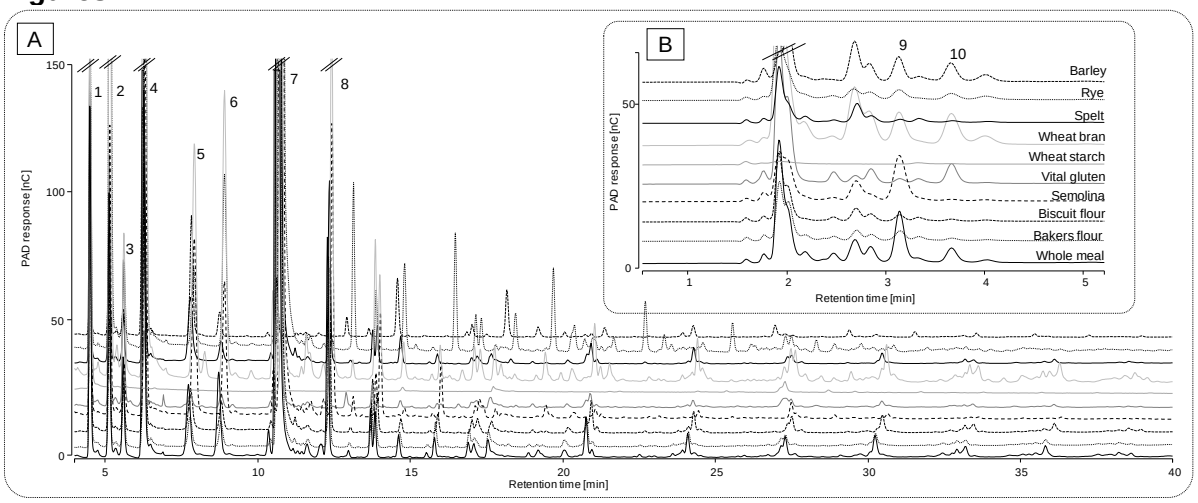


Figure 1. HPAEC-PAD (A) CarboPac PA200 and (B) CarboPac PA1 profiles of ingredients from Group I – fructan containing cereals with identical order of the profile in (A) and (B): (1) rhamnose [internal standard], (2) glucose, (3) fructose, (4) sucrose, (5) raffinose/stachyose, (6) kestose, (7) maltose, (8) DP3 fructan, (9) sorbitol, (10) mannitol. Peaks in (A) eluting after (8) are fructans with > DP3 and malto-dextrins

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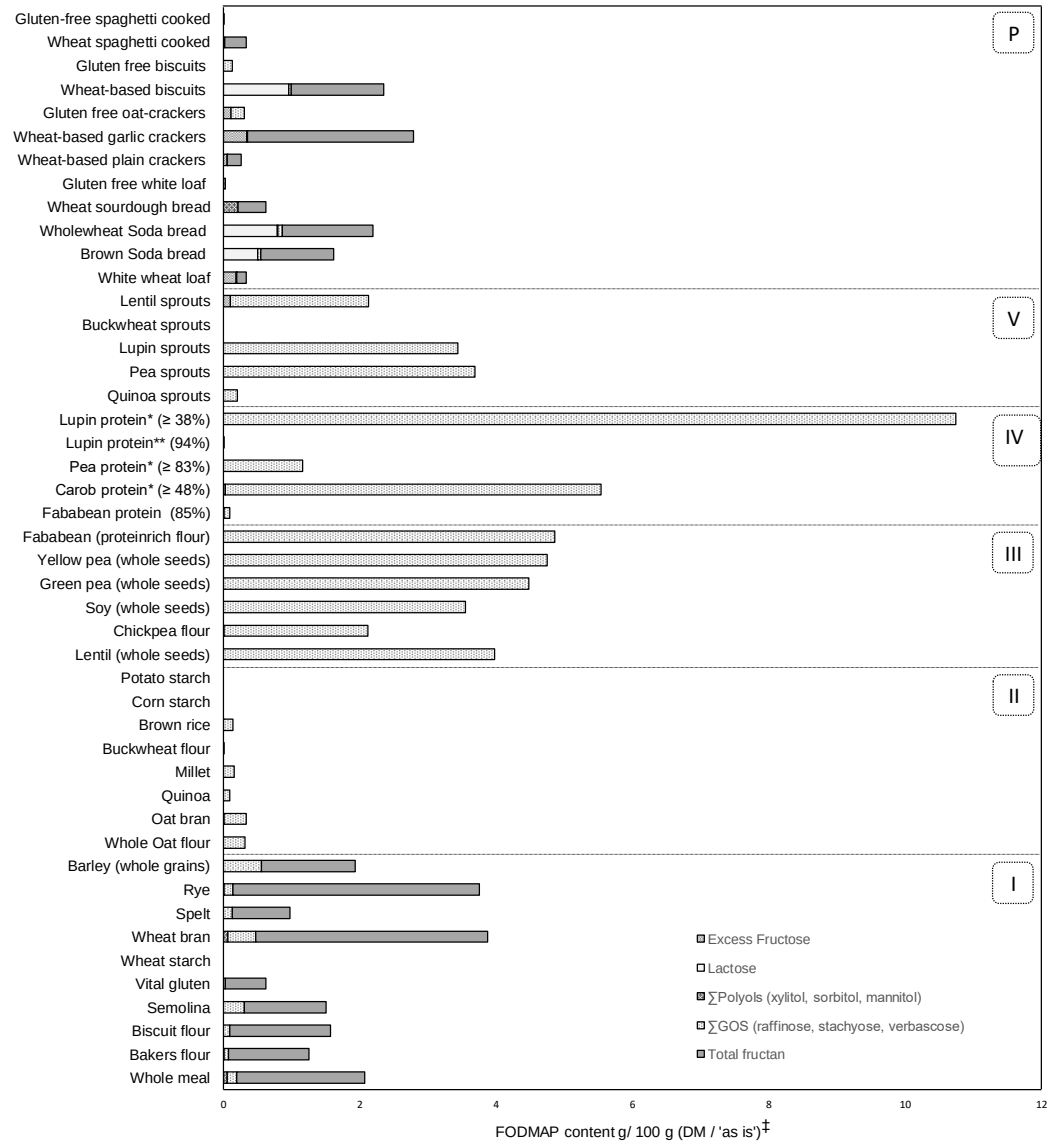


Figure 2. FODMAP contents of cereal-product ingredients divided into Group I – V. (Group I) – fructan containing cereals, (Group II) - low FODMAP and gluten-free cereals and pseudo-cereals, (Group III) - seeds and flours from pulses, (Group IV) – pulse protein ingredients, (Group V) – commercial sprouts and FODMAP levels of commercial products (P) quantified via HPAEC-PAD. Protein ingredients marked with one asterisk (*) are commercial protein ingredients, those marked with two asterisks (**) are protein ingredients delivered from EU project, provided by Fraunhofer IVV. (‡) FODMAP levels of ingredients are referred to the dry matter, whereas levels in products are referred to the fresh weight 'as is'.

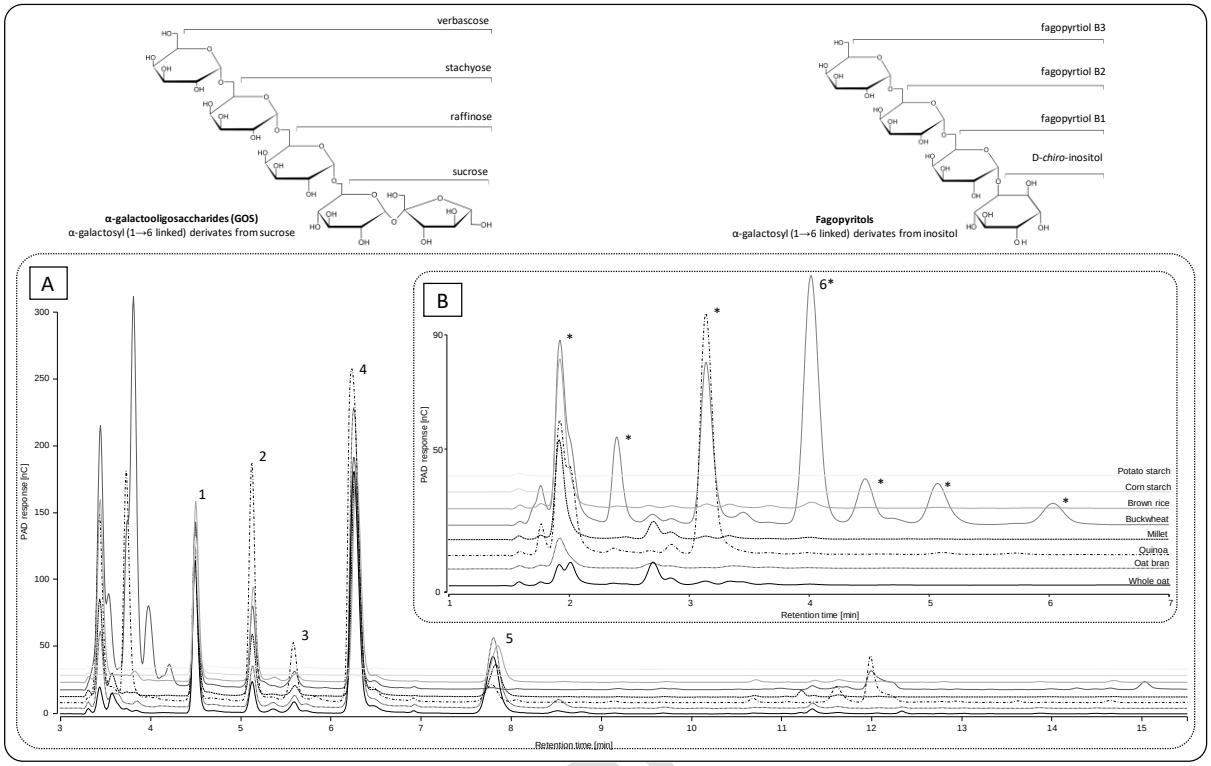


Figure 3. Chemical structures of α-galactooligosaccharides (GOS) and fagopyritols and HPAEC-PAD (A) CarboPac PA200 and (B) CarboPac PA1 profiles of ingredients from Group II - low FODMAP and gluten-free cereals and pseudo-cereals with identical order of the profile in (A) and (B): (1) rhamnose [internal standard], (2) glucose, (3) fructose, (4) sucrose, (5) raffinose/stachyose, (6*) fagopyritol B1. All peaks marked with an asterisk are unidentified compounds suspected to be cyclitols and fagopyritols

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Characterization of the FODMAP-profile in Cereal-product Ingredients

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Highlights

- screening of FODMAP contents in cereal product ingredients
- classification of ingredients as tool for low FODMAP product development
- analysis and characterization of commercial benchmark products
- highlight of relevant FODMAPs in ingredients and products

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Conflicts of interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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