Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali
in convenzione con l'Università degli Studi del Molise

Corso di Dottorato di Ricerca in
Scienze, Tecnologie e Biotecnologie per la Sostenibilità - XXX Ciclo

FRUCTAN AND β-GLUCAN ACCUMULATION IN BARLEY KERNEL AND DEVELOPMENT OF FLOURS ENRICHED IN BIOACTIVE COMPOUNDS

(**s.s.d. AGR/15**)

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A.A. 2016/17

Short abstract

The overall aim of this doctoral thesis was to characterise mature and immature barley (*Hordeum vulgare* L.) cultivars focusing on bioactive compounds fructan and β-glucan.

Developing barley kernels were studied to evaluate immature barley flour as an innovative functional ingredient. Seven barley cultivars grown in Italy were collected at different days after anthesis (DAA) until completed maturation and analysis were performed in order to assess variation of the content of fructan, β-glucan and total starch. Results indicate that fructan concentration in immature barley grain ranges from 3.3 to 5.5 times higher than at maturation and cultivar Scarlett stands out for the highest content of fructan (9.5 g/100 g flour- fresh weight) among the lines analysed.

Six barley varieties obtained in Sweden differing in starch composition and β-glucan content (normal starch, waxy, high amylose and shrunken endosperm barleys) were studied to evaluate β-glucan molecular features and effects of wheat β-glucanase hydrolysis on the structure correlated to baking process. Sifted flours samples underwent sequential extractions with water and alkali to isolate β-glucan fractions. On three varieties a separate extraction was performed obtaining only water-extractable and water-unextractable β-glucan isolates. These pellets were incubated with wheat flour (*Triticum aestivum*) extracts. The occurrence of oligosaccharides with DP 3-9 was analysed after lichenase digestion and analysis with high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). Results showed a higher ratio 3-O-β-cellobiosyl-D-glucose (DP3) to 3-O-β-cellotriosyl-D-glucose (DP4) in fractions of variety SLU 7 (shrunken endosperm barley) suggesting a more packed β-glucan structure compared to the other vareties. Moreover, it can be argued that wheat β-glucanase hydrolizes selectively β-glucan with lower DP3/DP4 ratio and that β-glucan of shrunken endosperm barley SLU 7 is composed of a population with higher DP3/DP4 ratio which may express higher resistance to enzymic action.

In this work, air classification was performed on four waxy and one non-waxy barley cultivars in order to obtain barley flour enriched in β-glucan. Barley varieties were collected in experimental fields in Italy, hammer milled, micronised and air classified to obtain a coarse fraction and a fine fraction respectively enriched and depleted of β-glucan. Proximate composition, β-glucan and dietary fibre content was assessed on each fraction to evaluate efficacy of the process and significant differences among cultivars. A maximum increase in β-glucan content of +84% in the coarse fraction was registered compared to the micronised flour. Cultivar CDC Fibar displayed the highest content of β-glucan in the coarse fraction (13.5% fw) and total dietary fibre enrichment was also achieved in all the coarse fractions.

Taken together the results obtained with this experimental work contribute to the characterization of barley to improve its inclusion in cereal-based functional foods production.

Keywords: air classification, barley, fructan, β-glucan, β-glucanase, lichenase, oligosaccharides

Extended abstract

Aim

The overall aim of this doctoral thesis was to characterise different barley (*Hordeum vulgare* L.) cultivars focusing on bioactive compounds fructan and β-glucan and contributing to the characterization of this cereal in the perspective of wider employment in cereal-based functional foods production.

Experimental procedure

The work is structured in three main experimental activities. E1) Study of fructan, β-glucan and total starch content in developing barley grains; E2) analysis of molecular characteristics of β-glucan in barley flours using lichenase digestion and HPAEC-PAD (High performance anion exchange chromatography-pulsed amperometric detection) and evaluation of the effects of wheat β-glucanase; E3) use of micronization and air classification technology to obtain β-glucan enriched fractions from waxy and non-waxy barley cultivars.

Material and methods

E1) Samples of ears from seven cultivars of waxy and non-waxy barley were collected at different developmental stages (3, 6-9, 10-11, 17, 20-21, 24-28, 31-38 days after anthesis-DAA) and at completed maturation. Kernels from immature ears were dried at 40 °C for 24 hours under vacuum (-660 Torr). Kernels were then ground with laboratory miller and analyzed. Mature barley kernels were also ground with a laboratory miller and analysed. Fructans, β-glucan and total starch content were determined by spectrophotometric methods. Protein content (N*x*6.25) was analyzed with Dumas method (Leco FP-528). Average kernel weight (fresh weight) was calculated as the ratio of total weight of kernels to the number of kernels.

E2) Six barley varieties differing in starch and dietary fibre composition (normal starch, waxy, high amylose and shrunken endosperm barleys) were obtained from Lantmännen Lantbruk (Sweden). The kernels were milled using a laboratory mill (Laboratoriums-mahlautomat model MLU 202, Genrunder Bühler Maschinenfabrik, Uzwill, Switzerland). Sifted flour fractions were pooled together and used for the analysis. β-Glucan was sequentially extracted with water and NaOH 50 mM obtaining three fractions: water extractable (WE), alkali-extractable (NaE) and residual (Res). A second extraction on sifted flour of three varieties was performed obtaining water extractable and water unextractable β-glucan. Pellets were incubated with wheat extract obtaining the following samples. WU-WhE-Ins is the insoluble fraction obtained after incubation of water unextractable β-glucan with wheat extract; WE-WhE-Sol is the soluble fraction obtained after incubation of water extractable β-glucan with wheat extract; WU-WhE-Sol is the soluble fraction obtained after incubation of water unextractable β-glucan with wheat extract. Analysis of oligomers released after lichenase digestion of β-glucan on the fractions fractions was performed using HPAEC-PAD (High performance anion exchange chromatography-pulsed amperometric detection). Separation was carried out on a Carbopac^{TM} PA100 (4x250mm) analytical column (Dionex, Sunnyvale USA) equipped with a guard column.

E3) Thirteen barley cultivars from year 2013/14-2014/15 were analysed to evaluate the β-glucan content. A selection of cultivars of waxy and non-waxy mature barley was collected, dehulled, hammer-milled, micronized (Separ microsystem mod. KMX-300) and air classified (Separ microsystem mod. SX/LAB), obtaining a coarse fraction and a fine fraction corresponding to the 40% and 60% of the total weight of the flour. These fractions were analyzed to assess their proximate composition and β-glucan enrichment and reduction respectively.

Results and Discussion

E1) The peak of average kernel fresh weight resulted at 21-28 DAA, while in Dingo at 38 DAA. These findings are in agreement with others work, as the decrease of kernel weight is the consequence of the dehydration process which is not balanced by reserve storage and accumulation. The peak of fructan concentration (g/100 g flour - fresh weight) resulted to be between 6 and 17 DAA. Cultivar Scarlett and Dingo had the highest concentration being 9.5 g/100 g flour and 6.6 g/100 g flour respectively. During the later stages of maturation, fructan content decreases steadily. Starch concentration tends to increase over maturation for all the cultivars analysed. β-Glucan substantially accumulates from 20-28 DAA in five cultivars corroborating previous findings. In Scarlett and Dingo, the content exceeds 1 g/100 g flour at 31-38 DAA and at maturation respectively. Linear accumulation (mg/kernel) of protein occurs coherently with previous works. The role of fructan metabolism in the first stages of maturation of the grain has been extensively studied. The synthesis aims at protecting tissues from oxidative stress and regulating the osmotic excess due to high quantity of sucrose, in order to allow photosynthesis to continue. Moreover, during maturation these polysaccharides help regulating the carbohydrate metabolism. On the other hand, the progressive reduction of fructan content is probably correlated to the accumulation of storage carbohydrate like starch.

E2) The occurrence of oligosaccharides yielded after lichenase hydrolysis on the three fractions (WE, NaE and Res) of β-glucan isolates was analysed. Data on the single fragments were calculated as normalised against the sum DP3+DP4 areas: DPx/(DP3+DP4). Information on the structure of β-glucan can be obtained by analysing DP3/DP4 ratio which was higher in the NaE and Res fractions compared to the WE, in all the barley varieties considered. Barley variety SLU 7 (shrunken endosperm barley, with high β-glucan and fructan content) had higher DP3/DP4 ratios in all the three fractions compared to the other varieties, while NGB 114602 had the lowest ratios. In the WE-WhE-Sol fractions, DP3/DP4 ratio is the highest compared to the other two β-glucan isolates. When looking at the data of fractions WU-Whe-Ins and WU-WhE-Sol, similar ratios values were obtained in these fractions in cultivar SW 28708 and Gustav differently to what observed in SLU 7. It can be speculated that in this variety the wheat β-glucanase released a group of polysaccharides with a different structure. These results can lead us to argue that wheat enzymes display a selective behaviour of hydrolysis towards specific β-glucan population, possibly with lower DP3/DP4 ratio and so with a more accessible structure to enzymic action. Barley varieties SLU 7 results to be different specifically in the insoluble fractions with a lower occurrence of β-glucan available to hydrolysis by wheat enzymes.

E3) The coarse fraction obtained by air classification technology is enriched in β-glucan compared with the starting micronized flour. Enrichment resulted more effective for cultivar Shangrila and CDC Alamo (+84%), but the starting high content in waxy barley resulted in coarse fractions with a marked higher concentration of β-glucan. In fact, the coarse fraction obtained from waxy cultivar CDC Fibar has a notable content of β-glucan (13.5% fw).

Conclusions and Future Perspectives

The aim of this thesis was to characterize barley flours focusing, specifically, on the functional polysaccharides fructan and β-glucan. Previous researches highlighted that fructan concentration is higher in the first stages of cereal maturation compared to mature grain. The evolution of the content of these compounds was evaluated in developing barley grains to study its potential application as innovative functional ingredient. Results showed that fructan concentration in immature barley grain can be from 3.3 to 5.5 folds higher than at maturation. However, at the peak of fructan concentration, β-glucan content is below 1 g/100g flour. It can be concluded that immature barley flour can be potentially employed as a source of fructan but not of β-glucan.

The study of the structure of β-glucan isolates with HPAEC-PAD showed that shrunken endosperm barley variety has a higher DP3/DP4 ratio compared to the other varieties (normal starch, high amylose and waxy). A higher ratio is related to a higher occurrence of (1→3) linkages in the β-glucan chain and a higher number of interchain interactions. Activity of wheat β-glucanase on water unextractable β-glucan in SLU 7 released polysaccharides with a more marked difference in DP3/DP4 ratio compared to the other analysed cultivars, leading to hypothesize that in this variety the enzyme acted more specifically on a selected β-glucan population probably more accessible to its action. Previous researches also showed that β-glucan in SLU 7 maintained a higher molecular weight (MW) during baking process compared to the other cultivars. A high β-glucan MW is related to greater positive physiological effects than low MW β-glucan. It can be speculated that structural features of these polysaccharides in SLU 7 found in this study may be a possible explanation, as the β-glucan chain may result less accessible to wheat β-glucanases. The results of this study improve knowledge on β-glucan structure in this cultivar which could be related to the feature “shrunken endosperm”. Since barley with shrunken endosperm showed a higher content of fructan and β-glucan, these findings can be of great interest to select barley cultivars with the aim to produce healthier food products.

Air classification of previously micronized flour is widely used to obtained fractions enriched in bioactive compounds. In this study, this technology was applied to waxy and non-waxy barley varieties in order to evaluate enrichment of β-glucan in the coarse fractions. Results clearly show that air classification, specifically of waxy barley varieties, but eventually of varieties with high β-glucan content like high amylose or shrunken endosperm, is valuable strategy to obtain flours with improved nutritional values, as in this study β-glucan content reached the maximum of 13.5% fw in the coarse fractions obtained.

The results obtained with this PhD thesis accentuate the potential role of barley flours as an innovative functional ingredient. In fact, immature barley flour, mature barley flour from selected varieties and

fractions obtained after physical enrichment in bioactive compounds, enhance the choices for inclusion of barley products in the formulations cereal-based foods (e.g. bread, pasta, biscuits and breakfast cereals) enriched in fructan and β-glucan.

Riassunto

Questa tesi di dottorato ha riguardato lo sviluppo e la caratterizzazione di farine d'orzo (*Hordeum vulgare* L.) arricchite in fruttani e β-glucani. Il lavoro è articolato in tre progetti.

L'evoluzione del contenuto di fruttani, β-glucani e amido totale è stata determinata in cariossidi di orzo ottenute a diversi stadi di maturazione da campi sperimentali in Italia. Il contenuto di fruttani nelle cariossidi immature è risultato da 3 a 5 volte più elevato rispetto alle cariossidi mature con la cultivar Scarlett che mostra il più elevato contenuto (9,5 g/100 g sfarinato-peso fresco). I β-glucani e l'amido totale mostrano un trend di accumulo lineare fino a maturazione completata.

Sei varietà di orzo ottenute in Svezia sono state selezionate per la diversa composizione di amido e fibre ed è stata valutata la struttura dei β-glucani e l'impatto dell'idrolisi da parte delle β-glucanasi del frumento su di essa, correlato al processo di panificazione. I β-glucani dalle farine sono stati estratti con acqua e NaOH ottenendo tre pellet (solubile in acqua, solubile in NaOH e residuo). Su tre varietà una estrazione successiva ha isolato solamente le frazioni di β-glucani solubili e insolubili in acqua, incubando i pellet con estratti di farina di frumento (*Triticum aestivum* L.) e separando le frazioni ottenute dopo l'idrolisi dei β-glucani. La frequenza di oligosaccaridi con grado di polimerizzazione (DP) 3-9 è stata valutata in tutti i pellet ottenuti dopo idrolisi con lichenasi ed analisi con HPAEC-PAD. I risultati ottenuti mostrano un più elevato rapporto trisaccaridi/tetrasaccaridi nei β-glucani della cultivar SLU 7 (con endosperma *shrunken*) suggerendo una struttura più compatta. Inoltre, si può ipotizzare che in questa varietà le β-glucanasi del frumento abbiano idrolizzato selettivamente una popolazione con più basso rapporto DP3/DP4 suggerendo la presenza di β-glucani con una maggiore resistenza all'azione idrolitica delle β-glucanasi.

La tecnologia di classificazione ad aria è stata utilizzata per ottenere frazioni arricchite in β-glucani, valutando altresì l'efficacia di questa tecnica. Cinque cultivars di orzo ottenute in Italia sono state macinate con mulino a martelli, micronizzate e sottoposte a classificazione ad aria ottenendo una frazione grossa e una fine rispettivamente arricchita e depauperata in β-glucani. Sulle frazioni ottenute è stata effettuata una analisi della composizione centesimale, del contenuto in β-glucani e in fibra alimentare. I risultati sottolineano un aumento massimo in β-glucani di +84% nella frazione grossa rispetto alla frazione micronizzata. La cultivar CDC Fibar ha riportato il più elevato contenuto in β-glucani nella frazione grossa (13,5%) mentre è risultato anche un aumento del contenuto di fibra alimentare in tutte le frazioni grosse.

I risultati di questo studio contribuiscono ad ampliare le conoscenze necessarie per sviluppare farine d'orzo arricchite in composti bioattivi e produrre alimenti funzionali innovativi.

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Abbreviations

ANOVA	Analysis of Variance
β-Glucan	Mixed linkage β-glucan
CF	Coarse Fraction
DAA	Days After Anthesis
DF	Dietary Fibre
DP	Degree of Polymerization
dw	dry weight
EFSA	European Food Safety Authority
FDA	US Food and Drug Administration
FF	Fine Fraction
fw	fresh weight
HPAEC-PAD	High Performance Anion Exchange Chromatography-Pulsed Amperometric Detector
IDF	Insoluble Dietary Fibre
LDL	Low Density Lipoprotein
MW	Molecular Weight
NSPs	Non-starch Polysaccharides
PCA	Principal Component Analysis
SDF	Soluble Dietary Fibre
TDF	Total Dietary fibre

1. State-of-the-art

1.1. Barley *(Hordeum vulgare* L.)

Barley (*Hordeum vulgare* L.) is a grass plant first domesticated in Fertile Crescent, in 8000 B.C. in the Israel-Jordan area (Badr *et al*., 2000) firstly with his wild relative *Hordeum spontaneum* L. The domestication occurred in the Fertile Crescent is responsible for the diversity of European and American cultivars, while a second domestication allowed a diversity from central Asia to Far East (Morrell & Clegg, 2007). The genus "*Hordeum*" belongs to the Gramineae (Poaceae) family.

Classification of barley occurs according to different parameters. Barley can be classified according to temperature requirements in:

- winter barley, that must be planted during cold exposure (vernalization) to initiate reproduction.
- Spring barley which does not require vernalization and can be sown in spring.

Barley can also be classified according to physical dispositions of kernels on the plant.

- Six-rowed barley has three kernels formed on each node of the head;
- two-rowed barley, instead, has one single kernel per side of the head.

Winter barley can be both six rowed and two rowed while spring barley only two rowed.

Another difference concerns the structure of the kernel (Newman & Newman, 2008).

- In hulled (covered) barley, the hulls firmly adhere to the caryopsis at maturity;
- in hulless (naked) barley the caryopsis is harvested free of the hull.

The characteristic "hulled-hulless" in barley is due to the single recessive gene (*nud*) in chromosome 7H and to a cementing substance causing hull adherence in hulled barley that is secreted by the caryopsis (Newman & Newman, 2005; Fettel *et al*., 2010).

1.1.1. Kernel anatomy and development

The botanical name of cereal fruit is "caryopsis", and consists of three main parts: pericarp, endosperm and germ. It is also identified as "grain" or "kernel" which in general consists of the husk, endosperm and embryo (Figure 1.1).

The barley grain has an elongated shape and is divided longitudinally by a crease.

The outer layer of the hulled grain is the husk, composed of the lemma and palea. In barley, the proportion of the husk is about 13% of the grain weight (Evers & Millar, 2002). The pericarp (fruit wall) is the outer layer that covers the caryopsis. It encloses the testa (seed coat) (Freeman & Palmer, 1984).

The endosperm consists of the aleurone layer and the starchy endosperm and it is the most important anatomical part in terms of proportion of the grain and food use. The aleurone is found under the testa and nucellus (Newman & Newman, 2008; Evers & Millars, 2002). The cells of the aleurone layer are rich in vitamin, lipid and minerals. The starchy endosperm is the largest proportion of the caryopsis

(about 75%) and is made of cells that are packed with starchy grains in a storage protein matrix (Evers & Millar, 2002). It surrounds the embryo and provides nutrients for its germination until the root system is sufficiently developed for photosynthesis. The main constituents of the endosperm cell walls are (1→3)(1→4)-β-glucan (~70% w/w) and arabinoxylan (~25% w/w) (Fincher, 1975).

The embryo arises from the fusion of male and female gametes and contains mostly lipid and lipid-soluble vitamins and is located on the side of the caryopsis attached to the rachis. It is comprised of the embryonic axis and scutellum (Evers & Millar, 2002). The scutellum separates the embryo from the endosperm and is a flat protective tissue.

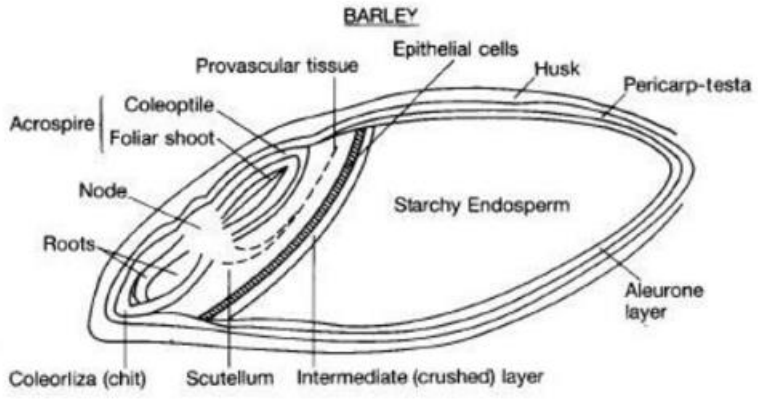

Figure 1.1. *Barley grain anatomy (Roberts T. presentation)*

For most cereals anthesis or pollination are often chosen as a starting point to study grain development. Pollination is hard to define as the precise moment when a successful pollen grain lands on stigma is difficult to detect (Evers & Millar, 2002). Anthesis is the time when anthers are first visible outside the floral parts. With this event, pollination takes place followed by fertilization, initiating growth of embryonic seed or kernel.

The time length between anthesis and maturity can be divided into three phases, according to growth characteristics and metabolites accumulation: pre-storage (0-8 days after anthesis -DAA), storage (8-24 DAA), desiccation (24-30 DAA) (Wobus *et al*., 2005; Sreenivasulu *et al*., 2010) (Figure 1.2; Figure 1.3).

The initial phase of kernel development consists of a progression of cell division and the formation of the seed able to produce a new generation. This stage is particularly important as it will determine the number of cells accumulating starch. Kernels that are crushed in this stage can release a watery substance that then becomes milky. This stage (until 14 DAA) is also called "watery ripe" and then "milky stage".

In the storage phase genes coding for carbohydrate synthesis are expressed and starch and proteins are accumulated (Figure 1.2). The weight increases and most of the final weight is set in this phase as well as the composition established. Kernels have a semi solid consistency so this phase is also called "soft dough".

In the desiccation phase the growth of the kernel declines, water content decreases consistently and this phase is also called "hard dough". This is also when the kernel loses the green color to reach the final

weight at maturation. When kernel reaches 30-40% moisture it is at physiological maturity and will not accumulate more dry matter (Anderson *et al*., 1995).

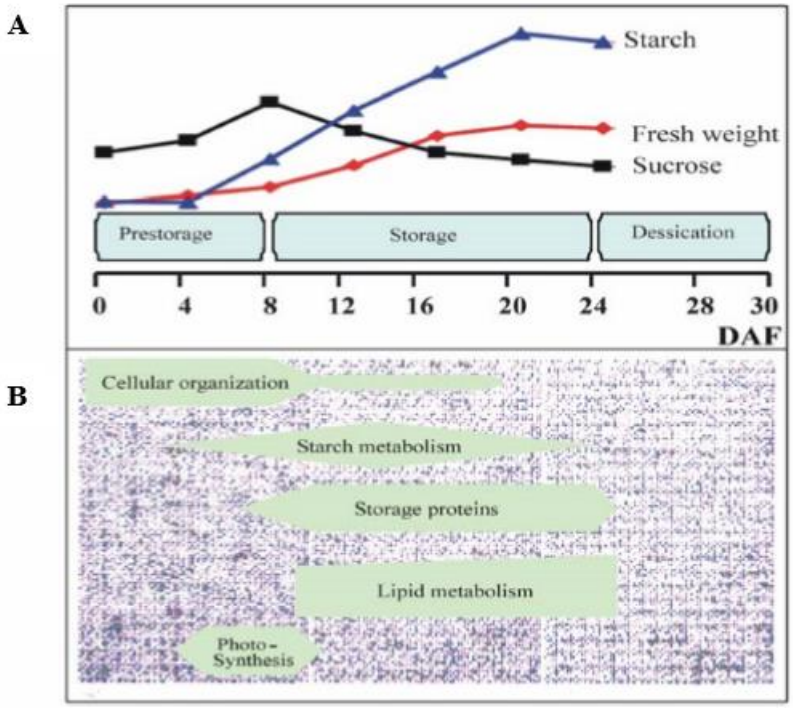

Figure 1.2. *Scheme of barley grain development. A-Biochemical parameters differentiating the developmental stages. B-Gene expression patterns underlying different developmental stages. DAF= Days after flowering (Wobus et al., 2005).*

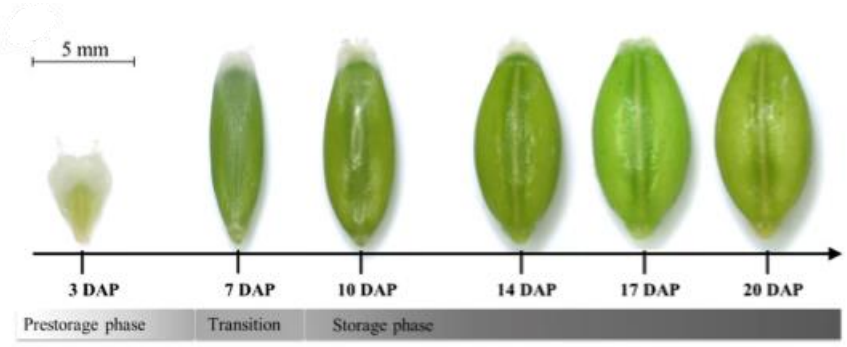

Figure 1.3. *Developmental stages of barley grain, DAP= Days after pollination (Peukert et al., 2014).*

1.1.2. Nutritional composition of barley grain

Whole barley grain is composed by 65-72% starch, 10-17% protein, 4-9% β-glucan, 2-3% free lipids and 1.5-2.5% minerals. Total dietary fibre (TDF) ranges from 11-34% and soluble dietary fibre from 3-20% (Holopainen *et al*., 2014; Baik & Ullrich 2008). Nutritional components of barley can vary according to genotype, agricultural practices and environmental conditions.

An overview of the general composition of a barley grain is reported in Table 1.1.

Table 1.1. *Composition of barley genotypes (g/100 g dm),*

	Hulless	Hulled
Item	Range	Range
Protein [a]	12.1-16.6	12.5-15.4
Starch	60.5-65.2	57.1-59.5
Sugars	2.0-4.2	2.8-3.3
Lipids	2.7-3.9	1.9-2.4
Fibre	12.6-15.6	18.8-22.6
Ash	2.3-3.5	2.3-3.0
Total dietary fibre	13.2-13.8	17.0-19.6
Total *β*-glucan	4.7-6.3	4.4-5.3

[a] *N x 6.25; (In: Newman and Newman, 2008).*

Starch

Starch is the energy reserve of plants and the most abundant biopolymer on earth (Schirmer *et al*., 2013). Starch consists of two main components, amylose and amylopectin. Amylose is a linear molecule of α-D-glucopyranosyl residues linked 1→4, while amylopectin is highly branched with chains of 1,4-linked α-D-glucose residues connected by 1,6-linkages.

Starch is the main constituent of barley grain followed by non-starch polysaccharides (Holtekjølen *et al*., 2006). In hull-less species lower TDF and higher starch content were found (Xue *et al*., 1997). Genotypes of barley can vary according to starch composition and amylose content. Normal starch genotypes have approximately 25% of amylose while waxy genotypes < 15% (2-10%) and high amylose more than 35% (Ajithkumar *et al.*, 2005; Oscarsson *et al*., 1997). The waxy feature in barley is controlled by a recessive *wax* gene on chromosome 7HS *(*Ajithkumar *et al*., 2005).

Barley starches are a mixture of large lenticular A-type granules (10-25μm) and smaller irregular B-granules (<10 μm) (Schirmer *et al*., 2013). The small granules account for the 90% of the total number of granules but only for the 10% of the total starch weight (Henry, 1988). Barley A- and B- granules differ for the amylopectin content and structure. Amylose content of small granule is lower than in large granules and the branch-chain length of amylopectin is longer in A-granules than in B-granules (Ao & Jane, 2007; MacGregor & Morgan, 1984; MacGregor & Ballance, 1980; Takeda *et al*., 1999). Normal barley varieties in general contain a higher amount of starch mainly composed of large granules, whereas genotypes with anomalous starch content contain less starch and smallest granules prevails in high amylose barley (You & Izydorczyk, 2002).

Non-starch polysaccharides

Non-starch polysaccharides (NSPs) in barley are structural elements in the cell walls of hull, aleurone and starchy endosperm tissues, and are mainly composed by arabinoxylans, pentosans, cellulose and β-glucan (Holtekjølen *et al*., 2006). Hulled varieties have significant higher content of NSPs than do the hulless. Insoluble NSPs content varies from 10.6% to 27.3%. Soluble NSPs is in the range of 4.5-26.9% dw corresponding to 16.4-69.7% of total non-starch polysaccharides.

The dominating barley dietary fibre are β-glucans and arabinoxylans (AX) located mainly in the cell walls of the endosperm. Arabinoxylans consist of a linear chain backbone of (1→4)-linked β-D-xylopyranosyl residues to which a α-L-arabinofuranose units are linked as side branches (Izydorczyk & Biliaderis, 1995). The content of AX in barley was found to be 2.89-4.76% (Zhang *et al*., 2013), while Holtekjølen *et al*. (2006) found a higher content of AX (7.4-15.7%), compared to other studies. The lowest amount was found among the hulless varieties, consistently with Knutsen & Holtekjølen, (2007), that reported that the hulled varieties had an average content of 6.7% compared to 4.4% in hull-less samples. Arabinose/Xylose ratio (which indicates degree of branching) in barley falls in the range of 0.3-1.1, typical of all cereals (Izydorczyk & Dexter, 2008) but was found to be higher for hulless varieties (Knutsen & Holtekjølen, 2007).

β-Glucans are extensively described in the following chapter.

In barley arabinogalactan content varies from 0.8-1.1% while the cellulose content is in the range 8.0-17.7% (Holtekjølen *et al*. 2006).

Monosaccharides and oligosaccharides

Glucose and fructose represent less than 10% of the total sugar in barley, being usually < 0.2%. The disaccharides sucrose and maltose have been reported in barley. Sucrose is involved in starch synthesis and accounts for 50% of the sugars in barley ranging 0.34-2% while maltose is < 0.2% (Henry, 1988). Henry & Saini (1989) found that barley had the highest level of sucrose compared to wheat, rye and oats (13.6 mg/g). Waxy gene is related to an increased level of sucrose, due to an altered starch synthesis (Xue *et al*., 1997). Low amount of raffinose is also detected in barley (Henry, 1988; MacLeod & Preece, 1954).

Protein

Protein content in barley is 10-17% on average. In Holtekolen *et al*. (2006) the highest amount of protein was found in the high amylose variety, while the normal normal starch hulless variety had higher protein content than the normal hulled varieties. On the other hand, according to Xue *et al*. (1997), hulless and waxy features do not affect the protein content.

Storage proteins support growing embryo providing nitrogen in case of germination and are disposed in a matrix surrounding endosperm starch granules (Fox *et al*., 2003). Hordeins are the most important storage proteins in barley. They are evolutionary related to glutenin and gliadin in wheat. Hordeins are classified according to their molecular size and aminoacid composition in B, C, D, γ hordeins (Holopainen *et al*., 2014). Non-storage proteins can be found in the cell walls and within the protein matrix, but few have been identified and have an impact on the grain quality (Fox *et al*. 2003). The analysis of composition of barley grains revealed that protein content gradually decreases toward the center of the grain at a pearling rate of 15%.

Proline is the most abundant aminoacid found in wholegrain barley followed by valine. Lower levels of glycine, threonin and arginine were also detected (Sullivan *et al*., 2010).

Lipids

The total lipids include 67-78% of non-polar lipids, 8-13% glycolipids, and 14-21% phospholipids (Morrison, 1993). In barley, the total amount of unsatured fatty acids is 77.09% of the total fatty acids. As for wheat and rye also for barley the major component other total fatty acids is linoleic acid (>50%), followed by palmitic (~20%) oleic (~10%), linolenic and stearic acid (Welch, 1975; Kan, 2015).

Ash

A barley grain contains on average approximately 498 μg of K, P, Mg and Ca combined, with potassium being the mineral present in greater amount, forming nearly 45% of the total content of the four elements of interest followed by phosphorus and magnesium (Kan, 2015; Stewart *et al*., 1988). Protein bodies of the aleurone layer typically contain Mg, Ca, K, P while Ca is in very low amounts.

Phytochemicals

The role of antioxidants for human health is crucial as they are able to prevent tissues and cellular damages by quenching radicals or preventing their formation. Cereals can be an important source of antioxidant compounds. Barley grain contains much greater amounts of phenolic compounds (0.2-0.4%) than other cereal grains, and they are represented by polyphenols, phenolic acids, proanthocyanidins and catechins that are concentrated in the hull, testa and aleurone (Baik & Ullrich, 2008). Ferulic acid is the most abundant phenolic acid (359-624 μg/g dw) followed by *p*-coumaric acid (79-260 μg/g) being mainly concentrate in the husk and outer layers (Andersson *et al*., 2008; Hernanz *et al*., 2001). Bellido & Beta, (2009) found that total anthocyanin content in purple and normal barley is 573-210 μg/g. Removal of 10% of the outer layers was a useful strategy to concentrate anthocyanins, as they increased in both purple and normal barley (3533 μg/g- 1587 μg/g). In the samples analyzed in the study, seventeen types of anthocyanin were detected.

The main carotenoids found in cereals are lutein and zeaxanthin. Lutein is abundant in wheat, and is predominant also in oat, barley while zeaxanthin is dominant in maize (Ndolo & Beta, 2013). In barley, the total carotenoid content was highest in germ fraction (57.3-60.3 mg/kg) and lower in aleurone and endosperm (28.6-28.9 mg/kg) (Masisi *et al*., 2015; Ndolo & Beta, 2013). Lutein levels was reported to be 1328 μg/kg in barley germ (while 72-431 μg/kg were reported in wheat and 72-1369 μg/kg in corn) while no trace was found in the endosperm. Zeaxanthin levels is 15139 μg/kg in barley germ while no trace was found in the endosperm, with higher level compared to corn (358-13671 μg/kg) and wheat (7-215 μg/kg) (Masisi *et al*., 2015).

Alkylresorcinols (AR) are amphiphilic 1,3-dihydroxy-5-alkyl phenolic lipids present in several plant families (Landberg *et al*., 2014). Alkylresorcinols are located in the outer layer of testa/inner layer of pericarp, mainly in wheat and rye, whereas barley contains a very small amount (Landberg *et al*., 2014), which can vary between 32 and 103 μg/g dm (Andersson *et al*., 2008). AR are stable during food processing, therefore they are used as biomarkers to determine the intake of whole grain cereals in the population. The most common AR found in cereal grains are homologue with odd alkyl chains of 17-25 carbon atoms. The source of whole grain can be identified by the relative homologue distribution,

specifically the ratio AR C17:0/C21:0, which is generally ~1 for rye, 0.1 for common wheat and 0.01 for durum wheat (Landberg *et al*., 2014). Alkylresorcinol C25:0 is the most abundant alkylresorcinol found in barley samples. Gómez-Caravaca *et al*. (2015) found that with air classification total alkylresorcinols increase in barley flour in the range 17.1%-30.2% from whole meal to coarse fraction and decreased from 19.5% to 32.6% in the fine fraction.

Tocols content in barley was found to be 46.2 to 68.8 μg/g dm in wholemeals (Andersson *et al*., 2008). α-Tocotrienol resulted the most abundant tocopherol, being ≥ 47.7% of total tocols.

1.1.3. Commercial use and economic data

Barley is a staple grown globally. In the year 2014-2015 the world total production was 144.3 million tonnes (International Grains Council[1]). The Russian federation is the top producer country in the world with 20 million tonnes, followed by France and Germany with about 11.5 million tonnes and Australia and Ukraine (about 9 million tonnes). In Europe, the total barley production is about 93.5 million tonnes, almost 65% of the world production (Figure 1.4). The total Italian production of barley in year 2014 was 846.142 tonnes (FAOSTAT, year 2014).

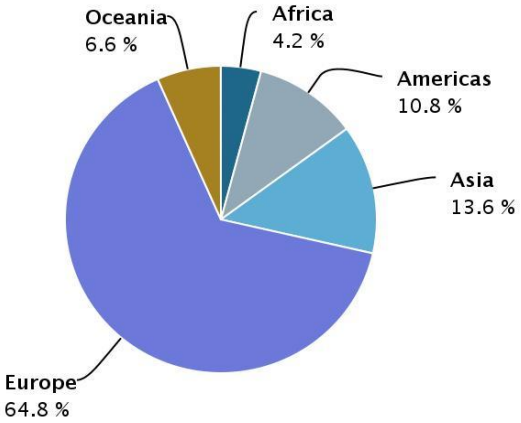

Figure 1.4. *Barley production share by region (year 2014) (FAOSTAT).*

Barley is produced for a wide range of end uses (Table 1.2). The main use is in the feed industry (60% of total barley production), in particular, for cattles, pigs and poultry, where only six-rowed barley is employed. The use of barley directly for human nutrition accounts for 4% of the whole production (International Grains Council, forecast for year 2016[2]). Finally, 20% of barley production is used for industry, specifically for malt production.

[1] http://www.igc.int/en/markets/marketinfo-sd.aspx (2017.04.03, 15.26)
[2] http://www.igc.int/en/markets/marketinfo-sd.aspx

Table 1.2. *End use of world barley production (International Grains Council, forecast year 2016)*

Barley end-use	Million tonnes
Food	7.4
Feed	98.1
Industrial	30.0
Other	10.2

About 90% of malt is used for beer while the rest is for food substitute (Taner *et al*., 2004). In year 2015 the European Union (EU) area for malting barley was about 12,200,000 hectars with a production of about 61 million tonnes of barley for malt, with an increase of about 10% compared to 2012. In Europe, Germany and France are the main producers of barley for malting (about 12,000,000 tonnes each), followed by UK, Spain, Denmark, and Poland[3].

The EU malting industry accounts for more than 60% of the world malt trade. European malt is exported to all continents: about 804,000 tons is exported to Africa; 650,000 to the America and 840,000 to Asia. About 253,000 tons is exported to European and non-EU countries (2015 data[4]).

As mentioned before, beer is the main end use of malt. EU is the second largest beer producer in the world after China and the brewing sector contributes to employment in Europe with 2.3 million jobs (Europe Economics 2016).

1.2. Non-starch polysaccharides: fructans and β-glucan

Non-starch polysaccharides (NSPs) include all the plant polysaccharides except for starch, as they differ from starch in the type and number of monomers, the type of linkages and occurrence in the chain. Moreover, starch can be digested by human enzymes as its linkages are hydrolized by pancreatic α-amylase which cleavages only α-1,4-glycosidic bonds, while NSPs cannot be digested and can only be fermented by gut bacteria. NSPs represent the major part of dietary fibre (DF) in grains and it can be considered as NSPs (Englyst, 1989). Many definitions of dietary fibre can be found. The European Union published the definition in the Commission Directive 2008/100/EC, in which dietary fibre means "*Carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine….*"

In 1999 the American Association of Cereal Chemists published the following definition: "*Dietary fiber is the edible parts and analogous carbohydrate that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine…*" (AACC, 2001).

From a structural and nutritional point of view, dietary fibre can be broadly classified as soluble and insoluble. Soluble dietary fibre (SDF) (which includes pectin, gums, mucillagines, β-glucan), delays the

[3]http://www.euromalt.be/data/14851931551_Spring%20and%20winter%20barley%20from%202012%20to%202015.pdf (2017.04.03, 15.32).

[4]http://www.euromalt.be/data/14851984045_Exports%20by%20destination%20from%202009%20to%202015.pdf (2017.04.03, 15.32).

postprandial gastric emptying, reducing the absorption of some nutrients in the small intestine, mainly starch and glucose. Insoluble dietary fibre (IDF) which includes cellulose, hemicellulose and lignin, have mainly a bulking effect increasing the fecal mass.

Despite they do not provide energy for human nutrition, NSPs have a wide variety of beneficial effects on human health, starting from the good functioning of human digestive system. In fact, dietary fiber promotes beneficial physiological effects including constipation prevention, blood cholesterol attenuation and reduced risk of the onset of diabetes (Kumar *et al*., 2012).

1.2.1. Fructans

1.2.1.1. Molecular structure

Fructans are linear or branched polysaccharides mainly composed of fructose units linked by β(2-1) or β(2-6) bonds (Livingston *et al*., 2009). The first part of the molecule can be formed by sucrose, but it is not necessary for the molecule be considered as fructan. In fact, fructans structure can be simplified as GFn (with glucose-fructose units) or Fn (with only fructose units) (Roberfroid, 2005). Fructans can be described by the degree of polymerization (DP). Inulin is a linear fructan with 2-1 linkages with a DP from 2 to 60. Inulin-type fructans include inulin and fructoligosaccharides (FOS) which instead have a DP between 2 and 10 (Niness, 1999). The shortest form is the trisaccharide 1-kestose. Levans are linear fructans with 2-6 linkages. Graminans are branched fructans reported in cereals with both 2-1 and 2-6 linkages. Inulin neoseries has 2-1 linkages but with glucose molecules with fructosyl subunits. Levan neoseries is based on 6G-kestotriose (Figure 1.5).

Fructans synthesis occurs in the vacuole and is achieved by a pool of enzymes named fructosyltransferase (FTs), whose characteristics are the following, despite the model may be more complicated (Livingston *et al*., 2009).

The enzyme sucrose-sucrose 1-fructosyl transferase (1-SST) forms 1-kestose from sucrose releasing a glucose. The enzyme fructan-fructan 1-fructosyltransferase (1-FFT) elongates the chain by adding fructose units, forming inulin. In cereals, sucrose-fructan 6-fructosyltransferase (6-SFT) transfers a fructose unit from sucrose to 1-kestose producing levan and branched fructans. The enzyme 6G-fructosyltransferase (6G-FFT) causes the elongation of 6G-kestose and produces inulin of neoseries.

Fructans hydrolysis, however, is carried out by fructan exohydrolase (FEH). The enzyme 1-FEH hydrolyzes 2-1 linkages, while 6-FEH mainly 2-6 linkages. Graminan-type fructans in cereals require both the enzymes to be hydrolyzed with the 6-kestose exohydrolase (6-KEH) catalyzing the hydrolysis of 6-kestose to sucrose and fructose (Van den Ende *et al*., 2004).

In this chapter, all type of fructans will be considered.

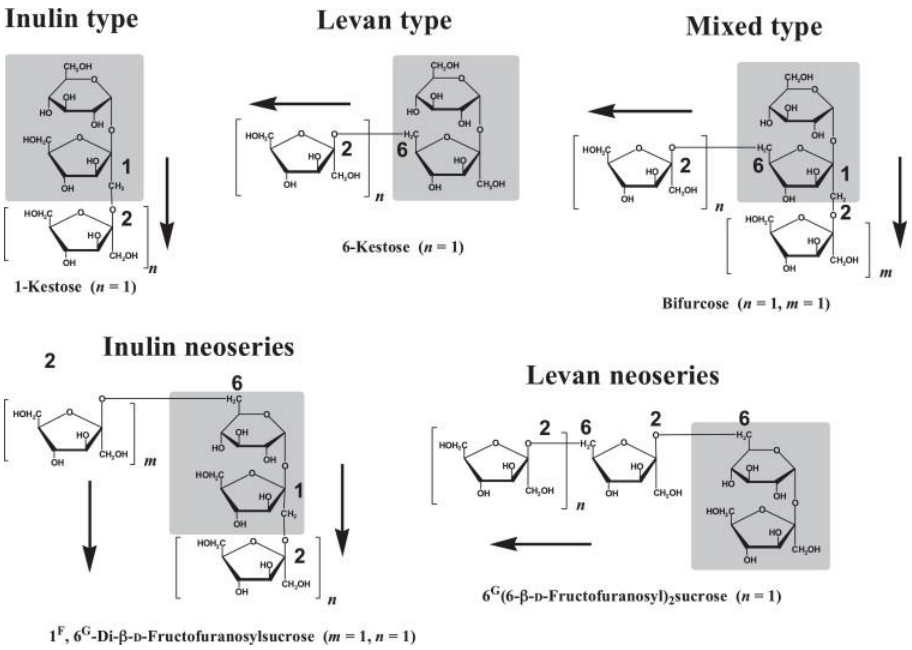

Figure 1.5. *Fructan types in plants (Benkeblia, 2013)*

1.2.1.2. Physiological role in plants and occurrence in cereal grains

Fructans are reserve carbohydrates, and are synthetized by 15% of higher plants (Hendry, 1993), but their physiological role in plants is wider. For instance, fructan synthesis lowers sucrose concentration in the cell and prevents negative feedback on photosynthesis induced by sucrose (Vijn & Smeekens, 1999).

Fructans are involved in kernel development and in resistance to abiotic stresses like freezing and drought (Livingston *et al*., 2009) as well as water stress during grain filling (Wardlaw & Willebrink, 2000; Yàñez *et al.*, 2017). In fact, fructans are highly soluble in water, and their synthesis pathway continues also at low temperatures. Fructans are a source of hexose sugars that can lower the water potential of intracellular liquids, allowing the resistance over drought. Cold stress activates FEH to degrade high DP fructans into lower DP fructans as it was demonstrated that in cold acclimatation plants showed increased fructans content (Bravo *et al*., 2001) and that an accumulation in roots system is a strategy to recover after cold stress (Abeynayake *et al*., 2015). The role of sugars oligosaccharides and fructans is to replace water during cold exposure and drying and binding phospholipids through hydrogen bonds thus maintaining the fluidity of the membrane (Crowe *et al*.,1987; Vereyken *et al*., 2001) with differences according to the type of family and DP (Cacela & Hincha, 2006).

Reactive oxygen species (ROS) are short-lived highly reactive molecules and their generation in the cell is in equilibrium with antioxidants defences such as enzymic and non-enzymic scavengers (Redza-Dutordoir & Averill-Bates, 2016). Recently, it was also demonstrated a role of fructans in hydroxyl radical scavenging in vitro, comparable to phenolic compounds (Peshev *et al*., 2013) suggesting an important role in quenching ROS species in the cell and contributing to abiotic stress tolerance (Keunen *et al*., 2013).

Mature cereal grains are not good source of fructan compared to other edible plants (Table 1.3). Nevertheless, the occurrence of their consumption make them important contributors to fructan intake as, for example, it is reported that wheat provides 70% of fructan intake in the diet of the U.S. population (Moshfegh *et al*., 1999). Mature wheat kernel contains fructans with both β(2-1) and β(2-6) fructosyl linkages belonging both to graminans and neo-type (Verspreet *et al*., 2015a; Verspreet *et al*., 2015b). In wheat, fructan content is higher in shorts and bran compared to flour and the maximum DP is 19 (Haskå *et al*., 2008). Fructan content in barley was originally quantified as between traces and 0.8 g/100 g dw (Åman *et al*., 1985) while more recent studies reported a much higher content. Nemeth *et al*. (2014) found a value between 0.9 and 4.2% dw, a maximum DP of 19-20 and a higher proportion of fragments DP ≤ 10. Also in Triticale the majority of fragments has a DP 3-9 (Rakha *et al*., 2010).

Immature cereal kernels contain a much higher quantity of fructan, which declines with maturation. The peak was reported at 9 DAA (days after anthesis) for wheat, rye, barley and triticale with a higher concentration found in barley (39 g/100 g meal at 4% moisture) (Nardi *et a.l*, 2003). De Gara *et al*. (2003) studied fructan accumulation in *Triticum durum* finding a maximum at 17 DAA (mg/kernel). In *Triticum aestivum*, fructan content is the highest at 16 DAA (2.5 mg/kernel) (Verspreet *et al*., 2013). All fructan synthetizing enzymes are active during the first two weeks, while fructan degrading enzymes are still active during maturation. The activity of FEH was also recorded at 7 DAA in *T. durum* kernels (Cimini *et al*., 2015). The pattern of fructan accumulation/degradation may be explained by their physiological role. The synthesis of fructan starting from sucrose may avoid a negative feedback on photosynthesis during kernel development, and their degradation may suggest a role in regulating the osmotic pressure when the kernel accumulates water (Cimini *et al*., 2015).

In barley in the pre-storage phase (until 6 DAP- days after pollination), it was reported an abundance of oligosaccharides of DP 4-7 in the pericarp. In total fructan content is 2.4% fw (fresh weight) (Peukert *et al*., 2014).

Table 1.3. *Fructan quantity in some consumed foods (dw= dry weight, fw= fresh weight).*

	Fructan (FOS +inulin)	**Reference**
Chicory root	55.3- 73.8 g/100 g fw	Moshfegh *et al*. (1999)
Jerusalem artichoke	28.0-35.0 g/100 g fw	Moshfegh *et al*. (1999)
Onions	2.2-15.0 g/100 g fw	Moshfegh *et al*. (1999)
Rye	3.6-4.6 g/100 g dw	Andersson *et al*. (2009)
Triticale	1.6-2.9 g/100 g dw	Rakha *et al*. (2011)
Barley	0.9-4.2 g/100 g dw	Nemeth *et al*. (2014)
Wheat (*Triticum aestivum*)	1.55-2.3 g/100 g dw	Huynh *et al.* (2008)
Einkorn wheat	1.90 g/100 g dw	Brandolini *et al*. (2011)

1.2.1.3. Effects on human health

The gastrointestinal tract is the primary endpoint of fructans action, influencing the gut microbiome composition of the colon which is involved in immunological and physiological functions contributing

to the homeostasis of the host (Meyer & Stasse-Wolthuis, 2009; Roberfroid, 2007; Schroeder *et al.*, 2009).

Fructans are considered prebiotics (Gibson, 1999; Roberfroid & Delzenne, 1998). A prebiotic is defined as a "*selectively fermented ingredients that allows specific changes both in the composition and/or the activity in the gastrointestinal microflora that confers benefits upon host well-being and health*" (Roberfroid, 2007a). Inulin-type fructans (ITF) are fermented in the large bowel and produce short chain fatty acids (acetate, propionate and butyrate) and lactic acid which are energy sources for microbic metabolism, for cell division and differentiation, and also reduce the pH of the gut (Roberfroid & Delzenne, 1998)[5]. Administration of fructan has a bifidogenic effect, modulating the growth of the beneficial species *Bifidobacterium* spp in the gut (Bouhnik *et al*., 2007; Dewulf *et al*., 2011; Guigoz *et al*., 2002; Salazar *et al*., 2015) with larger effects observed for high DP fructan (Van de Wiele *et al*., 2007). By influencing the colonic microbiome and the gut health, inulin-type fructans can improve the well-being and potentially reduce the risk of onset of different diseases (Guarner, 2005; Meyer & Stasse-Wolthuis, 2009; Roberfroid, 2007b). Fructans from cereals have similar fermentation profiles compared to extracts, so they can be potentially used as an alternative source of FOS (Belobrajdic *et al*., 2012; Jenkins *et al*., 2011).

Inulin-type fructans may improve calcium absorption and calcium balance probably due to both a transfer of calcium from the small intestine in the large bowel and an improved calcium availability in the colon as a result of their fermentation (Abrams *et al*., 2007; Roberfroid & Delzenne, 1998) therefore affecting bone health (Coxam, 2007). Supplementation with 8 g of ITF significantly increased bone mineralization in adolescents (Abrams *et al*., 2007). Inulin enriched oligofructose (10 g/day) increased calcium absorption in post-menopausal women and short term decrease of markers of bone resorption (Holloway *et al*., 2007).

The gut associated lymphoid tissue is the largest lymphoid tissue in the body, and one of its key function is the ability to identify harmful bacteria and elicit appropriate response. Colonization with bacteria is the event that allows and adequate development of mucosa immune system. Bacteria and epithelium communicate and this leads to metabolic and immunological reactions by the epithelial cells and its underlying lymphoid cells (Forchielli & Walker, 2005). Administration of fructoligosaccharides can induce immunological response by acting by signaling human immune cells (Vogt *et al*., 2013; Hosono *et al*., 2003).

In rats, it has also been proven tumoricidal effects of fructan on colon cancer (Choque *et al*., 2015).

In animal models, fructans and and fructans containing foods cause a reduction of body weight (Belobrajdic *et al*., 2012; Rendón-Huerta *et al*., 2012). Total energy intake was significantly lower in rats fed with inulin type fructans with effects mediated by the hormone GLP-1 (glucose-like peptide 1)

[5] The "prebiotic index" is the increase in bifidobacteria expressed as the absolute number (N) of new cfu/g of faeces divided by the daily dose (in g) of inulin type fructans ingested in each individual human nutrition trial. The prebiotic index of inulin type fructans is 10^8 N/g, comparable for the different types of inulin (Roberfroid, 2007b).

and the production of butyrate (Cani *et al*., 2004) also compared to a high fat diet (Cani et *al*., 2005). Increase of satiety was reported in healthy human subjects (Cani *et al*., 2006) mediated by hormonal regulation (Cani *et al*., 2009). In obese and overweight subjects, supplementation with oligofructose reduced body weight significantly (Parnell & Reimer 2009). Effects on body weight may be mediated by the lower caloric content of fructans. In fact, the energy content of fructans is 40-50% of that of a digestible carbohydrate giving a caloric value of 1-2 kcal/g (Kaur & Gupta, 2002; Roberfroid & Delzenne, 1998).

The meta-analysis of Liu *et al*. (2016) highlighted effects on fructan intake on LDL cholesterol (low density lipoprotein) reduction, while only two studies reported reduction of blood glucose levels. Dehghan *et al*. (2014) reported that oligofructose enriched inulin caused a decrease of fasting glucose levels in women with type-2 diabetes. Regulation of serum glucose may be explained by a delay of the carbohydrate absorption, or a modulation of hepatic gluconeogenesis (Roberfroid & Delzenne, 1998).

Reduction of serum triglycerides is one of the most important goal in order to prevent cardiovascular diseases. Feeding rats with 10% oligofructose significantly decreased serum triacylglycerols, mainly because of a reduction of the VLDL (very low density lipoprotein), with a reduced *de novo* lipogenesis rather than an increased catabolism (Delzenne & Kok, 2001). Merendino *et al*. (2006) demonstrated that rats fed with immature wheat kernels displayed a proliferation of lymphocytes and reduction of LDL cholesterol and triglycerides after several weeks of treatment. The meta-analysis reported in Brighenti (2007) over a 10 years period, revealed that the intake of 10 g/day inulin type fructans significantly decreases serum triacylglycerols in humans (-7.5%). It is reported that the effects are mainly mediated by gut fermentation, reduction of hepatic lipogenesis and synthesis of gastrointestinal hormones, rather than by blood glucose regulation. Chicory inulin (20 g/day) significantly reduces triglycerids in hypercholesterolemic men whereas association with cholesterol reduction resulted to be weaker (Causey *et al*., 2000).

Clinical trials reported that fructan intake may cause gastrointestinal symptoms. In general, the intake of inulin and short oligofructose in quantity lower than 15 g/day resulted to be well tolerated, eventually with mild transient events (Grabitske & Slavin, 2009). However, in a human trial 14 g of oligofructose caused gastrointestinal symptoms in healthy women (Pedernse *et al*., 1997).

Fructans are considered as "FODMAP", thus their consumption is reduced in specific diets targeting management of symptoms of the irritable bowel syndrome (abdominal pain, bloating and altered bowel habits) (Mansueto *et al*., 2015). FODMAP stands for "Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols" and are a group of poorly absorbed short chain carbohydrate including fructoligosaccharides, lactose, fructose, galactan and sorbitol. Barrett *et al*. (2010) provided evidences that the effects of FODMAP ingestion on irritable bowel syndrome (IBS) symptoms depend on poor absorption, on the increase of fermentable load and volume of liquid delivered to the proximal colon. This may be the mechanism explaining diarrhoea in some individuals (Barrett & Gibson, 2012). Reducing of FODMAP in the diet has been connected to a durable improvement in symptoms affected by IBS and quality life improvement (Gearry *et al*., 2009; Marsh *et al*., 2016) with effects on

fermentation and gas production, thus minimizing luminal distension and symptoms severity (Barrett & Gibson, 2012). The drawback of low FODMAP diet may be the reduction of intake of fermentable substrates for gut microflora. In fact, low FODMAP diet reduces the population of bifidobacteria after 4 weeks so a combination with probiotics is suggested (Staudacher & Whelan, 2016).

1.2.1.4. Physicochemical properties

Physical and chemical characteristics of inulin make it a food hydrocolloid used as an ingredient for multiple food applications. Hydrocolloids are generally defined as long chain polymers able to form viscous dispersion or gels in water (Milani & Maleki, 2012).

Inulin tends to aggregate in aqueous solutions forming supramolecular structure due to hydrogen bonding and hydrophobic interactions showing a critical aggregation concentration. Viscosity of inulin resulted to be proportional to molecular weight and concentration (Dan *et al*., 2009). In fact, above 15% concentration, inulin is able to form gel, by a network of small crystallites, showing a fat like texture. Concentration of inulin influences also the effect of temperature on gel formation. Inulin starts forming gels from 60 °C onward till 100% of gel at 70 °C. This is due to the kinetics energy that can allow contact between inulin chains (Kim *et al*., 2001). Inulin at 25 °C is almost insoluble, at 50 °C its solubility is only 1-2%, but increased temperature increases solubility reaching 34% at 90 °C (Kim *et al*., 2001). Glibowski (2010) demonstrated that combination of critical temperature and stirring time causes a reduction in viscosities increase due to inulin dissolution. Like other polymers, also inulin exhibits a glassy state which is described by the fundamental parameter of T_g (glass transition parameter). Basically, for temperature greater than T_g the material is a viscous liquid, whereas below this temperature, the glass transition itself forms an amorphous solid (Forrest *et al*., 1996). Glass transition temperature may vary according to moisture content (Zimeri & Kokini, 2002).

1.2.1.5. Effects of fructan addition on cereal-based products

Addition of inulin decreases water absorption of the dough, and increases time for dough development (Morris & Morris, 2012; Peressini & Sensidoni, 2009). Inulin delayed starch gelatinization and reduced water absorption during heating and in general replacement with up to 5% inulin resulted in acceptable characteristics of bread (Peressini & Sensidoni, 2009). In Juszczak *et al*. (2012) the presence of inulin decreased paste viscosity and increase gelatinization temperature of gluten-free dough.

In Ziobro *et al*. (2013) the addition of inulin with DP ≤ 10 caused an increase in loaf volume of gluten free breads, proportional to the concentration of inulin. Addition of inulin with DP ≥ 23 however did not affect leaf volume compared to control. The authors explain these data by a fact that mono and oligosaccharides released from low DP inulin compete with starch for unbound water, and at the same oligosaccharides and free sugars are easily used by yeasts.

More recently, Peressini *et al*. (2015) investigated the effect of inulin addition on extruded snacks, reporting a reduction of dough development due to inulin addition.

In pasta samples, Brennan *et al*. (2004) reported that inulin addition causes an increase in dry matter, a decrease of water absorption and loss of firmness at 10%. Aravind *et al*. (2012) however reported an increase in water absorption, a decrease of total gluten but not of the gluten index, optimal cooking time also increased with the higher concentration of inulin. Addition of inulin to formulations for spaghetti production had detrimental effects on sensory properties of the final product (Padalino *et al*., 2017).

1.2.1.6. Processing impacts

Because of their positive effects on human health, the impact of processing on fructan in foods should be evaluated. Bread making process has shown a negative effect on fructan composition probably due to enzymes of plants and microorganisms (Boskov Hansen *et al*., 2002). Morris *et al*. (2015) showed that inulin, which has a higher DP, was not degraded while FOS underwent degradation during bread making process. These findings are in agreements with Praznik *et al.* (2002). Baking process had a minor effect on long DP fructans in bread with substitution 10-12% with Jerusalem artichoke fructan. In the study of Gélinas *et al*. (2016) concentration of fructan dropped with or without baker's yeasts, while baking showed no effects on fructan structure. However Knez *et al*. (2014) reported no statistically significant loss of fructan in yeast free and unleavened wheat and rye bread while leavened bread significantly reduced the fructan content by 30%. Also, flour containing low DP fructan degraded more compared with fructan with a high DP (Andersson *et al*., 2009). Andersson *et al*. (2004) showed that air leavened non-fermented crisp bread had a similar amount of fructan as the flour, while yeast-fermented and especially sour dough bread had a reduced content. It is clear that yeasts activity is the primary cause of fructan drop in bread, therefore strategies to minimize fructan degradation mediated by yeast should be evaluated (Verspreet *et al*., 2013).

Pasta making also affects fructan content in the final product. Little effects of drying were observed, while a great amount of fructan, both FOS and inulin, were lost in boiling water compared with the uncooked samples, probably because of their higher solubility especially at high temperature (Bustos *et al*., 2011; Casiraghi *et al*., 2013; Gélinas *et al*., 2016).

Extrusion process, in particular when performed at high temperature, reduces the FOS levels in the final products, while inulin levels resulted to be more affected by the screw speed (Duar & Hoffman, 2015). These results are in agreement with Peressini *et al*. (2015) which also reports a loss of prebiotic compounds in extruded snacks.

Temperature and pH conditions during food processing may result in a degradation of fructan. Degradation was shown to occur at high temperature (60-120 °C), with a rate depending on pH (L'Homme *et al*., 2003; Matusek *et al*., 2009). FOS resulted to be more sensitive to acidic conditions (Courtin *et al*., 2009) while inulin has a higher thermostability at low pH (Duar & Hoffman 2015). For identical pH and temperature shortest oligomers are degraded faster (Blecker *et al*., 2002).

Dry heating of inulin at 165 °C reports an increase in low molecular weight compounds with DP below 13. After heating 30 minutes at 195 °C fructose oligomers tend to disappear (Böhm *et al*., 2005). Interestingly, dry heated inulin can improve the composition of gut microflora and preventing the growth

of harmful bacteria (Böhm *et al*., 2006). These effects are probably due to the formation of D-fructose dianhydride, a non-digestible and non-absorbable oligosaccharide which has shown positive effects on gut microbiota (Saito & Tomita 2000).

1.2.2. β-Glucan

1.2.2.1. Molecular structure

Mixed linkage β-glucan (hereafter referred to as β-glucan) are linear homopolymers of β-D-glucopyranosyl residues linked by two or three consecutives (1-4) linkages that are separated by a single (1-3) linkage (Figure 1.6) (Izydorczyk & Dexter, 2008). Approximately, the distribution of β-(1→4) and β-(1→3) linkages is 70% and 30% respectively. In barley, the average molecular weight of β-glucan is 1.5-1.8$x10^6$ g/mol. β-Glucan is part of the soluble dietary fiber, despite a proportion can be found in the insoluble fiber, in barley approximately 25% (Johansson *et al*., 2004). The structural features of β-glucan have been extensively studied through hydrolysis by lichenase. Lichenase is an endohydrolase which breaks specifically β(1-4) bonds which are linked at C-O-3, yielding mainly trisaccharides (3-O-β-D-cellobiosyl-D-glucose) and tetrasaccharides (3-O-β-D-cellotriosyl-D-glucose). The sum of tri and tetrasaccharides (DP3, DP4) after lichenase treatment was reported to be 90% of the total subunits (Cui *et al*., 2000). The specific ratio is also called DP3/DP4 ratio and it is very variable, depending on the source. In barley, the DP3/DP4 molar ratio is approximately 2-3 (Johansson *et al*., 2004; Knutsen & Holtekjølen, 2007). This ratio is a structure fingerprint of β-glucan as the occurrence of (1-3) linkages along the chain and their arrangement influences β-glucan tridimensional structure and thus behavior in solutions and functional properties. In fact, higher DP3/DP4 ratio leads to higher probability of interchain aggregation through hydrogen bonds and thus a lower solubility in water and a higher ability to form gels (Izydorczyk *et al*., 1998).

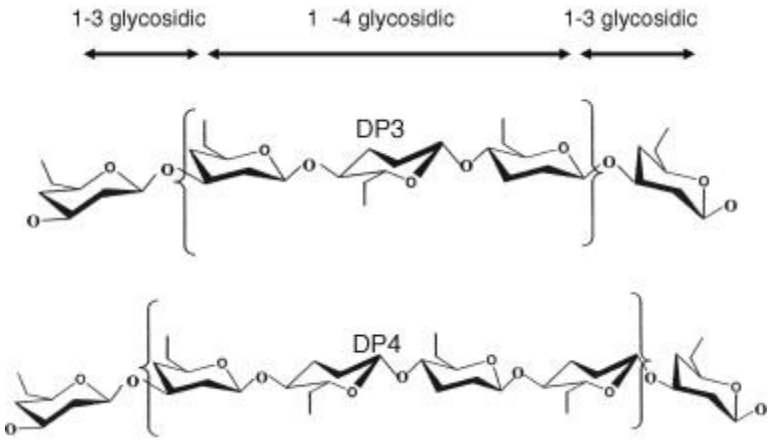

Figure 1.6. *Molecular structure of cereal β-glucan (Vasanthan & Temelli, 2008)*

1.2.2.2. Occurrence in cereal grain

β-Glucans are one of the major non-starch polysaccharides in barley and they are mainly located in the endosperm cell walls where they account for 70% of the total components (Fincher, 1975). In barley the

content of β-glucan falls in the range 2-11% (Baik & Ullrich, 2008; Holtekjølen *et al*., 2006; Izydorczyk *et al*., 2000) and it is influenced by both genetic and environmental factors. The highest content of β-glucan is normally reported in high amylose and waxy barley followed by zero amylose and normal barley (Andersson *et al*., 2008; Izydorczyk *et al*., 2000). Hulless barley contains a higher level of β-glucan compared to hulled barley, due to its localization in the endosperm cell walls (Holtekjølen *et al*., 2006; Izydorczyk *et al*., 2000).

β-Glucan are part of the dietary fiber but is the soluble fraction which has effects on glucose and cholesterol levels in blood. Therefore, in the evaluation of β-glucan quantity, the soluble fraction should always be determined (Izydorczyk *et al*., 2000). Extraction parameters such as temperature, pH and endogenous enzyme activity can greatly influence estimation of β-glucan yields (Åman & Graham, 1987). The water extractable β-glucan is higher in high amylose, and waxy barley than in normal barley (Ajithkumar *et al*., 2005; Izydorczyk *et al*., 2000).

Fractioning of the kernel showed that the distribution of β-glucan in the kernel is uneven. When bran, shorts and flour are analysed, the β-glucan result to be more concentrated in the shorts or middlings (Sullivan *et al*., 2010; Zheng *et al*., 2011) fractions which are between the bran and the endosperm.

1.2.2.3. Physicochemical properties and process impacts

Viscosity is the quantity that describes the resistance of a liquid to flow. Viscosity is represented by the symbol "η" and is the ratio between the shearing stress and the velocity gradient (shear rate). According to the Newton's law for an ideal viscous liquid the viscosity η is constant. The polymers that follow such equations are called Newtonian's fluids, otherwise they are called non-Newtonian's fluids. When the solution viscosity decreases with increase of shear rate, the solution is said to have a shear thinning flow behavior. On the other hand, if the solution viscosity increases with increasing shear rate, the solution has a shear thickening flow behavior (Bolmstedt, 2000). Specific viscosity describes the fractional increase in viscosity upon addition of polysaccharide. Intrinsic viscosity is defined as dividing the specific viscosity to the polymer concentration, extrapolated to zero concentration (cm^3/g, dl/g) and it is proportional to molecular weight. In a dilute solution, when concentration increases to a critical point (c*), molecules in the solution begin to overlap with each other. When the polymer concentration exceeds c* polymer chains starts to interact with each other forming networks.

Size and structural features of β-glucan are important for viscosity and rheological properties. Viscosity values in β-glucan samples was reported to be between 0.63 and 3.01 dl/g (Vaikousi *et al*., 2004). The intrinsic viscosity of β-glucan solutions in oat increased with the increase of molecular weight at 20°C (Lazaridou *et al*., 2003). The estimated coil overlap parameters "c*η" and "c** η" ranged between 0.19-0.56 and 2.12 and 2.86 (Vaikousi *et al.*, 2004).

In mixed linkage β-glucan solutions, the critical concentration and shear thinning properties are influenced mainly by the molecular size of polysaccharides and are less dependent on the structure (Lazaridou *et al*., 2004). The intrinsic viscosity values for β-glucans ranged between 2.98-3.11 dl/g for the high molecular weight and 1.56-1.86 (Lazaridou *et al*., 2004) for the low molecular weight.

Izydorczyk *et al*. (2000) monitored the changes in viscosity in a barley flour slurry during a period of 2 hours. The initial increase of viscosity is mainly attributed to solubilisation of β-glucans, while the decline is due to degradation by endogenous β-glucanases. Overall, it was found a better correlation between viscosity and soluble β-glucan.

Under optimal conditions β-glucan can gel also at low concentration (Makela *et al*., 2017). The specific ratio trisaccharides to tetrasaccharide obtained after β-glucan hydrolisis and the amount of cellulose like-fragments are important determinants of β-glucan aggregation in aqueous solution. The model exposed by Böhm & Kulicke (1999) proposes association of consecutive cellotriosyl units linked by (1-3) bonds forming a helical structure. In fact, gelling abilities of cereal β-glucans are in the order in wheat ≥ barley-rye ≥ and oat corresponding to the ratio of tri- to tetrasaccharides. Gelation rate increases with decrease of molecular weight, and the hypothesis may be that low molecular weight fragments are more mobile and prone to interact compared with the longer chains (Cui & Wang, 2009).

Viscosity is an important parameter affecting beneficial effects of β-glucan on human health and as reported is related to β-glucan solubility and molecular weight. Processes and storage can greatly influence β-glucan physicochemical characteristics like molecular weight and solubility therefore affecting its health impact. De Paula *et al*., (2017) reported that the steps of pasta making (extrusion and drying) negatively affected β-glucan physicochemical properties, while cooking improved its extractability. Bread making is reported to decrease the MW (molecular weight) of β-glucan by 50% in the final product (Flander *et al*., 2007; Tiwari *et al*., 2011). The baking process and yeasts addition itself did not influence the β-glucan molecular weight. Mixing barley and wheat flour causes a reduction of β-glucan molecular weight mainly due the activity of endo-β-glucanases in wheat flour. Nevertheless, reducing mixing and fermentation time and eventually adopt conditions that inactivate the enzymes can minimize β-glucan degradation during the baking process (Andersson *et al*., 2004; Gamel *et al*., 2015; Rieder *et al*., 2015).

Baking to muffin according to Beer *et al.* (1997) increased the extractable β-glucan by 3-4 fold but decreased the molecular weight peak by 50%. Extrusion process on oat cereals conducted at 131 °C and 18.7% water did not impact the molecular weight of β-glucan whereas increased solubility from 38.7 to 66.8% (Tosh *et al*., 2010). β-Glucan in extruded barley flour had a higher solubility compared to non-extruded flour (Jiang & Vasanthan 2000). Hu *et al*. (2010) also reported no significant losses in β-glucan content in oat kernels with normal pressure steaming, autoclave steaming, hot-air roasting and infrared roasting.

Beer *et al*. (1997) reported that storage of muffins at -20 °C for 8 weeks lead to a 50% decrease of solubilized β-glucan. These data are confirmed also by Lan-Pidhainy *et al*. (2007) which reported a reduced β-glucan extractability in oat muffin of more than 50% after 4 freeze-thaw cycles and an attenuated effectiveness reducing postprandial glycaemia.

1.2.2.4. Effects on human health

Intake of dietary fibre is recommended to reduce the onset of various diseases a maintain a good health status. Over the last years, many evidences have risen on additional specific beneficial effects of β-glucans on human health. Reduction in LDL cholesterol is the primary step to modify plasma lipid profile and reduce the risk of cardiovascular disease mortality (Briel *et al*., 2009). Rats fed with tortilla enriched in β-glucans showed significantly lower LDL cholesterol values in plasma but not total cholesterol. Excretion of fats was higher in the group fed with β-glucan enriched tortilla suggesting a role of β-glucan in interfering with fats absorption (Hecker *et al*., 1998). Effects were also achieved with hull less barley in hypercholesterolemic hamsters (Tong *et al*., 2015). Meta-analysis on humans suggested that oat and barley β-glucans can reduce total cholesterol and LDL cholesterol probably due to an increase bile excretion induced by β-glucan (Tiwari & Cummins, 2011). Results are confirmed also in hypercholesterolemic subjects, despite no significant results were observed for HDL (High density lipoprotein) cholesterol and triglycerids (Zhu *et al*., 2015). Studies *in vitro* demonstrate that β-glucan inhibits uptake of long chains fatty acids and downregulated expression of intestinal genes associated with fatty acid and cholesterol synthesis and fatty acid transport (Drozdowski *et al*., 2010).

Anomalous values of glucose and insulin concentration in blood can be indicators for insulin resistance and type-2 diabetes. Recommendations generally target a change in lifestyle dietary habits. Foods containing β-glucan in good amount are low glycaemic index (GI) foods and have been reported to decrease post-prandial insulin and glucose response in humans (AbuMweis *et al*., 2016; Ames *et al*., 2015; Tosh, 2013). In animal models the reduction in serum insulin could be linked to decrease to reduction in hepatic lipid content and an improved insulin clearance (Choi *et al*., 2010). In overweight subjects plasma insulin and glucose concentration are significantly lower after β-glucans load compared to treatment with resistant starch (Behall *et al*., 2006). In obese women Kim *et al*. (2009) reported that consumption of foods containing 10 g of β-glucan per serving significantly reduced insulin response. High molecular weight and high viscosity β-glucan are more effective in decreasing postprandial glucose response and in reduction of starch digestibility and alleviating the oxidative stress which may cause diabetes (Regand *et al*., 2011; Zhao *et al*., 2014).

Gut microbiota is implicated in regulating metabolic pathways and improve health status, but its composition can vary in response to different factors including changes in the diet and inclusion of food able to modulate gastrointestinal microbiome (Rebello *et al*., 2015). Changes in gut microbiota can also have impact on the regulation of host energy homeostasis (Delzenne *et al*., 2013). Barley and barley β-glucan containing foods can improve the composition of intestinal microbiota, positively affecting the growth of beneficial bacteria, increasing the levels of short chain fatty acids (SCFA) (De Angelis *et al*., 2015; Arena *et al*., 2014). Modulation of gut microbiota depends on the molecular weight which also affects the reducing potential of the risk of cardiovascular diseases (Wang *et al*., 2016). Obesity is a risk for the development of insulin resistance and heart diseases. In animal models, the intake of β-glucan was effective in reducing the body weight (Belobrajdic *et al*., 2016; Belobrajdic *et al*., 2015). Regular intake of high β-glucans foods can help managing the body weight through different mechanisms, like

reduction of nutrient absorption (Lifschitz *et al*., 2002), hormones regulations (Beck *et al*., 2009; Vitaglione *et al*., 2009) and appetite regulation.

Despite literature is controversial about regulation of food intake mediated by β-glucan, it was proposed that it may not be the main mechanism explaining managing of body weight (Clark & Slavin 2013). Vitaglione *et al*. (2009) reported a decrease in energy intake at lunch after breakfast containing 3% β-glucan. Kim *et al*. (2009) found marginal effects on satiety of both overweight men and women with a dose of 2 g/day. Pentikäinen *et al*. (2014) instead reported an in increase in postprandial satiety after consuming foods containing oat β-glucan. The increase in satiety after a meal containing β-glucan, may not influence energy intake at lunch (Schroeder *et al*., 2009; Vitaglione *et al*., 2010). Molecular weight was not reported to be influential for satiety or energy intake (Clegg & Thondre 2014),

Finally, microbial and cereal β-glucan may enhance the immune system (Daou & Zhang, 2012; Rieder & Samuelsen, 2012; Volman *et al*., 2008).

1.2.3. Fructans and β-glucan as functional food ingredients

There is increasing scientific evidence that beyond the nutritional properties, the intake of certain foods or food ingredients can improve physical and mental well-being and, moreover, reduce the risks of the onset of a wide range of diseases.

Nowdays, no universally accepted definition of functional foods exists. The concept of functional food proposed by the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE), coordinated by the International Life Sciences Institute (ILSI) considered specific features as follows:

- being a food or food-ingredient which is conventional or daily consumed in the diet.
- The food should be natural and no synthetic.
- It is beneficial for human health beyond its basic nutritional value, enhancing well-being or reducing the risk of diseases.

The Consensus Document on Scientific Concept of Functional Food in Europe published in 1999 proposed the following working definition "*A food can be considered as functional if it beneficially affects one or more target functions in the body, beyond its nutritional properties, in a way that can reduce the risk of disease or improve the health status*" (EU-ILSI 1999). Claims are used to communicate to the consumers the benefits of functional foods. According to the definition given by the Codex Alimentarius in 1991, a claim is "*Any representation which states suggest or implies that a food has certain characteristics relating to its origin, nutritional properties, nature, production, processing, composition or any other quality*" (EU-ILSI 1999).

Within the regulation on functional foods, claims can be broadly divided into nutrition and health claims. A health claim can be defined as a general statement that of a relationship between a food or a constituent of that food and health. Health claims can be divided into: function health claim, risk reduction claim and claims referring to children development. On the other hand, a nutrition claim is any representation

which states, suggest or implies that a food has a particular nutritional property including but not limited to the energy value and to the content of fat, carbohydrates as well as the content of vitamin and minerals (Codex Alimentarius 1997). In the European Union, health and nutrition claims are regulated by the "Regulation 2006/1924/EC on nutrition and health claims made on foods" and modifications.

As explained, fructans and β-glucan are considered part of the soluble dietary fiber. Adequate amount of these polysaccharides can allow foods to bear nutrition claims. In the case of dietary fibers, the following statements are allowed on foods (Regulation 2006/1924/EC):

- source of fibers: 3 g/100 g or 1.5 g/100 kcal.
- High fibers: 6 g/100 g or 3 g/100 kcal.

Fructans and β-glucan have been studied for their beneficial physiological functions. Results of studies on β-glucan lead to an approval process of health claims related also to barley-derived β-glucan. The European Food Safety Authority (EFSA) considered the quantity of 3 g/day of β-glucan from barley and > 1 g per quantified portion to obtain the claimed effects "Barley β-glucan have been shown to lower/reduce blood cholesterol" with the aim to reduce risk of coronary heart disease (EFSA 2011a). Reduction of post-prandial glycemic response was also regulated as a health claim with the quantity of 4 g/ 30 g available carbohydrates (β-glucan from oat and barley) (EFSA 2011b). The US Food and Drug Administration (FDA) set quantity of 0.75 g/portion of β-glucan to obtain the health claim related to reduction of the risk of cardiovascular disease.

At the moment, no health claims are allowed for cereal derived fructans. Despite this, health claims have been proposed and accepted regarding positive impact of fructans of different origin. One of the health claims concerns "native inulin" which has a DP ≥ 9 and is extracted by chicory. The statement is "*Chicory inulin contributes to normal defecation by increasing stool frequency*" and the claimed effect is obtained by consuming 12 g of inulin/day (EFSA, 2015). The other health claim involving fructan, was firstly proposed for fructooligosaccharides but then extended to non-digestible carbohydrate in general as having the ability to lower glycaemic response when replacing sugars in foods and beverages (EFSA, 2014). In order for foods to bear the claims, sugars should be replaced so that food or drinks contain reduced amount of sugars as stated in the annex to Regulation 2006/1924/EC (≤ 5 g/100 g for solids and ≤ 2.5 g/100 ml). Table 1.4. summarizes claims for fructan and β-glucan applicable within the US and European legislation.

Table 1.4. *Nutrition and health claims for fructan and β-glucan estabilished by EU-US legislation.*

Claim	β-glucan	Fructan	Quantity
[a]Source of fibers	●	●	3 g/100 g or 1.5 g/100 Kcal
[a]High fibers	●	●	6 g/100 g or 3 g/100 Kcal
[a]Barley β-glucans have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease	●		1 g/ portion and 3 g/day
[a]Consumption of β-glucans from oats or barley contributes to the reduction of the glucose rise after a meal	●		4 g/30 g available carbohydrates
[a]Chicory inulin contributes to normal defecation by increasing stool frequency		●	12 g/day
[a]Consumption of foods/drinks containing non-digestible carbohydrates instead of sugars induces a lower blood glucose rise after meals compared to sugar-containing foods/drinks	●	●	Sugars ≤ 5 g/100 g for solid foods and ≤ 2.5 g/100 mL in beverages
[b]Diets that are low in saturated fat and cholesterol and that include β-glucan from barley may reduce the risk of heart diseases	●		0.75 g /portion and 3 g/day

[a] EFSA, [b] FDA

1.3. References

AACC (2001). The definition of dietary fiber. *Cereal Foods World*, **46**: 112-126.

Abeynayake SW, Etzerodt TP, Jonaviciene K, Byrne S, Torben A, & Boelt B (2015). Fructan metabolism and changes in fructan composition during cold acclimation in perennial ryegrass. *Frontiers in Plant Science*, **6**(May): 1–13.

Abrams SA, Griffin IJ, & Hawthorne KM (2007). Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. *The Journal of Nutrition*, **137(**11 Suppl): 2524S–2526S.

Abrams SA, Hawthorne KM, Aliu O, Hicks PD, Chen Z, & Griffin IJ (2007). An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. *The Journal of Nutrition*, **137**(10): 2208–2212.

AbuMweis S, Thandapilly SJ, Storsley J, & Ames N (2016). Effect of barley β-glucan on postprandial glycaemic response in the healthy human population: a meta-analysis of randomized controlled trials. *Journal of Functional Foods*, **27**: 329–342.

Ajithkumar A, Andersson R, Christerson T, & Åman P (2005). Amylose and β-glucan content of new waxy barleys. *Starch/Staerke*, **57**(6): 235–239.

Åman P, & Graham H (1987). Analysis of total and insoluble mixed-linked (1-3),(1-4)-D- β-glucans in barley and oats. *Journal of Agricultural and Food Chemistry*, **35**: 704–709.

Åman P, Hesselman K, & Tilly A (1985). The variation in chemical composition of swedish barleys. *Journal of Cereal Science*, **3**: 73–77.

Ames N, Blewett H, Storsley J, Thandapilly SJ, Zahradka P, & Taylor C (2015). A double-blind randomised controlled trial testing the effect of a barley product containing varying amounts and types of fibre on the postprandial glucose response of healthy volunteers. *British Journal of Nutrition*, **113**(9): 1373–1383.

Anderson PM, Oelke EA. & Simmons SR (1995). Growth and development guide for spring barley. *University of Minesota, College of Agriculture*.

Andersson AAM, Armö E, Grangeon E, Fredriksson H, Andersson R, & Åman P (2004). Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*, **40**(3): 195–204.

Andersson AAM, Lampi, AM, Nyström L, Piironen V, Ward J, Li L, Gebruers K, Courtin CM, Delcour JA, Boros D, Fras A, Dynkowska W, Rakszegi M, Bedő Z, Shewry PR, & Åman P (2008). Phytochemical and fiber components in barley varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, **56**(21): 9777–9784.

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.

Ao Z, & Jane J (2007). Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydrate Polymers*, **67**(1): 46–55.

Aravind N, Sissons MJ, Fellows CM, Blazek J, & Gilbert EP (2012). Effect of inulin soluble dietary fibre addition on technological, sensory and structural properties of durum wheat spaghetti. *Food Chemistry*, **132**(2): 993–1002.

Arena MP, Caggianiello G, Fiocco D, Russo P, Torelli M, Spano G, & Capozzi V (2014). Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. International *Journal of Molecular Sciences*, **15**(2): 3025–3039.

Badr A, Müller K, Schäfer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, & Salamini F (2000). On the origin and domestication history of barley (*Hordeum vulgare*). *Molecular Biology of Evolution*, **17**(4): 499–510.

Baik BK, & Ullrich SE (2008). Barley for food: characteristics, improvement, and renewed interest. *Journal of Cereal Science*, **48**(2): 233–242.

Barrett, JS, Arry, Gearry RB, Muir JG, Irving PM, Rose R, Rosella O, Haines ML, Shepherd SJ, & Gibson PR (2010). Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon, *Alimentary Pharmacology & Therapeutics*, **31**(January): 874–882.

Barrett JS, & Gibson PR (2012). Fermentable disaccharides, monosaccharides and polyols (FODMAPs) and non-allergic food intolerance: FODMAPs or food chemicals?. *Therapeutic Advances in Gastroenterology*, **5**(4): 261–268.

Beck EJ, Tapsell LC, Batterham MJ, Tosh SM, & Huang XF (2009) Increases in peptide Y-Y levels following oat β-glucan ingestion are dose-dependent in overweight adults. *Nutrition Research*, **29**(10): 705–709.

Beer MU, Wood PJ, Weisz J, & Fillion N (1997). Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by an in vitro digestion system. *Cereal Chemistry*, **74**(6): 705–709.

Behall KM, Scholfield DJ, & Hallfrisch JG (2006). Barley β-glucan reduces plasma glucose and insulin responses compared with resistant starch in men. *Nutrition Research*, **26**(12): 644–650.

Bellido GG, & Beta T (2009). Anthocyanin composition and oxygen radical scavenging capacity (ORAC) of milled and pearled purple, black, and common barley. *Journal of Agricultural* and *Food Chemistry*, **57**(3): 1022–1028.

Belobrajdic DP, Hino S, Kondo T, Jobling SA, Morell MK, Topping, DL, Morita T, & Bird AR (2016). High wholegrain barley β-glucan lowers food intake but does not alter small intestinal macronutrient digestibility in ileorectostomised rats. *International Journal of Food Sciences and Nutrition*, **67**(6): 678–685.

Belobrajdic DP, Jenkins CLD, Bushell R, Morell MK, & Bird AR (2012). Fructan extracts from wheat stem and barley grain stimulate large bowel fermentation in rats. *Nutrition Research*, **32**(8): 599–606.

Belobrajdic DP, Jobling SA, Morell MK, Taketa S, & Bird AR (2015). Wholegrain barley β-glucan fermentation does not improve glucose tolerance in rats fed a high-fat diet. *Nutrition Research*, **35**(2): 162–168.

Benkeblia N (2013). Fructooligosaccharides and fructans analysis in plants and food crops. *Journal of Chromatography A*, **1313**: 54–61.

Blecker C, Fougnies C, Van Herck J, Chevalier J, & Paquot M (2002). Kinetic study of the acid hydrolysis of various oligofructose samples. *Journal of Agricultural and Food Chemistry*, **50**: 1602–1607.

Böhm A, Kaiser I, Trebstein A, & Henle T (2005). Heat-induced degradation of inulin. *European Food Research and Technology*, **220**: 466–471.

Böhm A, Kleessen B, & Henle T (2006). Effect of dry heated inulin on selected intestinal bacteria. *European Food Research and Technology*, **222**: 737–740.

Böhm N, & Kulicke W (1999). Rheological studies of barley (1-3)(1-4)-β-glucan concentrated solution: mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research*, **315**: 302–311.

Bolmstedt U (2000). Viscosity & rheology–theoretical and practical considerations in liquid food processing. *New Food*, **3**(2): nd.

Boskov Hansen H, Andreasen MF, Nielsen MM, Larsen L, Bach Knudsen K, Meyer A, Christensen LP, & Hansen Å (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *European Food Research and Technology*, **214**: 33–42.

Bouhnik Y, Achour L, Paineau D, Riottot M, Attar A, & Bornet F (2007). Four-week short chain fructo-oligosaccharides ingestion leads to elderly volunteers. *Nutrition Journal*, **6**(42): 1–7.

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**(1): 65–72.

Bravo LA, Ulloa N, Zuniga GE, Casanova A, Corcuera LJ, & Alberdi M (2001). Cold resistance in Antarctic angiosperms. *Physiologia Plantarum*, **111**: 55–65.

Brennan CS, Kuri V, & Tudorica CM (2004). Inulin-enriched pasta: effects on textural properties and starch degradation. *Food Chemistry*, **86**: 189–193.

Briel M, Ferreira-Gonzalez I, You JJ, Karanicolas PJ, Akl EA, Wu P, Blechacz B, Bassler D, Wei X, Sharman A, Whitt I, Alves da Silva S, Khalid Z, Nordmann AJ, Zhou Q, Walter SD, Vale N, Bhatnagar N, O'Regan C, Mills EJ, Bucher HC, Montori VC, & Guyatt GH (2009). Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. *BMJ (Clinical Research Ed.)*, **338**: 1-8.

Brighenti F (2007). Dietary fructans and serum triacylglycerols: a meta-analysis of randomized controlled trials. *The Journal of Nutrition*, **137**: 2552–2556.

Bustos M, Pérez G, & Leòn A (2011). Effect of four types of dietary fiber on the technological quality of pasta. *Food Science and Technology International*, **2011**: 1–9.

Cacela C, & Hincha DK (2006). Monosaccharide composition, chain length and linkage type influence the interactions of oligosaccharides with dry phosphatidylcholine membranes. *Biochimica et Biophysica Acta*, **1758**: 680–691.

Cani PD, Dewever C, & Delzenne NM (2004). Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *British Journal of Nutrition*, **92**: 521–526.

Cani PD, Joly E, Horsmans Y, & Delzenne NM (2006). Oligofructose promotes satiety in healthy human: a pilot study. *European Journal of Clinical Nutrition*, **60**: 567–572.

Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, & Delzenne NM (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *American Journal of Clinical Nutrition*, **90**: 1236–1243.

Cani PD, Neyrinck AM, Maton N, Delzenne NM, Patrice D, & Neyrinck AM (2005). Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obesity Research*, **13**(6): 1000-1007.

Casiraghi MC, Pagani MA, Erba D, Cecchini C, & D'Egidio MG (2013). Quality and nutritional properties of pasta products enriched with immature wheat grain. *International Journal of Food Science and Nutrition*, **65**(5): 544-550.

Causey JL, Feirtag, JM, Gallagher DD, Tungland BC, & Slavin J (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research*, **20**(2): 191–201.

Choi J., Kim H, Jung, MH, Hong S, & Song J (2010). Consumption of barley β-glucan ameliorates fatty liver and insulin resistance in mice fed a high-fat diet. *Molecular Nutrition & Food Research*, **54**(7): 1004–1013.

Choque Delgado GT, Tamashiro, Wirla MSC, & Pastore GM (2015). Immunomodulatory effects of fructans. *Food Research International*, **43**(5): 1231–1236.

Cimini S, Locato V, Vergauwen R, Paradiso A, Cecchini C, Vandenpoel L, Verspreet J, Courtin CM, D'Egidio MG, Van den Ende W, & De Gara L (2015). Fructan biosynthesis and degradation as part of plant metabolism controlling sugar fluxes during durum wheat kernel maturation. *Frontiers in Plant Science* **6**(Feb.): 1–10.

Clark MJ, & Slavin JL (2013). The effect of fiber on satiety and food intake: a systematic review. *Journal of the American College of Nutrition*, **32**(3): 200–211.

Clegg ME, & Thondre PS (2014). Molecular weight of barley β-glucan does not influence satiety or energy intake in healthy male subjects. *Appetite*, **83**: 167–172.

Codex Alimentarius (1997). *Guidelines for use of nutrition and health claims*. Cac/Gl 23-1997.

Courtin CM, Swennen K, Verjans P, & Delcour JA (2009). Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. *Food Chemistry*, **112**: 831–837.

Coxam V (2007). Current data with inulin-type fructans and calcium, targeting bone health in adults. *The Journal of Nutrition*, **137**(11 Suppl): 2527–2533.

Crowe JH, Crowe LM, Carpenter JF, & Wistrom CA (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochemistry Journal*, **242**: 1–10.

Cui SW, & Wang Q (2009). Cell wall polysaccharides in cereals: chemical structures and functional properties. *Structural Chemistry*, **20**(2): 291–297.

Cui W, Wood PJ, Blackwell B, & Nikiforuk J (2000). Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan-comparison with other cereal β-D-glucans. *Carbohydrate Polymers*, **41**(3): 249–258.

Dan A, Ghosh S, & Moulik SP (2009). Physicochemical studies on the biopolymer inulin: a critical evaluation of its self-aggregation, aggregate-morphology, interaction with water, and thermal stability. *Biopolymers*, **91**(9): 687–699.

Daou C, & Zhang H (2012). Oat β-glucan: its role in health promotion and prevention of diseases. *Comprehensive Reviews in Food Science and Food Safety*, **11**(4): 355–365.

De Angelis M, Montemurno E, Vannini L, Cosola C, Cavallo N, Gozzi G, Maranzano V, Di Cagno R, Gobbetti M, & Gesualdo L (2015). Effect of whole-grain barley on the human fecal microbiota and metabolome. *Applied and Environmental Microbiology*, **81**(22): 7945–7956.

De Gara L, De Pinto MC, Moliterni VMC, & D'Egidio MG (2003). Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany* **54**(381): 249–258.

Dehghan P, Pourghassem Gargari B, & Asghari Jafar-abadi M (2014). Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type-2 diabetes mellitus: a randomized controlled clinical trial. *Nutrition*, **30**(4): 418–423.

Delzenne NM, & Kok N (2001). Effects of fructans-type prebiotics on lipid metabolism. *The American Journal of Clinical Nutrition*, **73**: 456–458.

Delzenne NM, Neyrinck AM, & Cani PD (2013). Gut microbiota and metabolic disorders: How prebiotic can work? *The British Journal of Nutrition*, **109**: S81-S85.

Dewulf EM, Cani PD, Neyrinck AM, Possemiers S, Van Holle A, Muccioli GG, Deldicque L, Bindels LB, Pachikian BD, Sohet FM, Mignolet E, Francaux M, Larondelle Y, & Delzenne NM (2011). Inulin-type fructans with prebiotic properties counteract GPR43 overexpression and PPAR γ -related adipogenesis in the white adipose tissue of high-fat diet-fed mice. *The Journal of Nutritional Biochemistry*, **22**(8): 712–722.

Djurle S, Andersson AAM, & Andersson R (2016). Milling and extrusion of six barley varieties, effects on dietary fibre and starch content and composition. *Journal of Cereal Science*, **72**: 146–152.

Drozdowski LA, Reimer RA, Temelli F, Bell, RC, Vasanthan T, & Thomson ABR (2010). β-Glucan extracts inhibit the in vitro intestinal uptake of long-chain fatty acids and cholesterol and down-regulate genes involved in lipogenesis and lipid transport in rats. *Journal of Nutritional Biochemistry*, **21**(8): 695–701.

Duar RM, & Hoffman M (2015). Processing effects on four prebiotic carbohydrates supplemented in an extruded cereal and a low pH drink. *Food Science and Technology*, **1**: 1–11.

EFSA (European Food Safety Authority) (2011a). Scientific opinion on the substantiation of a health claim related to barley β-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) 1924/2006. *Efsa Journal*, **9**(12): 2471

EFSA (European Food Safety Authority) (2011b). Scientific opinion on the substantiation of health claims related to β-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236,1299), increase in satiety leading to a reduction in energy intake (ID 851,852), reduction of post-prandial glycemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *Efsa Journal*, **9**(6): 2207.

EFSA (European Food Safety Authority) (2014). Scientific opinion on the substantiation of a health claim related to non-digestible carbohydrates ad reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/006. *Efsa Journal*, **12**(1): 3513.

EFSA (European Food Safety Authority) (2015). Scientific Opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing

stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006 1. *Efsa Journal*, **13**(1): 3951.

Englyst H (1989). Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology*, **23**: 27–42.

EU-ILSI (1999). Scientific concepts of functional foods in Europe. Consensus document. *The British Journal of Nutrition*, **81**(Suppl 1): 1–27.

Europe Economics (2016). The contribution made by beer to the European economy. *The Brewers of Europe.*

Evers T, & Millar S (2002). Cereal grain structure and development: some implications for quality. *Journal of Cereal Science*, **36**: 261–284.

FDA (Food and Drug Administration) (2017) Health claims: Soluble fiber from certain foods and risk of coronary heart disease (CHD). Title 21, Chapter I, Part 101.81. Available at: https://www.ecfr.gov/cgi-bin/text idx?SID=ef11035c62ba01787fec18404f8070a2&mc=true&node=se21.2.101_181&rgn=div8.

Fettel N, Bowden P, McNee T, & Border N (2010). *Barley. Growth and Development*. (J. Edwards, Ed.), NSW Government, Industry and Investment.

Fincher GB (1975). Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brewing*, **81**: 116–122.

Flander L, Salmenkallio-Marttila M, Suortti T, & Autio K (2007). Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT - Food Science and Technology*, **40**(5): 860–870.

Forchielli ML, & Walker WA (2005). The role of gut-associated lymphoid tissues and mucosal defence. *The British Journal of Nutrition*, **93**: S41-S48.

Forrest JA, Dalnoki-Veress K, Stevens JR, & Dutcher JR (1996). Effect of free surfaces on the glass transition temperature of thin polymer films. *Physical Review Letters*, **77**(10): 8–11.

Fox GP, Panozzo JF, Li CD, Lance RCM, Inkerman PA, & Henry RJ (2003). Molecular basis of barley quality. *Australian Journal of Agricultural Research*, **54**(12): 1081-1101

Freeman PL, & Palmer GH (1984). The structure of the pericarp and testa of barley. *Journal of the Institute of Brewing*, **90**(2): 88–94.

Gamel TH, Abdel-Aal ESM, & Tosh SM (2015). Effect of yeast-fermented and sour-dough making processes on physicochemical characteristics of β-glucan in whole wheat/oat bread. *LWT - Food Science and Technology*, **60**(1): 78–85.

Gearry RB, Irving PM, Barrett JS, Nathan DM, Shepherd SJ, Gibson PR, & Hill B (2009). Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease- a pilot study. *Journal of Crohn's & Colitis*, **3**(1): 8–14.

Gélinas P, McKinnon C, & Gagnon F (2016). Fructans, water-soluble fibre and fermentable sugars in bread and pasta made with ancient and modern wheat. *International Journal of Food Science and Technology*, **51**: 555–564.

Gibson GR (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *The Journal of Nutrition*, **129**(7 Suppl): 1438S–1441S.

Glibowski P (2010). Effect of thermal and mechanical factors on rheological properties of high performance inulin gels and spreads. *Journal of Food Engineering*, **99**(1): 106–113.

Gómez-Caravaca AM, Verardo V, Candigliota T, Marconi E, Segura-Carretero A, Fernandez-Gutierrez A, & Caboni MF (2015). Use of air classification technology as green process to produce functional barley flours naturally enriched of alkylresorcinols, β-glucans and phenolic compounds. *Food Research International*, **73**: 88–96.

Grabitske HA, & Slavin JL (2009). Gastrointestinal effects of low-digestible carbohydrates gastrointestinal effects of low-digestible carbohydrates. *Critical Review in Food Science and Nutrition*, **49**: 327–360.

Guarner F (2005). Inulin and oligofructose: impact on intestinal diseases and disorders. *British Journal of Nutrition*, **93**: 61–65.

Guigoz Y, Rochat F, Rochat I, & Schiffrin EJ (2002). Effects of oligosaccharide on the fecal flora and non-specific immune system in elderly people. *Nutrition Research*, **22**: 13–25.

Haskå L, Nyman M, & Andersson R (2008). Distribution and characterisation of fructan in wheat milling fractions. *Journal of Cereal Science*, **48**(3): 768–774.

Hecker KD, Meier ML, Newman RK, & Newman CW (1998). Barley β-glucan is effective as a hypocholesterolaemic ingredient in foods. *Journal of the Science of Food and Agriculture*, **179**: 179–183.

Hendry GF (1993). Evolutionary origins and natural functions of fructans-a climatological, biogeographic and mechanistic appraisal. *New Phytologist*, **123**(1): 3–14.

Henry RJ (1988). The carbohydrates of barley grains-a review. *Journal of the Institute of Brewing*, **94**(2): 71–78.

Henry RJ, & Saini H (1989). Characterization of cereal sugars and oligosaccarides. *Cereal Chemistry*, **66**(5): 362-365.

Hernanz D, Nuñez V, Sancho AI, Faulds CB, Williamson G, Bartolomé B, & Gómez-Cordovés C (2001). Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. *Journal of Agricultural and Food Chemistry*, **49**(10): 4884–4888.

Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, & Friedlander AL (2007). Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. *British Journal of Nutrition*, **25**: 365–372.

Holopainen URM, Pihlava JM, Serenius M, Hietaniemi V, Wilhelmson A, Poutanen K, & Lehtinen P (2014). Milling, water uptake, and modification properties of different barley (*Hordeum vulgare*

L.) lots in relation to grain composition and structure. *Journal of Agricultural and Food Chemistry*, **62**(35): 8875–8882.

Holtekjølen AK, Uhlen AK, Bråthen E, Sahlstrøm S, & Knutsen SH (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. *Food Chemistry*, **94**(3): 348–358.

Hosono A, Ozawa A, Kato R, Ohnishi Y, Nakanishi Y, Kimura T, & Nakamura R (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Bioscience, Biotechnology and Biochemistry*, **64**(4): 758–764.

Hu X, Xing X, & Ren C (2010). The effects of steaming and roasting treatments on β-glucan, lipid and starch in the kernels of naked oat (*Avena nuda*). *Journal of the Science of Food and Agriculture*, **90**(4): 690–695.

Huynh B, Palmer L, Mather DE, Wallwork H, Graham RD, Welch RM, & Stangoulis JCR (2008). Genotypic variation in wheat grain fructan content revealed by a simplified HPLC method. *Journal of Cereal Science*, **48**: 369–378.

Izydorczyk MS, & Biliaderis CG (1995). Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers*, **28**(1): 33–48.

Izydorczyk MS, & Dexter JE (2008). Barley β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products-a review. *Food Research International*, **41**(9): 850–868.

Izydorczyk MS, Storsley J, MacGregor A, & Rossnagel BG (2000). Variation in total and soluble β-glucan content in hulless barley: effects of thermal, physical and enzymic treatments. *Journal of Agricultural and Food Chemistry*, **48**: 982–989.

Izydorczyk MS, Macri LJ, & MacGregor AW (1998). Structure and physicochemical properties of barley non-starch polysaccharides - I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, **35**: 249–258.

Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, & Rossnagel BG (2000). Variation in total and soluble β-glucan content in hulless barley: effects of thermal, physical, and enzymic treatments. *Journal of Agricultural and Food Chemistry*, **48**(4): 982–989.

Jenkins CLD, Lewis D, Bushell R, Belobrajdic DP, & Bird AR (2011). Chain length of cereal fructans isolated from wheat stem and barley grain modulates in vitro fermentation. *Journal of Cereal Science*, **53**(2): 188–191.

Jiang GS, & Vasanthan T (2000). Effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley. *Cereal Chemistry*, **77**(C): 396–400.

Johansson L, Tuomainen P, Ylinen M, Ekholm P, & Virkki L (2004). Structural analysis of water-soluble and -insoluble β-glucans of whole-grain oats and barley. *Carbohydrate Polymers*, **58**(3): 267–274.

Juszczak L, Witczak T, Ziobro R, Korus J, Cie E, & Witczak M (2012). Effect of inulin on rheological and thermal properties of gluten-free dough. *Carbohydrate Polymers*, **90**: 353–360.

Kan A (2015). Characterization of the fatty acid and mineral compositions of selected cereal cultivars from Turkey. *Records of Natural Products*, **9**(1): 124–134.

Katagiri M, Masuda T, Fumito T, & Kitabatake N (2011). Expression and development of wheat proteins during maturation of wheat kernel and the rheological properties of dough prepared from the flour of mature and immature wheat. *Food Science and Technology Research*, **17**(2): 111–120.

Kaur N, & Gupta AK (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of Bioscience*, **27**(7): 703–714.

Keunen ELS, Peshev D, Vangronsveld J, Van de Ende W, & Cuypers A (2013). Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant Cell and Environment*, **36**: 1242–1255.

Kim H, Stote KS, Behall KM, Spears K, Vinyard B, & Conway JM (2009). Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, β-glucan: a dose response study in obese women with increased risk for insulin resistance. *European Journal of Nutrition*, **48**(3): 170–175.

Kim Y, Faqih MN, & Wang SS (2001). Factors affecting gel formation of inulin. *Carbohydrate Polymers*, **46**: 135–145.

Knez M, Abbott C, & Stangoulis JCR (2014). Changes in the content of fructans and arabinoxylans during baking processes of leavened and unleavened breads. *European Food Research Technology*, **239**: 803–811.

Knutsen SH, & Holtekjølen AK (2007). Preparation and analysis of dietary fibre constituents in whole grain from hulled and hull-less barley. *Food Chemistry*, **102**(3): 707–715.

Kumar V, Sinha AK, Makkar HPS, de Boeck G, & Becker K (2012). Dietary roles of non-starch polysachharides in human nutrition: a review. *Critical Reviews in Food Science and Nutrition*, **52**(10): 899–935.

L'Homme C, Arbelot M, Puigserver A, & Biagini A (2003). Kinetics of hydrolysis of fructooligosaccharides in mineral-buffered aqueous solutions: influence of pH and temperature. *Journal of Agricultural and Food Chemistry*, **51**: 224–228.

Lan-Pidhainy X, Brummer Y, Tosh SM, Wolever TM, & Wood PJ (2007). Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycaemic effect. *Cereal Chemistry*, **84**(5): 512–517.

Landberg R, Marklund M, Kamal-Eldin A, & Åman P (2014). An update on alkylresorcinols-occurrence, bioavailability, bioactivity and utility as biomarkers. *Journal of Functional Foods*, **7**(1): 77–89.

Lazaridou A, Biliaderis CG, & Izydorczyk MS (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels. *Food Hydrocolloids*, **17**(5): 693–712.

Lazaridou A, Biliaderis CG, Micha-Screttas M, & Steele BR (2004). A comparative study on structure-function relations of mixed-linkage (1→3),(1→4) linear β-D-glucans. *Food Hydrocolloids*, **18**(5): 837–855.

Lifschitz CH, Grusak MA, & Butte NF (2002). Carbohydrate digestion in humans from a β-glucan-enriched barley is reduced. *The Journal of Nutrition*, **132**(9): 2593–2596.

Liu F, Prabhakar M, Ju J, Long H, & Zhou H (2016). Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials. *European Journal of Clinical Nutrition*, **71**(1): 1–12.

Livingston DP, Hincha, DK, & Heyer AG (2009). Fructan and its relationship to abiotic stress tolerance in plants. *Cellular and Molecular Life Sciences*, **66**: 2007–2023

MacGregor A, & Morgan J (1984). Structure of amylopectins isolated from large and small starch granules of normal and waxy barley. *Cereal Chemistry*, **61**(3): 222–228.

MacGregor AW, & Ballance DL (1980). Hydrolysis of large and small starch granules from normal and waxy barley cultivars by α-amylases from barley malt. *Cereal Chemistry*, **57**(1): 397–402.

MacLeod A, & Preece IA (1954). Studies on the free sugars of the barley grain. *Journal of Institute of Brewing*, **60**: 46–55.

Mäkelä N, Maina NH, Vikgren P, & Sontag-Strohm T (2017) Gelation of cereal β-glucan at low concentrations. *Food Hydrocolloids*, **73**: 60-66.

Mansueto P, Seidita A, Alcamo AD, & Carroccio A (2015). Role of FODMAPs in patients with irritable bowel syndrome. *Nutrition in Clinical Practice*, **30**(5): 665–682.

Marsh A, Eslick EM, & Eslick GD (2016). Does a diet low in FODMAPs reduce symptoms associated with functional gastrointestinal disorders? A comprehensive systematic review and meta - analysis. *European Journal of Nutrition*, **55**(3): 897–906.

Masisi K, Diehl-Jones WL, Gordon J, Chapman D, Moghadasian MH, & Trust B (2015). Carotenoids of aleurone, germ, and endosperm fractions of barley, corn and wheat differentially inhibit oxidative stress. *Journal of Agricultural and Food Chemistry*, **63**(10): 2715–2724.

Matusek A, Merèsz P, Thi Khanh D, & Orsi F (2009). Effect of temperature and pH on the degradation of fructo-oligosaccharides. *European Food Research and Technology*, **228**: 355–365.

Merendino N, D'Aquino M, Molinari R, De Gara L, D'Egidio MG, Paradiso A, Cecchini C, Corradini C, & Tomassi G (2006). Chemical characterization and biological effects of immature durum wheat in rats. *Journal of Cereal Science*, **43**: 129–136.

Meyer D, & Stasse-Wolthuis M (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *European Journal of Clinical Nutrition*, **63**(11): 1277–1289.

Milani J, & Maleki G (2012). Hydrocolloids in Food Industry, Food Industrial Processes – Methods and Equipment, Dr. Benjamin Valdez (Ed.), ISBN: 978-953-307-905-9, In Tech. Available at: http://www.intechopen.com/books/food-industrial-processes-methods-and-equipment/hydrocolloids-in-food- industry

Morrell PL, & Clegg MT (2007). Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. *Proceedings of the National Academy of Sciences of the United States of America*, **104**: 3289–3294.

Morris C, Lynn A, Neveux C, Hall AC, & Morris GA (2015). Function and effect of fructan enriched breads on breath hydrogen, satiety, energy intake, PYY and ghrelin. *Food and Function*, **6**: 2561–2567.

Morris C, & Morris GA (2012). The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: a review. *Food Chemistry*, **133**: 237–248.

Morrison WR (1993). *Barley lipids*, pp. 199-246. In: Mac Gregor AW, & Bhatty RS (eds.). Barley: chemistry and technology. American Association of Cereal Chemists.

Moshfegh AJ, Friday JE, Goldman JP, & Ahuja JKC (1999). Presence of inulin and oligofructose in the diets of americans. *The Journal of Nutrition*, **129**(7 Suppl): 1407–1411.

Nardi S, Calcagno C, Zunin P, D'Egidio MG, Cecchini C, Boggia R, & Evangelisti F (2016). Nutritional benefits of developing cereals for functional foods. *Cereal Research Communications*, **31**(3): 445–452.

Ndolo VU, & Beta T (2013). Distribution of carotenoids in endosperm, germ, and aleurone fractions of cereal grain kernels. *Food Chemistry*, **139**: 663–671.

Nemeth C, Andersson AAM, Andersson R, Mangelsen E, Sun C, & Åman P (2014). Relationship of grain fructan content to degree of polymerisation in different barleys. *Food and Nutrition Sciences*, **5**(Mar): 581–589.

Newman RK, & Newman CW (2008). *Barley for food and health. Science, technology and products*. Hoboken, New Jersey: John Wiley and Sons, Inc.

Niness KR (1999). Inulin and oligofructose: what are they? *The Journal of Nutrition*, **129**(7): 1402–1406.

Oscarsson M, Parkkonen T, Autio K, & Åman P (1997). Composition and microstructure of waxy, normal and high amylose barley samples. *Journal of Cereal Science*, **26**(2): 259–264.

Padalino L, Costa C, Conte A, Melilli MG, Sillitti C, Bognanni R, Raccuia SA, & Del Nobile MA (2017). The quality of functional whole-meal durum wheat spaghetti as affected by inulin polymerization degree. *Carbohydrate Polymers*, **173**: 84-90.

Parnell JA, & Reimer RA (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *The American Journal of Clinical Nutrition*, **89**: 1751–1759.

Pedernse A, Sandstrom B, & Van Amelsvoort JMM (1997). The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *British Journal of Nutrition*, **78**: 215–222.

Pentikäinen S, Karhunen L, Flander L, Katina K, Meynier A, Aymard P, Vinoy S, & Poutanen K (2014). Enrichment of biscuits and juice with oat β-glucan enhances postprandial satiety. *Appetite*, **75**: 150–156.

Peressini D, Foschia M, Tubaro F, & Sensidoni A (2015). Impact of soluble dietary fibre on the characteristics of extruded snacks. *Food Hydrocolloids*, **43**: 73–81.

Peressini D, & Sensidoni A (2009). Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. *Journal of Cereal Science*, **49**(2): 190–201.

Peshev D, Vergauwen R, Moglia A, Hideg É, & Van den Ende W (2013). Towards understanding vacuolar antioxidant mechanisms: a role for fructans? *Journal of Experimental Botany*, **64**(4): 1025–1038.

Peukert M, Thiel J, Peshev D, Weschke W, Van den Ende W, Mock H, & Matros A (2014). Spatio-temporal dynamics of fructan metabolism in developing barley grains. *The Plant Cell*, **26**: 3728–3744.

Praznik W, Cieślik E, & Filipiak-Florkiewciz A (2002). Soluble dietary fibres in Jerusalem artichoke powders: composition and application in bread. *Nahrung/Food*, **46**(3): 151–157.

Rakha A, Åman P, & Andersson R (2010). Characterisation of dietary fibre components in rye products. *Food Chemistry*, **119**(3): 859–867.

Rakha A, Åman P, & Andersson R (2011). Dietary fiber in triticale grain: variation in content, composition, and molecular weight distribution of extractable components. *Journal of Cereal Science*, **54**(3): 324–331.

Rebello CJ, Burton J, Heiman M, & Greenway FL (2015). Gastrointestinal microbiome modulator improves glucose tolerance in overweight and obese subjects: a randomized controlled pilot trial. *Journal of Diabetes and its Complications*, **29**(8): 1272–1276.

Redza-Dutordoir M, & Averill-Bates, DA (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta*, **1863**(12): 2977–2992.

Regand A, Chowdhury Z, Tosh SM, Wolever TMS, & Wood P (2011). The molecular weight, solubility and viscosity of oat β-glucan affect human glycaemic response by modifying starch digestibility. *Food Chemistry*, **129**(2): 297–304.

Regulation 2006/1924/EC of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods.

Rendón-Huerta JA, Juárez-Flores B, Pinos-Rodriguez JM, Aguirre-Rivera JR, & Delgado-Portales RE (2012). Effects of different sources of fructans on body weight, blood metabolites and fecal bacteria in normal and obese non-diabetic and diabetic rats. *Plant Food for Human Nutrition*, **67**: 64–70.

Rieder A, Ballance S, Løvaas A, & Knutsen SH (2015). Minimizing molecular weight reduction of β-glucan during barley bread making. *LWT - Food Science and Technology*, **64**(2): 767–774.

Rieder A, & Samuelsen AB (2012). Do cereal mixed-linked β-glucans possess immune-modulating activities? *Molecular Nutrition and Food Research*, **56**(4): 536–547.

Roberfroid MB (2007a). Inulin-type fructans: functional food ingredients. *The Journal of Nutrition*, **137**: 2493–2502.

Roberfroid MB (2007b). Prebiotics: the concept revisited. *The Journal of Nutrition*, **137**(3 Suppl 2): 830–937.

Roberfroid MB (2005). Introducing inulin-type fructans. *British Journal of Nutrition*, **93**: 13–25.

Roberfroid MB, & Delzenne NM (1998). Dietary fructans. *Annual Review of Nutrition*, **18**: 117–143.

Roberts T. Sorghum malting for future foods: University of Sidney. Power Point Presentation. Retrievable from: http://slideplayer.com/slide/3430689/.

Saito K, & Tomita F (2000). Difructose anhydrides: their mass-production and physiological functions. *Bioscience, Biotechnology and Biochemistry*, **64**(7): 1321–1327.

Salazar N, Dewulf EM, Neyrinck AM, Bindels LB, Cani PD, Mahillon J, de Vos WM, Thissen JP, Gueimonde M, de los Reyes-Gavilán CG, & Delzenne NM (2015). Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clinical Nutrition*, **34**: 501–507.

Schirmer M, Höchstötter A, Jekle M, Arendt E, & Becker T (2013). Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocolloids*, **32**(1): 52–63.

Schroeder N, Gallaher DD, Arndt EA, & Marquart L (2009). Influence of whole grain barley, whole grain wheat, and refined rice-based foods on short-term satiety and energy intake. *Appetite*, **53**(3): 363–369.

Sreenivasulu N, Borisjuk L, Junker BH, Mock HP, Rolletschek H, Seiffert U, Weschke W, & Wobus, U (2010). *Barley grain development: toward an integrative view*. In: International Review of Cell and Molecular Biology (Vol. 281, pp. 49–89).

Staudacher HM, & Whelan K (2016). Altered gastorintestinal microbiota in irritable bowel syndrome and its modification by diet: probiotics, prebiotics and the low FODMAP diet. *Proceedings of the Nutrition Society*, **75**(february): 306–318.

Stewart A, Nield H, & Lott JNA (1988). An investigation of the mineral content of barley grains and seedlings. *Plant Physiology*, **86**: 93–97.

Sullivan P, O'Flaherty J, Brunton N, Gee VL, Arendt E, & Gallagher E (2010). Chemical composition and microstructure of milled barley fractions. *European Food Research and Technology*, **230**(4): 579–595.

Takeda Y, Takeda C, Mizukami H, & Hanashiro I (1999). Structures of large, medium and small starch granules of barley grain. *Carbohydrate Polymers*, **38**(2): 109–114.

Taner A, Muzaffer A, & Fazil D (2004). Barley: post-harvest operations. Food and Agriculture Organization of the United Nations, (Ed. AGST/FAO), Retrieved from: http://www.fao.org/3/a-au997e.pdf

Tiwari U, & Cummins E (2011). Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels. *Nutrition*, **27**(10): 1008–1016.

Tiwari U, Cummins E, Sullivan P, Flaherty JO, Brunton N, & Gallagher E (2011). Probabilistic methodology for assessing changes in the level and molecular weight of barley β-glucan during bread baking. *Food Chemistry*, **124**(4): 1567–1576.

Tong LT, Zhong K, Liu L, Zhou X, Qiu J, & Zhou S (2015). Effects of dietary hull-less barley β-glucan on the cholesterol metabolism of hypercholesterolemic hamsters. *Food Chemistry*, **169**: 344–349.

Tosh SM (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition*, **67**(4): 310–317.

Tosh SM, Brummer Y, Miller SS, Regand A, Defelice C, Duss R, Wolever TMS, & Wood PJ (2010). Processing affects the physicochemical properties of β-glucan in oat bran cereal. *Journal of Agricultural and Food Chemistry*, **58**(13): 7723–7730.

Vaikousi H, Biliaderis CG, & Izydorczyk MS (2004). Solution flow behaviour and gelling properties of water-soluble barley (1→3,1→4)-β-glucans varying in molecular size. *Journal of Cereal Science*, **39**(1): 119–137.

Van de Wiele T, Boon N, Possemiers S, Jacobs H, & Verstraete W (2007). Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of Applied Microbiology*, **102**: 452–460.

Van den Ende W, Yoshida M, Clerens S, Vergauwen R, & Kawakami A (2004). Cloning, characterization and functional analysis of novel 6-kestose exohydrolase (6-KEHs) from wheat (*Triticum aestivum*). *New Phytologist*, **166**: 917–932.

Vasanthan T, & Temelli F (2008). Grain fractionation technologies for cereal β-glucan concentration. *Food Research International*, **41**(9): 876–881.

Vereyken IJ, Chupin V, Demel RA, Smeekens SCM, & De Kruij B (2001). Fructans insert between the headgroups of phospholipids. *Biochimica et Biophysica Acta*, **1510**: 307–320.

Verspreet J, Cimini S, Vergauwen R, Dornez E, Locato V, Le Roy K, De Gara L, Van den Ende W, Delcour JA, & Courtin CM (2013). Fructan metabolism in developing wheat (*Triticum aestivum* L.) kernels. *Plant Cell Physiology*, **54**(12): 2047–2057.

Verspreet J, Dornez E, & Courtin CM (2015a). Cereal grain fructans: structure, variability and potential health effects. *Trends in Food Science & Technology*, **43**(1): 32–42.

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

Vijn I, & Smeekens S (1999). Fructan: more than a reserve carbohydrate? **120**: 351–359.

Vitaglione P, Lumaga RB, Montagnese C, Messia MC, Marconi E, & Scalfi L (2010). Satiating effect of a barley β-glucan-enriched snack. *Journal of the American College of Nutrition*, **29**(2): 113–121.

Vitaglione P, Lumaga RB, Stanzione A, Scalfi L, & Fogliano V (2009). β-Glucan-enriched bread reduces energy intake and modifies plasma ghrelin and peptide YY concentrations in the short term. *Appetite*, **53**(3): 338–344.

Vogt L, Ramasamy U, Meyer D, Pullens G, Venema K, Faas MM, Schols HA, & de Vos P (2013). Immune modulation by different types of β2→1-fructans is toll-like receptor dependent. *PlosOne*, **8**(7): 1–12.

Volman JJ, Ramakers JD, & Plat J (2008). Dietary modulation of immune function by β-glucans. *Physiology and Behavior*, **94**(2): 276–284.

Wang Y, Ames NP, Tun HM, Tosh SM, Jones PJ, & Khafipour E (2016). High molecular weight barley β-glucan alters gut microbiota toward reduced cardiovascular disease risk. *Frontiers in Microbiology*, **7**(Feb): 1–15.

Wardlaw I, & Willebrink J (2000). Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. *New Phytologist*, **148**: 413–422.

Welch RW (1975). Fatty acid composition of grain from winter and spring sown oats, barley and wheat. *Journal of the Science of Food and Agriculture*, **26**(4): 429–435.

Wobus U, Sreenivasulu N, Borisjuk L, Rolletschek H, Panitz R, Gubatz S, & Weschke W (2005). Molecular physiology and genomics of developing barley grains. *Recent Research Development in Plant Molecular Biology*, **2**(2): 1–29.

Xue Q, Wang L, Newman RK, Newman CW, & Graham H (1997). Influence of the hulless, waxy starch and short-awn genes on the composition of barleys. *Journal of Cereal Science*, **26**(2): 251–257.

Yàñez A, Tapia G, Guerra F, & del Pozo A (2017) Stem carbohydrate dynamics and expression of genes involved in fructan accumulation and remobilization during grain growth on wheat (*Triticum aestivum* L.) genotypes with contrasting tolerance to water stress. *Plos ONE*, **12**(5): 1-18.

You SG, & Izydorczyk MS (2002). Molecular characteristics of barley starches with variable amylose content. *Carbohydrate Polymers*, **49**(1): 33–42.

Zhang X, Xue D, Wu F, & Zhang G (2013). Genotypic and environmental variations of arabinoxylan content and endoxylanase activity in barley grains. *Journal of Integrative Agriculture*, **12**(8): 1489–1494.

Zhao Q, Hu X, Guo Q, Cui SW, Xian Y, You S, Chen X, Xu C, & Gao X (2014). Physicochemical properties and regulatory effects on db/db diabetic mice of β-glucans extracted from oat, wheat and barley. *Food Hydrocolloids*, **37**: 60–68.

Zheng X, Li L, & Wang Q (2011). Distribution and molecular characterization of β-glucans from hull-less barley bran, shorts and flour. *International Journal of Molecular Sciences*, **12**(3): 1563–1574.

Zhu X, Sun X, Wang M, Zhang C, Cao Y, Mo G, Liang J, & Zhu S (2015). Quantitative assessment of the effects of β-glucan consumption on serum lipid profile and glucose level in hypercholesterolemic subjects. *Nutrition, Metabolism and Cardiovascular Diseases*, **25**(8): 714–723.

Zimeri JE, & Kokini JL (2002). The effect of moisture content on the crystallinity and glass transition temperature of inulin. *Carbohydrate Polymers*, **48**: 299–304.

Ziobro R, Korus J, Juszczak L, & Witczak T (2013). Influence of inulin on physical characteristics and staling rate of gluten-free bread. *Journal of Food Engineering*, **116**: 21–27.

Websites

http://www.fao.org/faostat/en/#home

http://www.igc.int/en/

http://www.euromalt.be/

2. Variation of fructan, β-glucan and total starch content in developing kernels of different barley cultivars

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Abstract

Fructans are fructose-based oligo and polysaccharides with proven prebiotic effects and further studied positive health outcomes. In the kernel, these compounds accumulate in the first phases of maturation, then the content progressively declines under the influence of signalling pathways regulating carbohydrate metabolism. Barley is a cereal with known positive effects on human health due to the content of bioactive molecules like β-glucan. To evaluate the potential of immature barley grain as a source of bioactive compounds and to elucidate the correlations between polysaccharides accumulation during barley kernel maturation, samples of barley grains (*Hordeum vulgare* L.) were collected at different stages after anthesis, including maturation, and accumulation of fructan, β-glucan, total starch and proteins was studied. Fructan concentration in immature barley grain reaches a peak between 6 and 17 DAA with cultivar Scarlett accumulating the highest fructan content (9.5 g/100 g flour) compared to the other lines. β-Glucan and total starch showed a linear increase over ripening.

Results obtained highlight physiological changes in polysaccharide accumulation during barley development and also supported the concept of immature barley kernel as an innovative functional ingredient contributing to fructan intake.

Keywords: barley, developing grain, fructans, β-glucans, immature kernel, polysaccharides.

2.1. Introduction

Barley (*Hordeum vulgare* L.) has long been appreciated as a healthy food, particularly because it is a source of bioactive molecules like non-starch polysaccharides and phenolic compounds (Baik & Ullrich, 2008; De Paula *et al*., 2017).

During maturation, physical and chemical composition of cereal kernel undergo substantial modifications that can be interesting from a nutritional point of view. Barley kernel maturation can be broadly divided into three phases (Wobus *et al*., 2005). The first stage is prestorage phase (0-8 Days after anthesis - DAA). The second phase is the storage phase when the kernel accumulates mainly starch and storage proteins (until 24 DAA). The final stage is dessiccation and maturation. Changes in polysaccharides accumulation during cereal kernel development have been observed. Specifically, the fructose-based oligo and polysaccharides named fructans are much more abundant in the first stages of kernel maturation, then the content progressively declines (Nardi *et al*., 2003; Paradiso *et al*., 2008; De Gara *et al*., 2003; Verspreet *et al*., 2013). Chemically, fructans are linear or branched polysaccharides mainly composed of β(2-1) or β(2-6) frucosyl-fructose glycosidic (Livingston *et al*., 2009). These oligo and polysaccharides are reserve carbohydrates, and are synthetized by 15% of higher plants (Hendry, 1993). Among fructans types, inulin has a linear chain with 2-1 linkages and a degree of polymerization (DP) from 2 to 60 and include inulin and fructoligosaccharides (FOS) which instead have a DP 2-10 (Niness, 1999). Improved composition of gut microbiota is the most important positive effect of fructans on human health as they are universally defined as prebiotics (Gibson, 1999; Roberfroid & Delzenne, 1998). Furthermore, trials with fructan supplementation reported increased calcium absorption and bone mineralization (Abrams *et al*., 2007; Holloway *et al*., 2007) and reduction of blood tryglicerids (Brighenti, 2007). Inulin also showed a protective role in human gut mucosa against oxidative stress (Pasqualetti *et al*., 2014).

β-Glucans are among the major non starch polysaccharides in mature barley and they are mainly located in the aleurone layer and starchy endosperm cell walls where they account for 70% of the total components (Fincher, 1975). In mature caryopsis the content of β-glucan falls in the range 2-11.3% (Baik & Ullrich, 2008; Holtekjølen *et al*., 2006; Izydorczyk & Dexter, 2008). Positive impacts of β-glucan and β-glucan containing foods on human health include reduction of blood cholesterol (Tiwari & Cummins, 2011), flattering of postprandial blood glucose (AbuMweis *et al*., 2016; Ames *et al*., 2015; Tosh, 2013) and improved composition of intestinal microbiota (De Angelis et al. 2015; Arena et al. 2014). During barley endosperm development the content of these compounds increases linearly starting from 11-12 DAA (Coles, 1979; Tsuchiya *et al*., 2005).

Studies on fructan accumulation were carried out mainly in developing wheat grain, while little investigation was performed on the concomitant variation of fructan and β-glucan content in barley kernel. In order to elucidate the variation of polysaccharides accumulation, in this study evolution of the content of fructan and β-glucan and total starch was assessed in barley kernels collected at different DAA. Our objective is also to determine whether immature barley flour can be used as a potential functional ingredient for the production of foods enriched in fructan and contributing to β-glucan intake.

Moreover, results of this study can be a useful starting point for further researches in order to identify the moment with the maximum yield of these bioactive compounds and suitable technological properties to include immature barley fractions in cereal-based products.

2.2. Materials and methods

Samples

Five barley (*H. vulgare* L.) cultivars (Alimini, Cometa, Calanque, CDC Alamo and CDC Fibar) were grown in experimental fields in Perugia (Italy) in the year 2013-2014 in randomized blocks. Cultivars Alimini, Calanque and CDC Alamo were collected at the following DAA: 3, 7, 10, 17, 21, 28, 38. Cultivar Cometa and CDC Fibar were collected at the following DAA: 3, 7, 10, 17, 21, 28, 35. Samples of all cultivars were then harvested at completed maturation (55 DAA). Other two barley cultivars, Scarlett and Dingo, were grown in experimental fields in Montenero and Montecilfone (Italy) in year 2015-2016. Ears of cultivar Scarlett were collected at 3, 6, 11, 17, 20, 24, 31 DAA while cultivar Dingo was collected at 3, 8, 10, 17, 21, 24, 31 DAA. Samples of ears were then obtained at completed maturation (62 DAA for cultivar Scarlett and 67 DAA for Dingo). To simplify, all results will be presented according to the following DAA: 3, 6-8, 10-11, 17, 20-21, 24-28, 31-38, 55-67.

Ears were stored at -18 °C until analysed. Immature kernels are characterised by a high moisture content which can reach up to 70% fw -fresh weight (Koga *et al*., 2017). To reduce the percentage of moisture, prevent microbial and enzymatic spoilages and preserve the bioactive molecules, kernels were incubated at 40 °C for 24 hours under vacuum (-660 Torr), and samples used for analysis. Mature kernels were instead employed without any pre-treatment. After that, samples were milled with a laboratory miller and stored at +4 °C. Both immature and mature kernels did not undergo any dehulling process. Barleys characteristics are shown in Table 2.1.

Table 2.1. *Characteristics of barley cultivars considered in this study.*

Cultivar	Hulled	Hulless	Two-row	Six-row	Waxy	Normal starch
Alimini	●			●		●
Cometa	●		●			●
Calanque	●		●			●
CDC Alamo		●	●		●	
CDC Fibar		●	●		●	
Scarlett	●		●			●
Dingo	●			●		●

Kernel weight

In order to calculate the average kernel fresh weight, spikes were thawed and kernels at each stage of maturation were manually separated from ears, grouped, counted and weighed.

Chemical analysis

Fructan content was determined using the enzymatic kit (K-FRUC 03/14 Megazyme™ International Ltd, Ireland), AACC method 32.32. β-Glucan assessment was performed using the enzymatic detection kit (K-BGLU 06/11 Megazyme™ International Ltd, Ireland), AACC method 32-23. Total starch content analysis was performed using the enzymatic assay kit (Megazyme™ International Ltd, Ireland), AACC method 76-13.01. Protein content (N x 6.25) was determined by combustion nitrogen analysis with Leco FP-528. Data are expressed as "g/100 g flour (fresh weight)" and as "mg/kernel".

2.3. Results and discussion

In Table 2.2 the variation of kernel fresh weight during grain ripening is reported. This data increased steadily reaching the peak at 24-28 DAA in five cultivars and then decreased until completed maturation. In cultivar CDC Alamo grain weight started to decline from 21 DAA onward with a maximum value of 75.4 mg (fw). In cultivar Dingo, instead, maximum rate of fresh weight accumulation is at 31-38 DAA. At the peak of kernel weight the average value recorded is 72.5 mg. In cultivar CDC Fibar and CDC Alamo the average kernel weight reduction at maturation is substantial compared to the other lines. This may be due to the fact that in hulless cultivars CDC Fibar and CDC Alamo the hull is loosely attached to the grain and it comes off at harvesting. These results are in agreement with results obtained in wheat by others work (De Gara *et al*., 2003; Katagiri *et al.*, 2011). From a physiological point of view, the reduction of kernel weight coincides with the desiccation phase. In this stage of kernel development, the dehydration process is not balanced by reserve storage and accumulation and dry seed is in a preparation for a quiescent period and then for germination (Angelovici *et al*., 2010; De Gara *et al*., 2003).

The amount of fructan in barley cultivars collected at different times after anthesis is presented in Table 2.3. Fructan concentration expressed on g/100 g flour fw (fresh weight) showed a peak in the cultivar considered between 6 and 17 DAA. Cultivar CDC Alamo and Calanque reached the peak in the period 6-8 DAA, Dingo at 17 DAA while all the other cultivars between 10-11 DAA. Barley cultivars collected in Montenero and Montecilfone in year 2016 showed a higher fructan concentration compared to the other lines, being 9.5 ± 0.07 g/ 100 g flour in Scarlett and 6.6 ± 0.05 g/100 g flour in cultivar Dingo. Among the cultivars obtained in year 2013-14, Alimini showed the highest level with 5.5 ± 0.04 g/100 g flour, followed by the two waxy cultivars sampled in this study.

Table 2.2. *Average fresh kernel weight (mg) at different stages of maturation (mean value ± sd; DAA = Days after anthesis).*

Cultivar	DAA							
	Prestorage		***Storage***				***Dessiccation-Maturation***	
	3	6-8	10-11	17	20-21	24-28	31-38	55-67
Alimini	13.0 ± 0.9	15.7 ±2.9	30.8 ± 7.1	48.1 ± 5.3	58.2 ± 3.2	76.7 ± 8.3	44.8 ± 4.1	47.8 ± 1.2
Cometa	16.0 ± 2.3	18.4 ± 4.3	27.9 ± 7.8	61.8 ± 3.2	66.3 ± 4.8	81.5 ± 3.3	72.3 ± 7.1	50.1 ± 1.2
Calanque	13.5 ± 1.3	23.4 ± 3.2	36.5 ± 6.5	61.3 ± 4.6	66.9 ± 4.3	70.3 ± 5.7	61.1 ± 1.8	51.7 ± 1.1
CDC Alamo	17.4 ± 3.6	32.3 ± 5.6	38.3 ± 5.7	62.1 ± 3.6	75.4 ± 2.2	67.4 ± 4.0	47.8 ± 1.4	38.4 ± 1.4
CDC Fibar	16.3 ± 0.9	19.0 ± 3.3	28.2 ± 4.9	50.9 ± 5.1	65.2 ± 6.4	76.7 ± 8.3	72.3 ± 3.6	36.1 ± 2.0
Scarlett	14.8 ± 2.4	23.4 ± 3.7	30.0 ± 6.7	54.1 ± 5.1	n.d.*	63.5 ± 3.1	48.7 ± 1.7	45.4 ± 3.1
Dingo	9.0 ± 0.4	13.0 ± 0.5	16.9 ± 2.5	35.3 ± 4.3	46.1 ± 5.0	54.5 ± 5.9	63.6 ± 3.2	48.6 ± 4.5

*not determined

Fructan content tends to decline during the last stages of grain development amounting to 1.1-1.6 g/100 g flour at maturation in line with other findings (Nemeth *et al*., 2014) with cultivar Scarlett showing the highest value.

Data considered on a per-kernel basis showed the same trend, with a peak detected at the same or later stage, between 10 and 17 DAA, in agreement with previous results obtained in wheat (De Gara *et al*., 2003). Again, cultivars Scarlett and Dingo reported higher values, with 3.1 ± 0.03 mg and 2.3 ± 0.02 mg/ kernel respectively. At maturation, fructan content is between 0.4 and 0.7 mg with the lowest value detected in CDC Fibar and the highest value detected in Scarlett.

The values of fructan content obtained in this study are lower than those reported in one barley cultivar by Nardi *et al*. (2003) which found a peak of fructan concentration of 39 g/100 g meal (4% moisture) at 9 DAA. In our study, values expressed on a fresh weight basis are instead higher than those reported by Peukert *et al.* (2014) in barley whole grain. At the peak of fructoligosaccharides concentration (considering only oligosaccharides with DP 3-4), the content claimed by Peukert *et al.* (2014) is approximately 2.2% fw while in our study total fructan content varies between 4.3 and 9.5 g/100 g flour. In the study by De Gara et *al*. (2003) performed on wheat (*Triticum durum* L.*)*, maximum accumulation of fructan was recorded at 17 DAA with 1.87 mg/ kernel. According to our data, the value of fructan content ranges between 1.4 and 1.7 mg/ kernel among the cultivars collected in 2013-14. On the other hand, when considering cultivar Scarlett and Dingo our data suggest a higher accumulation compared to De Gara *et al*. (2003). Our data are overall comparable also with the large variation of maximum fructan accumulation reported by Paradiso *et al.* (2008) (7-25% dw) among samples of wheat (*Triticum durum* L.). Only the values of maximum fructan accumulation obtained in cultivar Scarlett and Dingo are instead close to those reported by Cimini *et al*. (2015) (35% dw) and by Verspreet *et al*. (2013) (2.5 mg/ kernel) in *Triticum aestivum* samples.

The physiological role of accumulation and further degradation of fructan for grain development has been broadly investigated in previous studies. Before starch synthesis, carbon is mainly used to build short chain fructans (Peukert *et al*., 2016). Fructan synthesis during the prestorage and storage phase regulates osmotic impacts due to excess amount of sucrose and inhibits negative feedbacks on photosynthesis, while accumulation of inulin-type fructan during the grain filling phase may protect

tissues from oxidative stress (Peukert *et al*., 2016). It is suggested that these short-chain polysaccharides help diluting sucrose and regulate carbohydrate metabolism during kernel maturation (Verspreet *et al*., 2015; Cimini *et al.*, 2015). The reduction of fructoligosaccharides and monosaccharides is correlated to the accumulation of storage carbohydrate in the grain like starch (Cerning & Guilbot, 1973; Iametti *et al*,. 2006). In fact, in this study starch synthesis starts massively when fructan content declines (Figure 2.1 and Table.2.5).

Table 2.3. *Fructan content in developing barley cultivars (mean value ± sd).*

Cultivar	**DAA**							
	Prestorage		***Storage***				***Dessiccation - Maturation***	
	3	6-8	10-11	17	20-21	24-28	31-38	55-67
Alimini								
g/100 g flour	1.6 ± 0.03	5.5 ± 0.04	5.0 ± 0.25	3.0 ± 0.06	1.5 ± 0.02	0.8 ± 0.00	1.3 ± 0.02	1.1 ± 0.03
mg/kernel	0.2 ± 0.00	0.9 ± 0.01	1.5 ± 0.08	1.4 ± 0.03	0.9 ± 0.01	0.6 ± 0.00	0.6 ± 0.01	0.5 ± 0.01
Cometa								
g/100 g flour	2.3 ± 0.06	3.0 ± 0.06	4.3 ± 0.03	2.3 ± 0.07	1.5 ± 0.02	0.9 ± 0.05	0.9±0.05	1.1 ± 0.03
mg/kernel	0.4 ± 0.01	0.5 ± 0.01	1.2 ± 0.01	1.4 ± 0.05	1.0 ± 0.01	0.7 ± 0.04	0.7 ± 0.04	0.6 ± 0.01
Calanque								
g/100 g flour	1.4 ± 0.00	4.5 ± 0.01	3.8 ± 0.07	1.7 ± 0.01	1.0 ± 0.01	0.8 ± 0.00	1.5 ± 0.03	1.1 ± 0.02
mg/kernel	0.2 ± 0.00	1.0 ± 0.00	1.4 ± 0.02	1.1 ± 0.01	0.6 ± 0.01	0.6 ± 0.00	0.9 ± 0.02	0.6 ± 0.01
CDC Alamo								
g/100 g flour	4.8 ± 0.29	4.9 ± 0.00	4.5 ± 0.12	2.1 ± 0.03	1.0 ± 0.07	1.0 ± 0.04	1.7 ± 0.01	1.5 ± 0.12
mg/kernel	0.8 ± 0.05	1.6 ± 0.00	1.7 ± 0.05	1.3 ± 0.02	0.7 ± 0.05	0.7 ± 0.03	0.8 ± 0.00	0.6 ± 0.05
CDC Fibar								
g/100 g flour	1.9 ± 0.01	4.4 ± 0.09	5.0 ± 0.10	2.3 ± 0.06	1.4 ± 0.01	1.1 ± 0.08	0.9 ± 0.04	1.1 ± 0.04
mg/kernel	0.3 ± 0.002	0.8 ± 0.02	1.4 ± 0.03	1.2 ± 0.03	0.9 ± 0.01	0.8 ± 0.06	0.7 ± 0.03	0.4 ± 0.02
Scarlett								
g/100 g flour	4.2 ± 0.05	8.7 ± 0.27	9.5 ± 0.07	5.8 ± 0.05	n.d.*	2.9 ± 0.21	1.5 ± 0.02	1.6 ± 0.04
mg/kernel	0.6 ± 0.01	2.0 ± 0.06	2.9 ± 0.02	3.1 ± 0.03	n.d.*	1.8 ± 0.13	0.7 ± 0.01	0.7 ± 0.02
Dingo								
g/100 g flour	1.0 ± 0.02	3.5 ± 0.02	5.6 ± 0.01	6.6 ± 0.05	4.9 ± 0.06	2.6 ± 0.03	1.9 ± 0.03	1.2 ± 0.04
mg/kernel	0.1 ± 0.00	0.4 ± 0.00	0.9 ± 0.00	2.3 ± 0.02	2.2 ± 0.03	1.4 ± 0.02	1.2 ± 002	0.6 ± 0.02

*not determined; g/100 g flour is referred to fresh weight.

Despite a notable decrease in fructan content, at maturation these polysaccharides are retained in the kernel. The presence of fructan in mature grain may be due to their ability to stabilise the membranes and the possibility to save metabolic energy from their breakdown (Cimini *et al*., 2015). Furthermore, the physiological role of fructan in response to abiotic stresses like drought and freezing has been illustrated (Livingston *et al*., 2009; Wardlaw & Willebrink, 2000).

For the purpose of identifying interesting varieties accumulating fructan, in this study cultivar Scarlett definetely stands out. At the peak of fructan concentration (g/100 g flour) levels are 1.4-2.2-fold higher

compared to the other cultivars considered. Analysis of values expressed on mg/kernel highlights remarkable fructan synthesis taking place in the kernel in absolute values (Table 2.3).

In Table 2.4 β-Glucan content variation is reported. Overall, it tends to increase over barley kernel maturation in all the cultivars considered.

Table 2.4.*β-Glucan content in developing barley kernels (mean value ± sd).*

Cultivar	**DAA**							
	Prestorage		***Storage***				***Dessiccation-Maturation***	
	3	6-8	10-11	17	20-21	24-28	31-38	55-67
Alimini								
g/100 g flour	0.2 ± 0.03	0.1 ± 0.02	0.3 ± 0.08	0.3 ± 0.01	0.9 ± 0.05	2.1 ± 0.03	4.4 ± 0.03	4.3 ± 0.01
mg/kernel	0.03 ± 0.00	0.02 ± 0.00	0.1 ± 0.03	0.2 ± 0.00	0.5 ± 0.03	1.6 ± 0.02	2.0 ± 0.01	2.0 ± 0.01
Cometa								
g/100 g flour	0.1 ± 0.05	0.2 ± 0.00	0.2 ± 0.03	0.6 ± 0.03	0.9 ± 0.02	2.6 ± 0.04	3.5 ± 0.06	5.3 ± 0.03
mg/kernel	0.02 ± 0.01	0.04 ± 0.001	0.1 ± 0.01	0.4 ± 0.02	0.6 ± 0.01	2.1 ± 0.04	2.5 ± 0.04	2.7 ± 0.01
Calanque								
g/100 g flour	0.2 ± 0.03	0.3 ± 0.003	0.2 ± 0.02	0.3 ± 0.01	0.8 ± 0.00	2.0 ± 0.03	2.3 ± 0.03	3.2 ± 0.03
mg/kernel	0.03 ± 0.00	0.1 ± 0.001	0.1 ± 0.01	0.2 ± 0.00	0.5 ± 0.00	1.4 ± 0.02	1.4 ± 0.02	1.6 ± 0.01
CDC Alamo								
g/100 g flour	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.003	0.8 ± 0.01	2.0 ± 0.03	4.0 ± 0.17	5.7 ± 0.04	6.3 ± 0.19
mg/kernel	0.04 ± 0.01	0.1 ± 0.02	0.1 ± 0.001	0.5 ± 0.01	1.5 ± 0.02	2.7 ± 0.12	2.7 ± 0.02	2.4 ± 0.07
CDC Fibar								
g/100 g flour	0.1 ± 0.02	0.2 ± 0.01	0.3 ± 0.10	0.9 ± 0.04	1.4 ± 0.04	4.1 ± 0.17	4.8 ± 0.09	8.5 ± 0.18
mg/kernel	0.02 ± 0.00	0.04 ± 0.00	0.1 ± 0.03	0.4 ± 0.02	0.9 ± 0.03	3.2 ± 0.13	3.4 ± 0.06	3.1 ± 0.07
Scarlett								
g/100 g flour	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.00	0.3 ± 0.00	n.d.*	0.6 ± 0.02	1.4 ± 0.05	4.3 ± 0.04
mg/kernel	0.03 ± 0.01	0.04 ± 0.01	0.1 ± 0.00	0.15 ± 0.00	n.d.*	0.4 ± 0.01	0.7 ± 0.02	1.9 ± 0.02
Dingo								
g/100 g flour	0.3 ± 0.01	0.1 ± 0.05	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.02	0.3 ± 0.01	0.5 ± 0.02	3.6 ± 0.01
mg/kernel	0.03 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	1.7 ± 0.01

*not determined; g/100 g flour is referred to fresh weight.

β-Glucan substantially accumulates from 24-28 DAA in cultivar Alimini, Cometa and Calanque and at 20-21 DAA in cultivars CDC Alamo and CDC Fibar corroborating previous findings (Coles, 1979; Tsuchiya *et al*., 2005). In Scarlett and Dingo the synthesis is delayed since the content exceeds 1 g/100 g flour from 31-38 DAA onward and at maturation respectively. The trend of β-glucan accumulation displayed in Dingo is noteworthy since the β-glucan content sharply increases (7-fold) while in all the other cultivars, β-glucan is synthetized more gradually. Waxy barley cultivars (CDC Alamo and CDC Fibar) displayed an earlier accumulation of β-glucan and they store a higher content at maturation as expected according to previous studies (Holtekjølen *et al*., 2006). β-Glucan represents the predominant component of the endosperm cell walls in barley (Fincher, 1975). Before 5 DAP (Days after pollination)

β-glucan is present in maternal cell walls, while after 5 DAP it was shown that its accumulation continues in the cell walls of the cellularizing endosperm (Wilson *et al*., 2006).

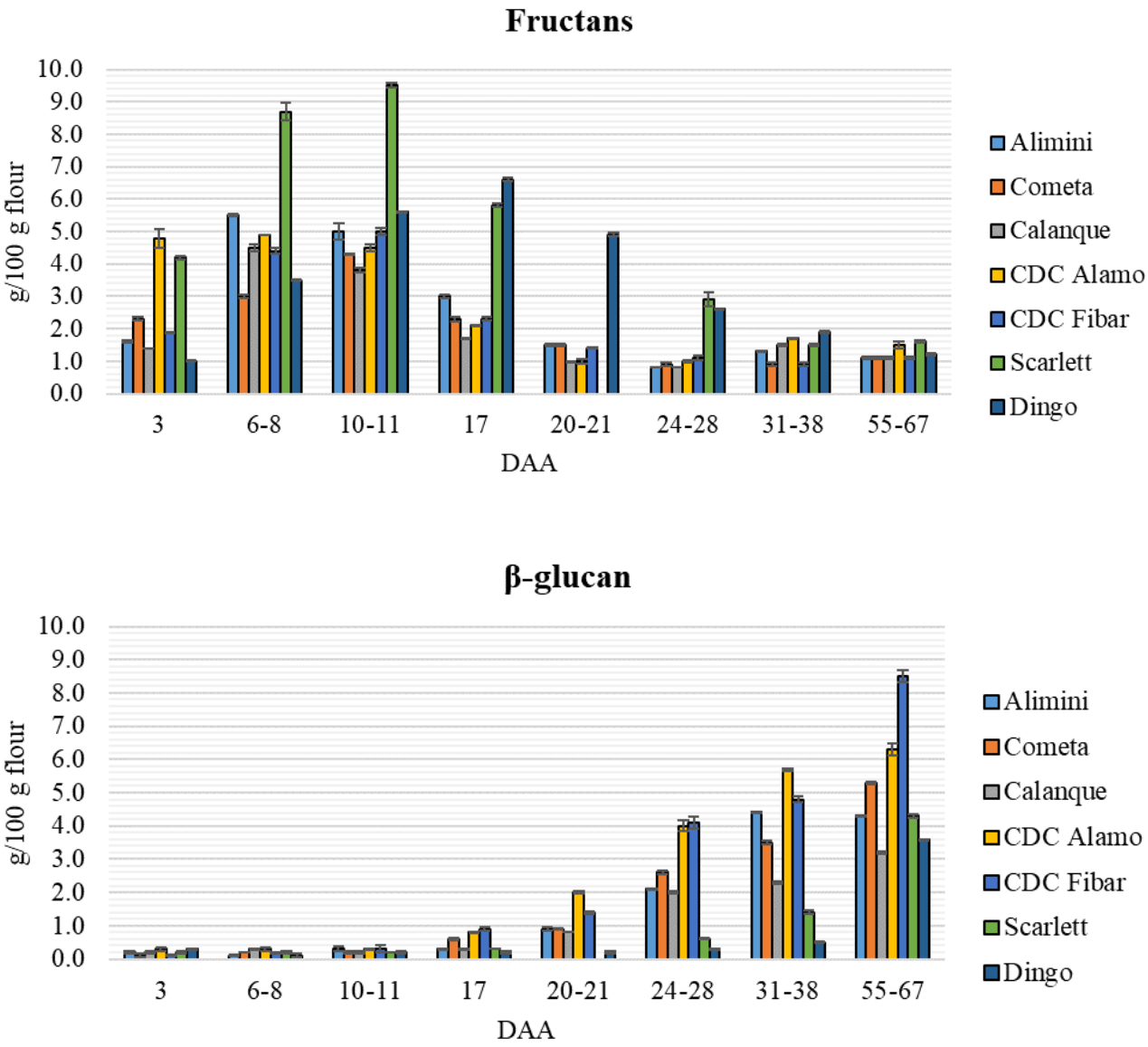

Figure 2.1. *Variation of fructans and β-glucan accumulation during barley kernel development. Error bars refer to standard deviation; g/100 g flour is referred to fresh weight.*

In Table 2.5 total starch variation is reported. As expected the accumulation of total starch tends to increase over ripening and is overall linear when data are considered as g/100 g flour in agreement with other studies (Zheng *et al*., 2017). The content at 3 DAA is already between 1.0 and 2.1 g/100 g flour. In the early stages of maturation (6-8 DAA) the content is the highest in waxy barley CDC Alamo (7.5 g/100 g flour) compared to the other lines. At 17 DAA a sharp increase is recorded for all the cultivars analysed with a concomitant decline of fructan content but following the same trend of β-glucan accumulation (Figure 2.1)

Table 2.5. *Total starch content in developing barley kernels (mean value ± sd).*

Cultivar	DAA							
	Prestorage		***Storage***				***Dessiccation -Maturation***	
	3	6-8	10-11	17	20-21	24-28	31-38	55-67
Alimini								
g/100 g flour	1.0 ± 0.04	2.3 ± 0.47	5.3 ± 0.09	16.0 ± 0.32	19.2 ± 0.12	29.4 ± 0.53	42.6 ± 0.15	48.6 ± 0.71
mg/kernel	0.1 ± 0.01	0.4 ± 0.07	1.6 ± 0.03	7.7 ± 0.15	11.2 ± 0.07	22.5 ± 0.41	19.1 ± 0.07	23.2 ± 0.34
Cometa								
g/100 g flour	1.6 ± 0.14	1.7 ± 0.31	4.0 ± 0.36	15.7 ± 2.9	22.0 ± 0.4	29.4 ± 0.64	36.0 ± 1.74	49.8± 1.15
mg/kernel	0.2 ± 0.02	0.3 ± 0.06	1.1 ± 0.10	9.7 ± 1.79	14.6 ± 0.27	23.9 ± 0.52	26.0 ± 1.26	24.9 ± 0.58
Calanque								
g/100 g flour	2.1 ± 0.01	2.6 ± 0.09	7.4 ± 0.31	19.3 ± 0.61	26.2 ± 0.58	35.9 ± 0.62	47.9 ± 0.07	53.9 ± 1.7
mg/kernel	0.3 ± 0.00	0.6 ± 0.02	2.7 ± 0.11	11.8 ± 0.37	17.5 ± 0.39	25.3 ± 0.44	29.2 ± 0.04	27.9 ± 0.9
CDC Alamo								
g/100 g flour	1.9 ± 0.04	7.5 ± 0.21	10.5 ± 0.17	18.7 ± 0.43	25.4 ± 0.20	30.0 ± 1.03	45.4 ± 1.65	52.4 ± 1.8
mg/kernel	0.3 ± 0.01	2.4 ± 0.07	4.0 ± 0.07	11.6 ± 0.26	19.2 ± 0.15	20.2 ± 0.69	21.7 ± 0.79	20.1 ± 0.7
CDC Fibar								
g/100 g flour	1.3 ± 0.02	2.4 ± 0.09	7.3 ± 0.07	16.5 ± 0.27	20.6 ± 0.15	23.8 ± 0.21	27.7 ± 1.36	44.8 ± 1.69
mg/kernel	0.2 ± 0.00	0.5 ± 0.02	2.0 ± 0.02	8.4 ± 0.14	13.4 ± 0.10	18.3 ± 0.16	20.0 ± 0.98	16.2 ± 0.61
Scarlett								
g/100 g flour	1.4 ± 0.07	3.0 ± 0.02	5.9 ± 0.15	12.2 ± 0.64	n.d.*	19.5 ± 0.49	27.2 ± 1.09	55.2 ± 1.23
mg/kernel	0.2 ± 0.01	0.7 ± 0.01	1.8 ± 0.05	6.6 ± 0.34	n.d.*	12.4 ± 0.31	13.3 ± 0.53	25.1 ± 0.56
Dingo								
g/100 g flour	1.9 ± 0.02	1.6 ± 0.01	1.9 ± 0.04	5.4 ± 0.08	10.0 ± 0.04	13.8 ± 0.28	20.9 ± 1.15	52.6 ± 0.75
mg/kernel	0.2 ± 0.00	0.2 ± 0.00	0.3 ± 0.01	1.9 ± 0.03	4.6 ± 0.02	7.5 ± 0.15	13.3 ± 0.73	25.6 ± 0.36

*not determined; g/100 g flour is referred to fresh weight.

In Table 2.6 the evolution of protein content in developing barleys is reported. When considering the protein content on a per-kernel basis, overall linear accumulation occurs coherently with previous works (De Gara *et al*., 2003; Koga *et al*., 2017) despite at maturation four cultivars display a reduction of protein content (Cometa, Calanque, CDC Alamo and CDC Fibar). On the other hand, data expressed on g/100 g flour basis showed a non-linear trend before 20-28 DAA (Table 2.6).

Table 2.6. *Protein[a] content in developing barley kernels (mean value ± sd).*

Cultivar	DAA							
	Prestorage		***Storage***				***Dessiccation-Maturation***	
	3	6-8	10-11	17	20-21	24-28	31-38	55-67
Alimini								
g/100 g flour	2.9 ± 0.02	3.8 ± 0.04	3.3 ± 0.00	3.2 ± 0.00	3.0 ± 0.03	4.1 ± 0.07	7.2 ± 0.01	8.8 ± 0.13
mg/kernel	0.4 ± 0.00	0.6 ± 0.01	1.0 ± 0.00	1.5 ± 0.00	1.8 ± 0.02	3.1 ± 0.05	3.2 ± 0.01	4.2 ± 0.06
Cometa								
g/100 g flour	3.5 ± 0.02	3.2 ± 0.02	3.4 ± 0.02	3.5 ± 0.01	3.7 ± 0.06	4.6 ± 0.05	6.6 ± 0.03	8.8 ± 0.05
mg/kernel	0.5 ± 0.00	0.6 ± 0.00	0.9 ± 0.01	2.2 ± 0.01	2.5 ± 0.04	3.8 ± 0.04	4.8 ± 0.02	4.4 ± 0.02
Calanque								
g/100 g flour	3.5± 0.02	3.7 ± 0.03	3.4 ± 0.04	3.5 ± 0.02	4.2± 0.05	5.5 ± 0.02	8.4 ± 0.06	9.0 ± 0.06
mg/kernel	0.5± 0.00	0.9 ± 0.01	1.2 ± 0.02	2.2 ± 0.01	2.8±0.03	3.9 ± 0.02	5.1 ± 0.03	4.6 ± 0.03
CDC Alamo								
g/100 g flour	3.5 ± 0.03	3.8 ± 0.00	3.6 ± 0.01	3.7 ± 0.02	4.8 ± 0.02	6.8 ± 0.05	9.4 ± 0.005	10.5 ± 0.00
mg/kernel	0.6 ± 0.01	1.2 ± 0.00	1.4 ± 0.003	2.3 ± 0.01	3.6 ± 0.01	4.6 ± 0.03	4.5 ± 0.00	4.0 ± 0.00
CDC Fibar								
g/100 g flour	2.6 ± 0.00	2.9 ± 0.01	3.6 ± 0.05	3.7 ± 0.02	4.2 ± 0.01	5.3 ± 0.04	6.1 ± 0.00	12.0 ±0.02
mg/kernel	0.4 ± 0.00	0.6 ± 0.00	1.0 ± 0.01	1.9 ± 0.01	2.7 ± 0.01	4.1 ± 0.03	4.4 ± 0.00	4.3± 0.01
Scarlett								
g/100 g flour	3.4 ± 0.06	3.2 ± 0.04	2.6 ± 0.06	2.5 ± 0.00	n.d.*	2.7 ± 0.01	3.2 ± 0.02	6.9 ± 0.05
mg/kernel	0.5 ± 0.01	0.7 ± 0.01	0.8 ± 0.02	1.3 ± 0.00	n.d.*	1.7 ± 0.01	1.6 ± 0.01	3.1 ± 0.02
Dingo								
g/100 g flour	3.7 ± 0.08	3.4 ± 0.004	3.2 ± 0.05	2.7 ± 0.05	2.5 ± 0.03	2.8 ± 0.00	3.4 ± 0.00	9.5 ± 0.07
mg/kernel	0.3 ± 0.01	0.4 ± 0.00	0.5 ± 0.01	0.9 ± 0.02	1.2 ± 0.01	1.5 ± 0.00	2.2 ± 0.00	4.6 ± 0.03

[a]*N x 6.25;* g/100 g flour is referred to fresh weight; *not determined.

2.4. Conclusion

The potential of immature cereals has been studied for industrial application. Immature cereals can undergo either wet fractionation (for green crops) or dry fractionation (for near-maturity plants) to obtain a range of products for different purposes (feed, starch industry, cellulose industry etc.) (Carlsson, 1997). Moreover, another application of immature crops can be the industrial production of fructose (D'Egidio *et al.*, 1997). Fructan are non-starch polysaccharides with prebiotic effects that accumulates in immature kernels at a higher rate compared to mature grain. The inclusion of flour from immature grains in cereal-based products could be potential application to achieve the improvement of prebiotic intake of the population. Among cereals, barley has been extensively studied as a source of a wide variety of bioactive compounds especially β-glucan. For this reason, evaluating immature barley kernel as a source of both fructan and β-glucan was the purpose of this study.

The highest fructan concentration in immature barley grain was recorded between 6 and 17 DAA being from 3.3 to 5.5 times higher than at maturation. Scarlett accumulated the highest fructan content (9.5 g/100 g flour) compared to the other lines. By contrast, at the peak of fructan concentration, β-glucan content was below 1 g/100 g flour in all the cultivars, being higher in waxy mature barley cultivars compared to non-waxy cultivars. Substantial accumulation of β-glucan takes place between 20 and 28 DAA in five barley cultivars, while in Scarlett and Dingo a higher synthesis occurs in the later stages. A simultaneous increase of total starch accumulation and decline of fructan concentration was observed. Among all the barley cultivars considered in this study, Scarlett stands out to be potentially employed for the production of prebiotic cereal-based foods (bread, pasta, breakfast cereals etc). On the other hand, to achieve both fructan and β-glucan enrichment, combination of immature barley flour with mature waxy barley flour is suggested. The drawback of the use of immature ears is definetely managing the elevated content of water of the grain, which in the first stage of maturation can be over 60% w/w. To obtain immature barley flours for industrial application, we may suggest harvesting whole crops of immature plants and apply a mild heating (30-40 °C) to reduce moisture content. After drying, fractionation techniques can be applied to separate the kernels from the straw, and subsequently mill them to obtain flour as a fructan-enriched ingredient. Identifying barley cultivars accumulating high levels of fructan (e.g. in this study cultivar Scarlett) is also of vital importance since harvesting can eventually take place at the end of prestorage phase or at the beginning of the grain filling phase, to obtain kernels with a lower moisture content but still acceptable levels of fructan, in order to reduce costs associated with manipulations of immature grains (specifically related to the drying process).

2.5. References

Abrams SA, Griffin IJ, & Hawthorne KM (2007). Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. *The Journal of Nutrition*, **137**(10): 2524–2526.

AbuMweis S, Thandapilly SJ, Storsley J, & Ames N (2016). Effect of barley β-glucan on postprandial glycaemic response in the healthy human population: a meta-analysis of randomized controlled trials. *Journal of Functional Foods*, **27**: 329–342.

Ames N, Blewett H, Storsley J, Thandapilly SJ, Zahradka P, & Taylor C (2015). A double-blind randomised controlled trial testing the effect of a barley product containing varying amounts and types of fibre on the postprandial glucose response of healthy volunteers. *British Journal of Nutrition*, **113**(9): 1373–1383.

Angelovici R, Galili G, Fernie AR, & Fait A (2010). Seed desiccation: a bridge between maturation and germination. *Cell Press*, **15**(4): 211–218.

Arena MP, Caggianiello G, Fiocco D, Russo P, Torelli M, Spano G, & Capozzi V (2014). Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. International *Journal of Molecular Sciences*, **15**(2): 3025–3039.

Baik BK, & Ullrich SE (2008). Barley for food: characteristics, improvement, and renewed interest. *Journal of Cereal Science*, **48**(2): 233–242.

Brighenti F (2007). Dietary fructans and serum triacylglycerols: a meta-analysis of randomized controlled trials. *The Journal of Nutrition*, **137**: 2552–2556.

Carlsson R (1997). Food and non-food uses of immature cereals. In: Cereals. Novel uses and processes. Ed. Campbell GM & McKee SL. Plenum Press, New York and London, pp.159-167.

Cerning J, & Guilbot A (1973). Changes in carbohydrate composition during development and maturation of the wheat barley kernel. *American Association of Cereal Chemistry*, **50**: 220–232.

Cimini S, Locato V, Vergauwen R, Paradiso A, Cecchini C, Vandenpoel L, Verspreet J, Courtin CM, D'Egidio MG, Van den Ende W, & De Gara L (2015). Fructan biosynthesis and degradation as part of plant metabolism controlling sugar fluxes during durum wheat kernel maturation. *Frontiers in Plant Science*, **6**(Feb.): 1–10

Coles G (1979). Relationship of mixed-link β-glucan accumulation to accumulation of free sugars and other glucans in the developing barley endosperm. *Carlsberg Research Communication*, **44**: 439–453.

D'Egidio MG, Cecchini C, Corradini C, Donini V, Pignatelli V, & Cervigni T (1997). Innovative uses of cereals for fructose production, pp143-151 In: Cereals. Novel uses and processes. Ed. Campbell GM & McKee SL. Plenum Press, New York and London.

De Angelis M, Montemurno E, Vannini L, Cosola C, Cavallo N, Gozzi G, Maranzano V, Di Cagno R, Gobbetti M, & Gesualdo L (2015). Effect of whole-grain barley on the human fecal microbiota and metabolome. *Applied and Environmental Microbiology*, **81**(22): 7945–7956.

De Gara L, De Pinto MC, Moliterni VMC, & D'Egidio MG (2003). Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany*, **54**(381): 249–258.

De Paula R, Rabalski I, Messia MC, Abdel-Aal E-SM, & Marconi E (2017). Effect of processing on phenolic acids composition and radical scavenging capacity of barley pasta. *Food Research International*, **102**: 136-143.

Fincher GB (1975). Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brewing*, **81**: 116–122.

Gibson GR (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *The Journal of Nutrition*, **129**(7 Suppl): 1438–1441

Hendry GF (1993). Evolutionary origins and natural functions of fructans-a climatological, biogeographic and mechanistic appraisal. *New Phytologist*, **123**(1): 3–14.

Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, & Friedlander AL (2007). Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. *British Journal of Nutrition*, **25**: 365–372.

Holtekjølen AK, Uhlen AK, Bråthen E, Sahlstrøm S, & Knutsen SH (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. *Food Chemistry*, **94**(3): 348–358.

Iametti S, Bonomi F, Pagani MA, Zardi M, Cecchini C, & D'Egidio MG (2006). Properties of the protein and carbohydrate fractions in immature wheat kernels. *Journal of Agricultural and Food Chemistry*, **54**: 10239–10244.

Izydorczyk MS, & Dexter JE (2008). Barley β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products-a review. *Food Research International*, **41**(9): 850–868.

Katagiri M, Masuda T, Fumito T, & Kitabatake N (2011). Expression and development of wheat proteins during maturation of wheat kernel and the rheological properties of dough prepared from the flour of mature and immature wheat. *Food Science and Technology Research*, **17**(2): 111–120.

Koga S, Böcker U, Wieser H, Koehler P, Uhlen AK, & Moldestad A (2017). Polymerisation of gluten proteins in developing wheat grain as affected by desiccation. *Journal of Cereal Science*, **73**: 122–129.

Livingston DP, Hincha, DK, & Heyer AG (2009). Fructan and its relationship to abiotic stress tolerance in plants. *Cellular and Molecular Life Sciences*, **66**: 2007–2023

Nardi S, Calcagno C, Zunin P, D'Egidio MG, Cecchini C, Boggia R, & Evangelisti F (2003). Nutritional benefits of developing cereals for functional foods. *Cereal Research Communications*, **31**(3): 445–452.

Nemeth C, Andersson AAM, Andersson R, Mangelsen E, Sun C, & Åman P (2014). Relationship of grain fructan content to degree of polymerisation in different barleys. *Food and Nutrition Sciences*, **5**(Mar): 581–589.

Niness KR (1999). Inulin and oligofructose: what are they? *The Journal of Nutrition*, **129**(7 Suppl): 1402–1406.

Paradiso A, Cecchini C, Greco E, D'Egidio MG, & De Gara L (2008). Variation in fructooligosaccharide contents during plant development and in different cultivars of durum wheat. *Plant Biosystem-An International Journal Dealing with all Aspects of Plant Biology*, **142**(3): 656–660.

Pasqualetti V, Altomare A, Guarino MPL, Locato V, Cocca S, Cimini S, Palma R, Alloni R, De Gara L, & Cicala M (2014). Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. *Plos One*, **9**(5): 1–9.

Peukert M, Thiel J, Mock HP, Marko D, Weschke W, & Matros A (2016). Spatiotemporal dynamics of oligofructan metabolism and suggested functions in developing cereal grains. *Frontiers in Plant Science*, **6**: 1–13.

Peukert M, Thiel J, Peshev D, Weschke W, Van den Ende W, Mock H, & Matros A (2014). Spatio-temporal dynamics of fructan metabolism in developing barley grains. *The Plant Cell*, **26**: 3728–3744.

Roberfroid MB, & Delzenne NM (1998). Dietary fructans. *Annual Review of Nutrition*, **18**: 117–143.

Tiwari U, & Cummins E (2011). Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels. *Nutrition*, **27**(10): 1008–1016.

Tosh SM (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition*, **67**(4): 310–317.

Tsuchiya K, Urahara T, Konishi T, Kotake T, Tohno-oka T, Komae K, Kawada N, & Tsumuraya Y (2005) Biosynthesis of (1→3),(1→4)-β-glucan in developing endosperms of barley (*Hordeum vulgare* L.). *Physiologia Plantarum*, **125**: 181-191.

Verspreet J, Cimini S, Vergauwen R, Dornez E, Locato V, Le Roy K, De Gara L, Van den Ende W, Delcour JA, & Courtin CM (2013). Fructan metabolism in developing wheat (*Triticum aestivum* L.) kernels. *Plant Cell Physiology*, **54**(12): 2047–2057.

Verspreet J, Dornez E, & Courtin CM (2015). Cereal grain fructans: structure, variability and potential health effects. *Trends in Food Science & Technology*, **43**(1): 32–42.

Wardlaw I, & Willebrink J (2000). Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. *New Phytologist*, **148**: 413–422.

Wilson SM, Burton R, Doblin MS, Stone BA, Newbigin EJ, Fincher GB, & Bacic A (2006). Temporal and spatial appearance of cell wall polysaccharides during cellularization of barley (*Hordeum vulgare*) endosperm. *Planta*, **224**: 655-667.

Wobus U, Sreenivasulu N, Borisjuk L, Rolletschek H, Panitz R, Gubatz S, & Weschke W (2005). Molecular physiology and genomics of developing barley grains. *Recent Research Development in Plant Molecular Biology*, **2**(2): 1–29.

Zheng X, Qi J, Hui H, Li L, & Wang F (2017). Starch accumulation in hulless barley during grain filling. *Botanical Studies*, **58**: 1-12.

3. Structure analysis of β-glucan in barley and effects of wheat β-glucanase

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Abstract

In this study, β-glucan in samples of sifted flour from six barley varieties was sequentially extracted with water and NaOH obtaining three fractions: water extractable (WE), NaOH extractable (NaE) and residual (Res). β-Glucan isolates were incubated with lichenase and oligomers released were analysed with high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). A higher ratio 3-O-β-cellobiosyl-D-glucose (DP3) to 3-O-β-cellotriosyl-D-glucose (DP4) is reported in WE, NaE and Res fractions in variety SLU 7 (shrunken endosperm) compared to the other lines, suggesting a more packed β-glucan structure. Water-extractable and water-unextractable β-glucan fractions were incubated with wheat extract and effects of wheat β-glucanase on the structure was assessed after lichenase digestion and analysis with HPAEC-PAD. Findings suggest that wheat β-glucanase manifests a selective hydrolysis towards β-glucan with lower DP3/DP4 ratio and that β-glucan of SLU 7 is composed of a population with higher DP3/DP4 ratio and hence with a more tighten structure which may be more resistant to enzymic action. Overall, the results obtained are of interest to characterise barleys differing in starch and dietary fibre composition, specifically shrunken endosperm barley SLU 7 in the perspective of its inclusion in the production of β-glucan enriched foods.

Key words: barley, extraction, β-glucan, β-glucanase, lichenase, oligosaccharides.

3.1. Introduction

Cereal mixed linkage β-glucan (hereafter referred to as "β-glucan") are linear homopolysaccharides of β-D-glucopyranosyl consecutives residues linked by (1→4) bonds separated by single (1→3) linkages. Barley grain (*Hordeum vulgare* L.) is a valuable source of β-glucan and its incorporation in cereal-based products contributes also to the intake of other bioactive compounds like phenolic compounds (De Paula *et al*., 2017a). The content of β-glucan in barley generally ranges between 2% and 11% by weight which is higher compared to other cereals like wheat, rye, spelt and emmer *(*Andersson *et al.*, 2009*;* Messia *et al.*, 2017). In barley, these polysaccharides represent the predominant constituent of the endosperm cell walls, accounting for ~70% of the total components (Fincher, 1975). β-Glucan from barley and oats demonstrated positive impact on human health, primarily the reduction of postprandial blood glucose and blood LDL cholesterol (AbuMweis *et al*., 2016; Ames *et al*., 2015; Tiwari & Cummins, 2011; Tosh, 2013; Wood *et al*., 1994). Due to these beneficial effects, barley can, therefore, be considered a functional ingredient. The US Food and Drug Administration set values for β-glucan contents (0.75 g/ portion and 3g/day) to allow foods to bear health claims on lowered risk of coronary heart diseases (FDA, 2017). On the other hand, the European Food Safety Authority (EFSA) approved health claims related to β-glucan and reduction of postprandial blood glucose (4 g/30 g available carbohydrates) and cholesterol management (≥ 1 g/ quantified portion and 3 g/day) on foods containing barley and oat fibre (EFSA, 2011).

Barley starch is normally composed of 25-30% amylose (Morrison *et al*., 1984) but genotypes can differ according to starch content and composition. In "waxy" barleys (low-amylose) the amylose content is 0-10%, while in "high-amylose" barleys it is higher than 35% (Ajithkumar *et al*., 2005; Oscarsson *et al*., 1997; Yanagisawa *et al*., 2006). The highest content of β-glucan is reported in high amylose and waxy starches (~6-7%) because of thicker endosperm cell walls and smaller starch granules respectively, while normal starch barleys contain approximately 4% of β-glucan (Andersson *et al*., 2008; Izydorczyk *et al*., 2000; Xue *et al*., 1997; Oscarsson *et al*., 1997). Shrunken endosperm phenotype in barley can be caused by mutations in genes expressed in either maternal tissues (*seg*) or in the kernel itself (*sex*) (Ma *et al.*, 2014) while *shx* mutation is a recessive trait that is generated spontaneously (Schulman & Ahokas, 1990). Starch content in these genotypes is reduced (Morell *et al*., 2003; Schulman *et al*., 1995) and the chemical composition of the grain can be different from a normal barley kernel as a result of a switch from starch synthesis to e.g. oligofructan and β-glucan (Clarke *et al*., 2008; Schulman & Ahokas, 1990) Physicochemical properties of β-glucan, like molecular weight (MW), are critical for the effects on human health (Keogh *et al*., 2003; Wolever *et al*., 2010; Wood, 2002). Studies confirmed that high MW β-glucan are more effective than low MW β-glucan for cholesterol reduction and prevention of cardiovascular diseases (Wang *et al*., 2016a; Wang *et al*., 2016b). Food processing can have impacts on β-glucan physicochemical characteristics, thus affecting the physiological properties. For instance, steps of extrusion and drying in pasta making reduced viscosity, solubility and the peak molecular weight of β-glucan, while cooking increased its extractability (De Paula *et al*., 2017b). Bread making is reported

to decrease the MW of β-glucan mainly because of the activity of endo β-glucanase in wheat flour (Andersson *et al*., 2004; Flander *et al*., 2007; Tiwari *et al.*, 2011). Reducing mixing and fermentation time and eventually inactivating the enzymes have been proposed as strategies to minimize β-glucan degradation during the baking process (Åman *et al*., 2004; Andersson *et al.*, 2004; Rieder *et al*., 2015). In the study of Djurle *et al*. (2018) it was reported that in bread containing flour from barley variety SLU 7 (shrunken endosperm barley), the molecular weight of β-glucan was higher compared to breads containing the other varieties considered in the study.

The molecular structure of β-glucan has been extensively studied using lichenase hydrolysis. Lichenase (β-(1→3), β-(1→4) -D-glucan-4-glucanohydrolase) specifically hydrolyses β-(1→4) bonds which are linked at C-O-3 yielding mainly tri-and tetrasaccharides, representing 90% of the oligomers released (Cui *et al*., 2000; Izydorczyk *et al*., 1998a, 1998b). The ratio between oligomers with degree of polymerization (DP) 3 and those with DP4 released after lichenase hydrolysis is specific for each type of grain, being 4.5, 3.3, and 2.2 for wheat, barley and oat, respectively (Cui *et al*., 2000). DP3/DP4 ratio also affects β-glucan behaviour in solutions. In fact, higher DP3/DP4 leads to a higher interchain aggregation with hydrogen bonds with decreased solubility in water and greater ability to form gels (Izydorczyk & Dexter, 2008).

The objective of this study was to investigate the oligosaccharide composition describing the molecular structure of β-glucan in the barley samples differing in starch and dietary fibre composition and evaluate the impact of the action of wheat β-glucanase.

3.2. Materials and methods

Samples

Six barley samples were obtained from Lantmännen Lantbruk (Sweden): Gustav, NGB 114602 SLU 7, KVL 301, SW 28708 and Karmosè. The kernels were milled using a laboratory mill (Laboratoriums-mahlautomat model MLU 202, Genrunder Bühler Maschinenfabrik, Uzwill, Switzerland), producing six fractions of sifted flour and two bran fractions from each variety (Djurle *et al*., 2016). All the fractions of sifted flour were pooled and used for the analysis. Composition and dietary fibre content of sifted flour was assessed previously (Djurle *et al*., 2016) and reported in Table 3.1.

Table 3.1. *Characteristics, composition (% dw) and amylose content (% of starch) in barley varieties (flour samples) as reported by Djurle et al. 2016.*

Variety	Characteristics	Starch	Amylose	β-glucan	Fructan
Gustav	Commercial, feed use	66.8	29	3.6	0.9
NGB 114602	Anthocyanin-rich	66.8	31	3.7	0.7
SLU 7	Shrunken endosperm, high β-glucan, high fructan	53.9	31	7.5	2.5
Karmosè	High amylose	55.9	47	5.3	1.7
KVL 301	Low β-glucan, low fructan	56.2	28	3.0	1.2
SW 28708	Hulless, low amylose (waxy)	58.0	2	4.7	2.4

Reagents

α-Amylase solution - Thermostable α-amylase (Megazyme International Ireland Ltd) for TDF (Total Dietary Fibre) and starch assay (3,000 U/mL)

Amyloglucosidase solution - Amyloglucosidase (Megazyme International Ireland Ltd) for TDF and starch assay (3,260 U/mL) was diluted 1:10 with sodium acetate buffer (0.1 M, pH 5.0).

Lichenase 50 U/mL - Enzyme from Megazyme International Ireland Ltd (E-LICH 1,000 U/mL) was diluted 1:20 with sodium phosphate buffer (20 mM, pH 6.5).

β-Glucan extraction process

A schematic overview of the extraction process to evaluate β-glucan structure in the six varieties considered is illustrated in Figure 3.1. Flour (0.5 g) was weighed in glass test tube. The extraction process started by adding aqueous ethanol 50% v/v (20 mL) and then the content was stirred and the tubes incubated in a boiling water bath for 15 minutes to inactivate endogenous β-glucanase. When starch is heated in excess of water, gelatinization occurs, the granules swell and form gel particles resulting in an increased viscosity. Waxy starches generally swell more than normal starches providing higher peak viscosity (Tester & Morrison, 1990; Varavinit *et al.*, 2003). Indeed, variety SW 28708 had a lower pasting and peak temperature compared to non-waxy barley lines (Källman, 2013) and this probably explains why this flour was swelling in 50% ethanol at 95 °C and forming lumps that were hard to disperse. To avoid difficulties in pellet dispersion, the inactivation of endogenous enzymes in this variety was performed by using ethanol 80% v/v (20 mL). After that, the tubes were centrifuged at 1,000 g for 15 minutes and the supernatant discarded. To the pellet, 15 mL water was added along with 50 µL of heat stable α-amylase and stirred. The tubes were incubated for 1 hour in a boiling water bath stirring 2-3 times. After centrifugation, the supernatant was collected in new tubes (S1) and the pellet (P1) was washed twice with water (10 mL) that was discarded. Amyloglucosidase solution (500 µl) was added to S1 and incubated at 60 °C overnight shaking. Ethanol was added to S1 to obtain 60% v/v, centrifuged and the supernatant discarded to obtain the pellet WE (water extractable). NaOH (50 mM, 20 mL) was added to the pellet P1 and incubated for 17 hours at room temperature shaking. After centrifugation, the supernatant (S2) was collected in tubes and neutralised with glacial acetic acid. The residual pellet (Res) was washed twice with 15 mL water which was then discarded. Ethanol was added in S2 to obtain 60% v/v and, after centrifugation, the pellet was collected and named NaE (NaOH extractable). All the pellets were stored at -18 °C until analysed. The extraction was performed in duplicate. To prepare samples for β-glucan quantification, the same procedure was used but 0.25 g of barley flour from cultivar SLU 7 and SW 28708 was weighed since concentration in the WE fraction resulted excessively high for accurate absorbance values determinations.

To evaluate the impact of wheat β-glucanase on β-glucan structure only two fractions of β-glucan were considered: water extractable (WE) and water unextractable (WU). Barley flour from cultivar SLU 7, SW28708 and Gustav was weighed (0.5 g) in glass tubes. Ethanol 50% v/v (20 mL) was added to cultivar

SLU 7 and Gustav, while ethanol 80% v/v (20 mL) was added to cultivar SW 28708 and stirred. The tubes were incubated in a boiling water bath for 15 minutes to inactivate the enzymes. After centrifugation (1,000 g, 15 minutes) the supernatant was discarded and 15 mL water was added to the pellets with 50 µL of heat stable α-amylase. The content was stirred and the tubes were incubated in a boiling water bath for 1 hour stirring 2-3 times. After centrifugation, the pellet was washed two times with 10 mL water, that was then discarded, obtaining the pellet named "WU". The supernatant was collected in new tubes and 500 µL of diluted amyloglucosidase solution was added and incubated overnight shaking at 60 °C. Ethanol was added to reach 60% v/v. After centrifugation, the pellet WE was recovered. The extraction was performed in duplicate and all the pellets were stored at -18 °C for the same number of days.

Wheat extract preparation

Wheat flour (*Triticum aestivum*) was weighed in a beaker (5 g) and 150 mL water was added. The mixture was stirred on a magnetic stirrer for 15 minutes and let stand for another 15 minutes. Aliquots were collected in tubes and centrifuged (1,000 g, 15 minutes). The supernatants were pooled together in one beaker and stirred to homogenize the solutions and obtain the wheat extract (WhE).

Incubation of β-glucan isolates with wheat extract

Water (1 mL) was added to the pellet WE and WU, stirred and incubated in a boiling water bath for 1 minute, stirred and incubated for further 2-3 minutes and stirred until completely dispersed. Wheat extract (5 mL) was added to the pellets, stirred and incubated for 1 hour at 37 °C shaking. After that, the tubes were immediately incubated in a boiling water bath for 15 minutes to inactivate the enzymes. To all samples 250 µL amyloglucosidase solution was added and mixed and then the tubes were placed in a water bath at 60 °C overnight shaking. After centrifugation, only the pellet from the sample WU-WhE was recovered washed twice with 10 mL water and named WU-Whe-Ins. To the supernatants, ethanol was added to obtain a final concentration of 60% v/v. The solutions were centrifuged and the pellets were collected and named WE-WhE-Sol, WU-WhE-Sol. All the pellets were stored at -18 °C. The schematic process to obtain these fractions is illustrated in Figure 3.2.

Wheat β-glucanase assessment

The Megazyme kit "Malt & bacterial β-glucanase & cellulase assay procedure (Azo-barley glucan method, K-MBGL 03/11)" was used to assess β-glucanase level in the wheat extract, with some modifications. Briefly, aliquots of wheat extracts were incubated with Azo-Barley glucan substrate solution for 4 hours at 30 °C. This substrate was depolimerised to fragments which are soluble when precipitant solution is added. After centrifugation, the absorbance of supernatant is read at 590 nm and correlated to β-glucanase occurrence. Correction factors for concentration and incubation time were applied.

Chromatographic analysis

Aqueous ethanol (50%, 0.2 mL) was added to the pellets "Res" and "WU" to aid dispersion. Then all the pellets (WE, NaE, Res, WE-WhE-Sol, WU-Whe-Sol, WU-WhE-Ins) were suspended in 4 mL sodium phosphate buffer (20 mM, pH 6.5) and stirred. The samples were immediately incubated in a boiling water bath for 60 seconds, stirred and incubated for further 2 minutes and stirred again. After that, they were equilibrated for 5 minutes at 50 °C and 0.2 mL of lichenase (10 U) was added to each tube, stirred and incubated at 50 °C for 1 hour stirring 2-3 times. After centrifugation (1,000 g, 15 minutes), 2 mL of supernatant was transferred in new tubes and placed for 5 minutes in a boiling water bath to inactivate the enzymes, then 2.5 mL of water was added to all samples stirred and centrifuged. The supernatant was recovered, filtered through 0.45 µm filter (Millex millipore) and Ultrapure water (18 megohm-cm) was used to dilute samples. WE, NaE, Res samples were diluted 2-fold; samples WE-WhE-Sol were diluted 1:50 while WU-Whe-Sol, and WU-WhE-Ins were diluted 1:5.

Aliquots (10 µL) of the diluted samples were analysed on high performance anion exchange chromatography. High viscosity β-glucan from barley flour (Megazyme International Ireland Ltd) was used as a standard. Separation was carried out on a Carbopac^{TM} PA100 (4x250mm), analytical column (Dionex, Sunnyvale USA) equipped with a guard column. The elution was performed at 25 °C with a flow rate of 0.4 mL /min, using 0.1 M NaOH (A) and 1 M NaOAc+0.1 M NaOH (B) with the following gradient elution: 0 min (92% A, 8% B), 30 min (77% A, 23% B), 31 min (92% A, 8% B) held until 60 min. Detection was performed with a pulsed amperometric detector (Thermo scientific) using the following potential waveform: 0.00-0.20 s, + 0.10 V; 0.40 s, +0.10 V; 0.41-0.42 s, -2.00V; 0.43 s, 0.60 V; 0.44-0.45 s, -0.10 V. Chromatograms of the fragments generated after lichenase digestion were analysed with Chromeleon V6.80 software. Data on the peaks areas related to the oligosaccharides were normalised as: Area (DP*x*)/Area (DP3+DP4).

β-Glucan quantification in the obtained fractions

The β-glucan analysis was performed on samples WU and WU-WhE-Ins according to the AACC method 32-23 (Megazyme International Ltd). For β-glucan isolates WE, NaE, Res the method was modified as the following. The samples "Res" were wet with 0.2 mL aqueous ethanol to aid dispersion. Sodium phosphate buffer (8 mL, 20 mM, pH 6.5) was added and stirred. On stirring, the tubes were placed in a boiling water bath for 60 seconds, stirred and incubated for further 2 minutes and stirred again. The samples were equilibrated at 50 °C for 5 minutes and lichenase (0.4 mL, 20 U) was added and stirred. The tubes were incubated at 50 °C for 1 hour, mixing them 2-3 times. Sodium acetate buffer (10 mL, 200 mM, pH 4.0) was added and mixed. The tubes were allowed to equilibrate to room temperature and centrifuged (1,000 g, 10 minutes). Aliquots of the supernatant (0.1 mL) were dispensed in three glass test tubes. Enzyme β-glucosidase (0.1 mL) was added to two of these tubes, while to the third sodium acetate buffer (0.1 mL, 50 mM, pH 4.0) was added. All the tubes were incubated at 50°C for 10 minutes. Glucose oxidase/peroxidase reagent (3 mL) was added to each tube and incubated at 50

°C for further 20 minutes. After cooling to room temperature, the absorbance values were measured at 510 nm against the reagent blank within 1 hour.

Moisture content

Samples of flour were weighed (0.1 g) and incubated at 105 °C for 16 hours. The analysis was performed in triplicate.

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's pairwise comparison test were performed on the data obtained by chromatographic analysis using Minitab 16 (Minitab 16 Statistical Software, 2010, Minitab Inc., State College, Pennsylvania) and significant difference was set at $p < 0.05$. Principal component analysis (PCA) was performed using The Unscrambler X 10.1 (CAMO Software AS, Norway) software.

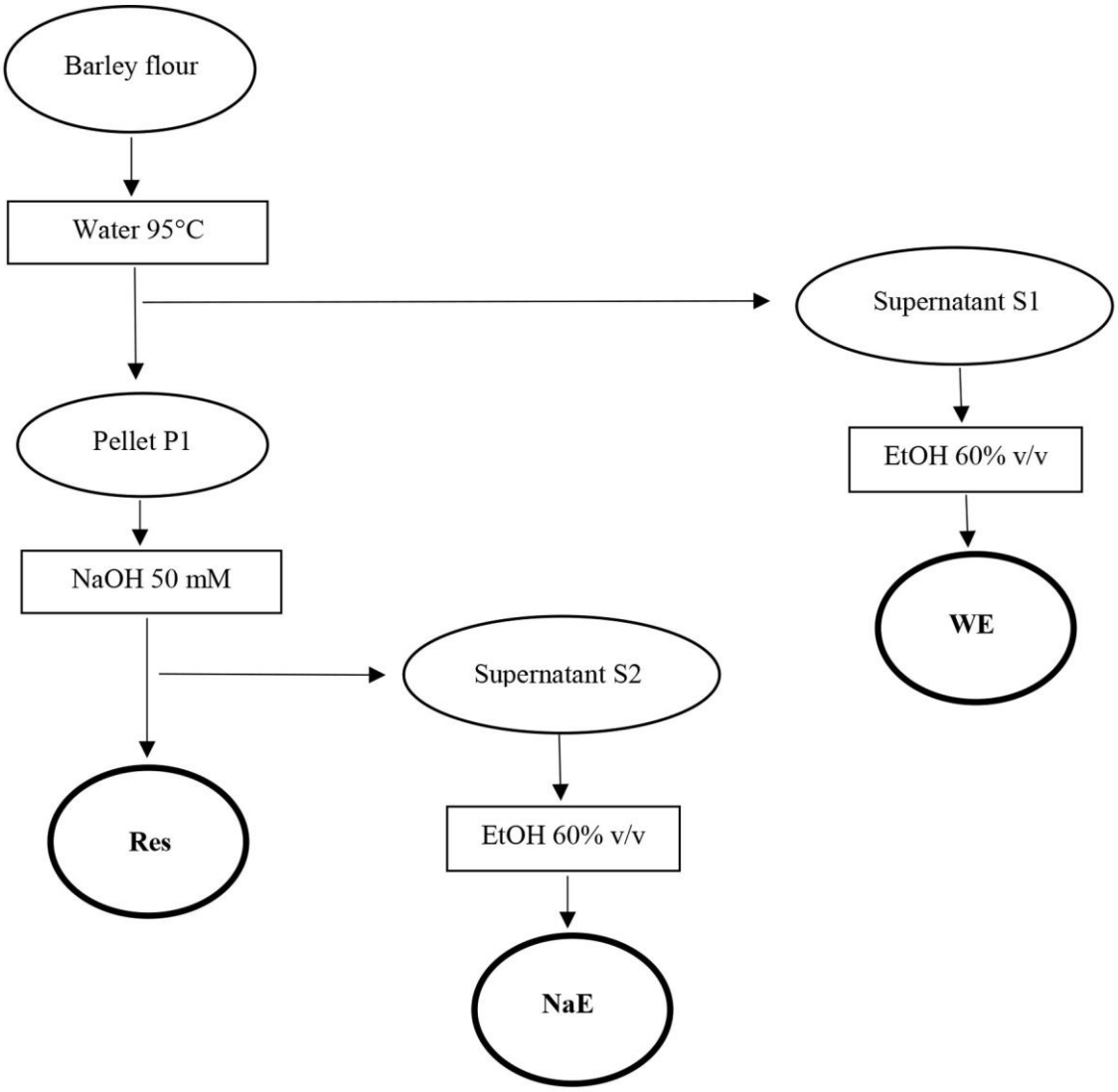

Figure 3.1. *Sequential extraction of barley β-glucan in the six varieties with water and alkali.*

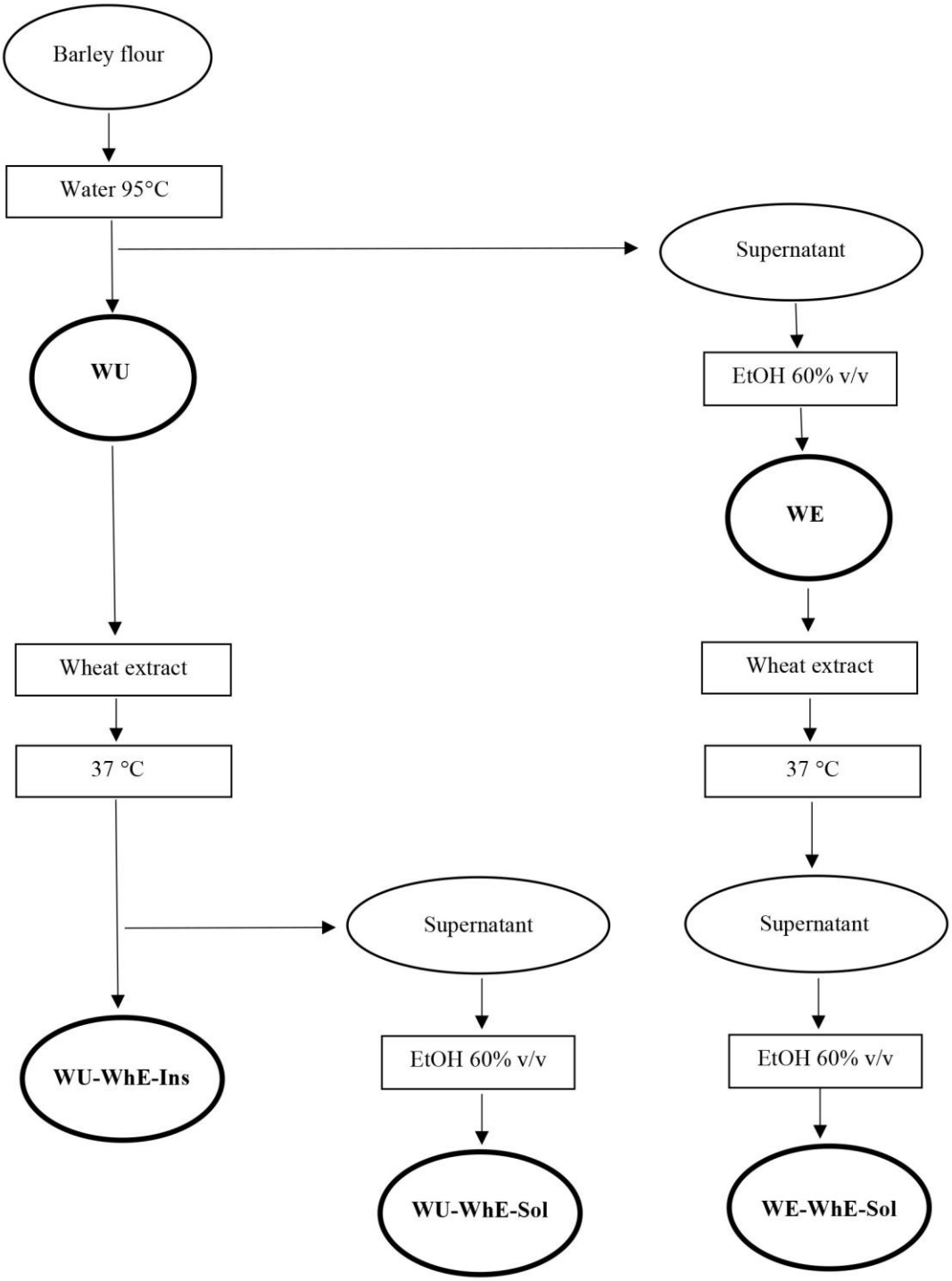

Figure 3.2. *Diagram of the extraction of water-soluble and water-insoluble β-glucan and further incubation with wheat extract.*

3.3. Results and discussion

β-Glucan structure in barley cultivars

The molecular structure of the β-glucan of different barley cultivars was investigated performing sequential extraction of β-glucan with water and alkali and then undergoing the obtained fractions (water and alkali-extractable fractions and residuals) to enzymic digestion by lichenase. Chromatographic analysis with HPAEC-PAD (high performance anion exchange chromatography-pulsed amperometric detector) was finally used to evaluate the distribution of oligosaccharides released from β-glucan after lichenase treatment.

The distribution of oligosaccharides generated after lichenase hydrolysis of the three fractions is presented in Table 3.2. Data on the single fragments are reported as normalised against the sum of DP3+DP4 areas. As previously mentioned, the digestion of β-glucan with lichenase generates mainly tri- and tetrasaccharides but also a smaller proportion of oligosaccharides with DP up to 20 (Izydorczyk & Dexter, 2008; Lazaridou *et al*., 2008a). In this study, only areas of peaks of fragments DP3-9 were considered, despite minor amounts of oligosaccharides with DP > 9 were also detectable after chromatographic analysis (Figure 3.3). The content of oligosaccharides decreased with the increase of DP, until DP7 then it increases again (Table 3.2), coherently with others work (Izydorczyk *et al*., 1998a, 1998b; Papageorgiou *et al*., 2005). The proportion of the sum tri- and tetrasaccharides on the total amount of oligosaccharides in this study varied between 77 and 84% in the WE fraction, 89-92% in the NaE fraction and 86-90% in the residual pellet. As a consequence, the amount of fragments with DP ≥ 5 was higher in the water-soluble β-glucan, a result which is not in agreement with previous works (Lazaridou *et al*., 2008b). Nevertheless, it is important to mention that in the cited study analysis was performed on endosperm cell walls isolated from fibre rich fractions obtained by roller milling of pearled barley and that the highest proportion of fragments with DP ≥ 5 was found in fractions obtained after sequential extractions with $Ba(OH)_2$ and then NaOH.

Important information on the structure of β-glucan are obtained by analysing the ratio DP3/DP4 which was higher in the NaE and Res fractions compared to the WE, in all the varieties analysed. In WE fractions DP3/DP4 ratios were on average lower compared to previous findings (Izydorczyk *et al*., 1998b; Lazaridou *et al.*, 2008a, 2008b). Differences were observed among varieties. SLU 7 reported the highest DP3/DP4 ratios in all the three fractions compared to the other varieties, while NGB 114602 was characterized by the lowest ratios. ANOVA revealed statistically significant differences (p<0.05) of DP3/DP4 ratios of SLU 7 from all the varieties except SW 28708 (Table 3.2). Figure 3.4 reports the magnitude of data differences between replicates. On average, the percent difference in WE extract is 1.5%, while in NaE ad Res is 7.3% and 4.8% respectively. Isolates of cultivar SLU 7 showed the highest percent difference among samples.

Higher ratios of tri- to tetrasaccharides in the β-glucan chain is related to conformational features, specifically multiple intermolecular associations and hence decreased solubility (Böhm & Kulicke, 1999). From these results, it can be concluded that the structure of β-glucan in SLU 7 may be compact

enough to reduce effects of wheat endo-β-glucanase leading to a decreased breakdown during the baking process.

Table 3.2. *Distribution of oligosaccharides released from β-glucans in the three fractions after lichenase digestion (mean values of normalised data: Area DPx/ Area(DP3+DP4); WE= water-extractable, NaE= alkali-extractable, Res= residual).*

Sample		DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP3/DP4 [1]	DP5+DP6
Gustav	WE	0.64	0.36	0.10	0.05	0.011	0.016	0.033	1.79	0.15
	NaE	0.68	0.32	0.05	0.02	0.003	0.006	0.017	2.17	0.07
	Res	0.66	0.34	0.06	0.03	0.006	0.007	0.020	1.98	0.09
	Average								*1.98^{c}*	
NGB 114602	WE	0.64	0.36	0.10	0.06	0.013	0.017	0.038	1.74	0.16
	NaE	0.67	0.33	0.05	0.03	0.004	0.007	0.021	1.99	0.08
	Res	0.66	0.34	0.06	0.03	0.006	0.007	0.021	1.96	0.09
	Average								*1.90^{c}*	
SLU 7	WE	0.66	0.34	0.14	0.08	0.013	0.024	0.033	1.91	0.22
	NaE	0.71	0.29	0.06	0.02	0.005	0.006	0.012	2.50	0.08
	Res	0.70	0.30	0.07	0.03	0.005	0.007	0.012	2.36	0.10
	Average								*2.25^{a}*	
Karmosè	WE	0.64	0.36	0.13	0.07	0.020	0.023	0.047	1.80	0.20
	NaE	0.68	0.32	0.06	0.03	0.005	0.007	0.019	2.12	0.09
	Res	0.67	0.33	0.07	0.03	0.006	0.008	0.021	2.00	0.10
	Average								*1.97^{c}*	
KVL 301	WE	0.65	0.35	0.09	0.04	0.009	0.015	0.032	1.86	0.13
	NaE	0.70	0.30	0.05	0.02	0.003	0.006	0.018	2.28	0.07
	Res	0.65	0.35	0.07	0.04	0.006	0.012	0.030	1.88	0.11
	Average								*2.01^{bc}*	
SW 28708	WE	0.65	0.35	0.13	0.08	0.020	0.024	0.044	1.86	0.21
	NaE	0.70	0.30	0.05	0.02	0.003	0.003	0.015	2.39	0.07
	Res	0.69	0.31	0.06	0.02	0.003	0.007	0.017	2.20	0.08
	Average								*2.15^{ab}*	

[1] Average values followed by different letters are significantly different ($p<0.05$).

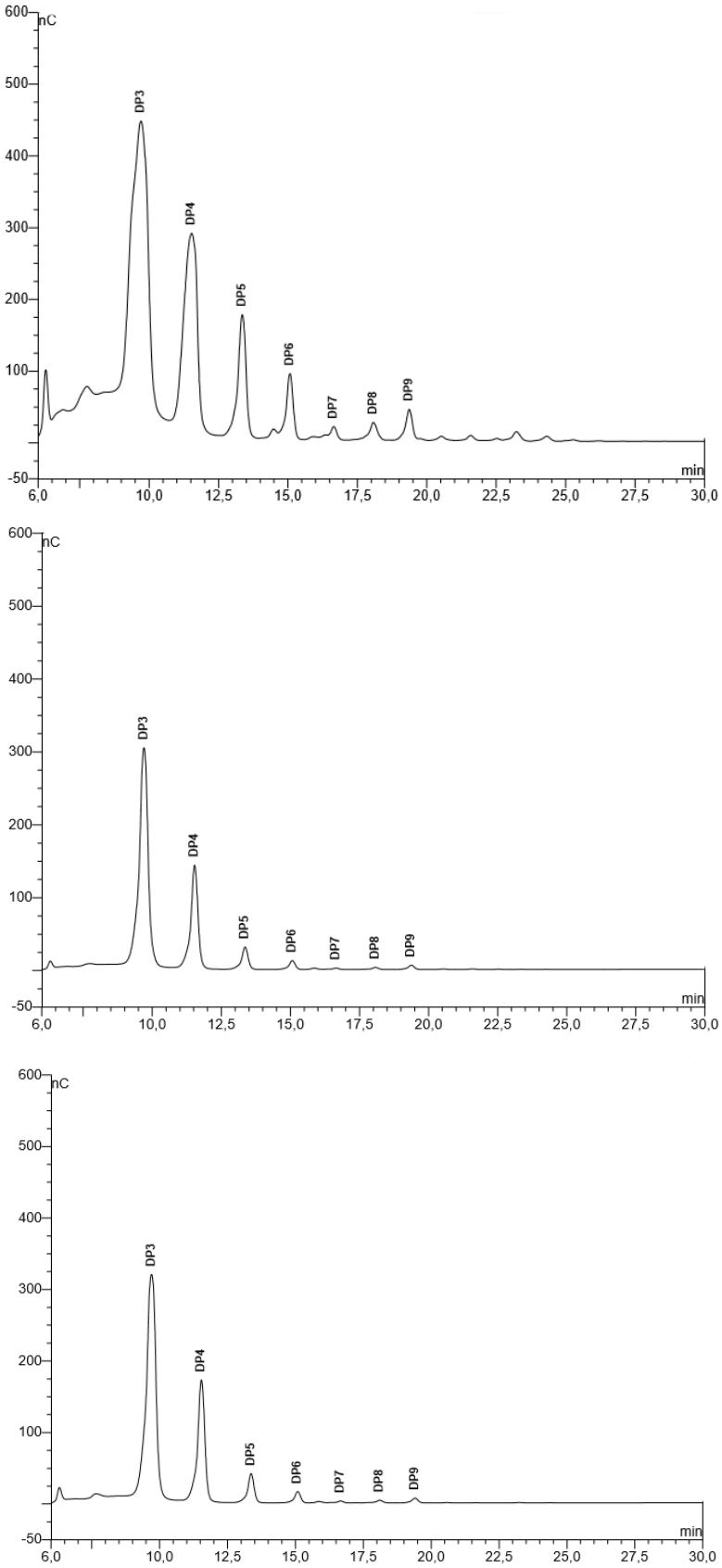

Figure 3.3. *Chromatograms of oligosaccharides released after lichenase digestion of β-glucan in variety SLU 7. WE (top), NaE (middle), Res (bottom).*

Results of this study give additional information on the structure of β-glucan in the varieties analysed. Fragments with DP5 were more abundant in water soluble extracts, and the sum DP5+DP6 is related to WE fractions ($p<0.05$) with no statistical differences among varieties.

To better understand relations between the pattern of oligosaccharides and the fractions of β-glucan analysed, a principal component analysis was performed. The scores and loadings plots, considering the normalized data on oligosaccharides, the DP3/DP4 ratio and DP5+DP6 sum, are shown in Figure 3.5. The variable DP3/DP4 mainly contribute to the first principal component, while fragments with DP ≥ 5 and sum DP5+DP6 were explained by the second principal component with DP5+DP6 being the most influential variable. In the scores plot a specific clustering is observed as the water extractable β-glucans of variety Karmosè, SW 28708 and SLU 7 were found in the upper left quarter of the plot. When comparing the loading plot and the scores plot interesting relationship are observed. DP3/DP4 ratio is higher in NaE extracts of SLU 7 and SW 28708 but also in the Res extract of SLU 7. On the other hand, it can be stated that oligosaccharides with DP5 and DP6 are higher in water extractable fractions and specifically the sum of DP5+DP6 explains the characteristics in variety SW 28708, Karmosè and SLU 7. Overall, similarities were found between variety SLU 7 and SW 28708. This may be due to the waxy feature of SW 28708 that is in general correlated to a higher DP3/DP4 ratio compared to non-waxy genotypes (Lazaridou & Biliaderis, 2007), which is a characteristic shared with SLU 7.

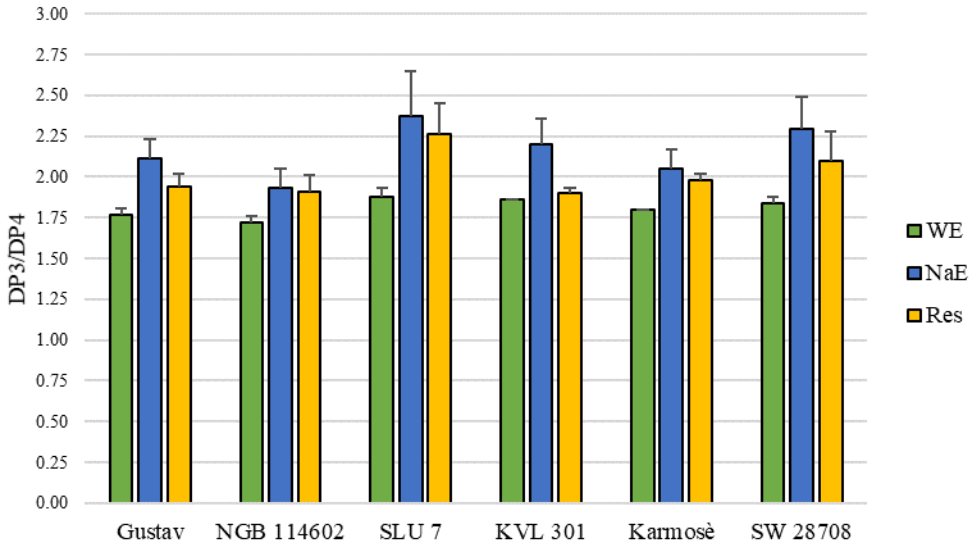

Figure 3.4. *Variation of DP3/DP4 ratio in barley cultivars, fractions WE, NaE, Res. Error bars refer to difference between replicates.*

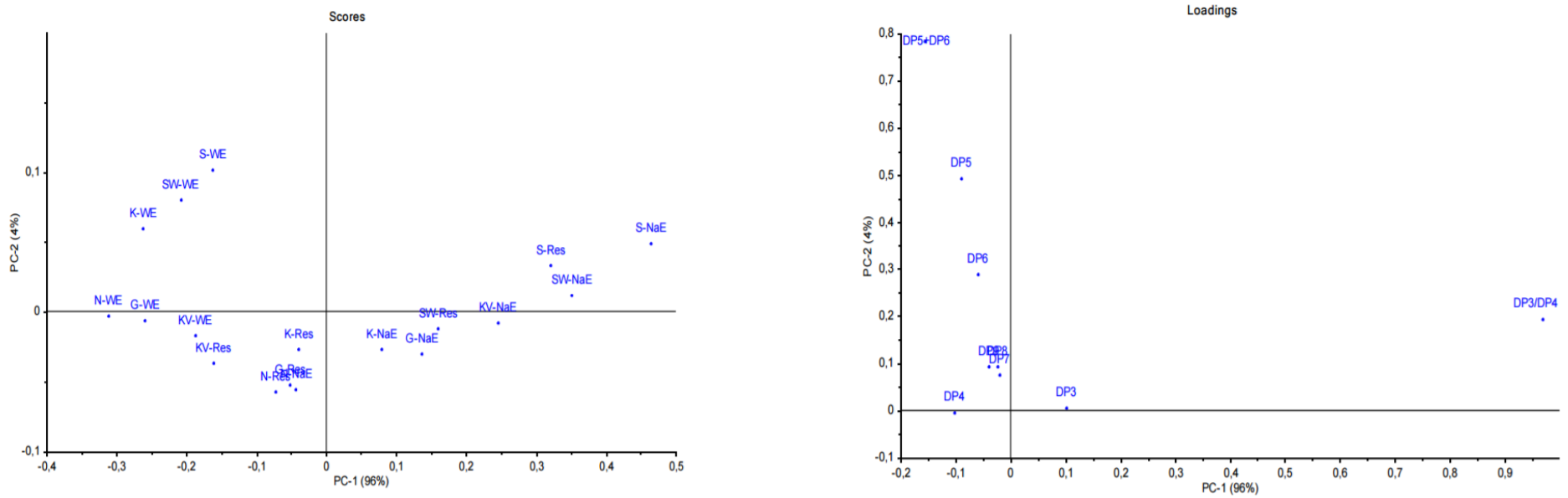

Figure 3.5. *PCA scores and loadings plots of β-glucan fragments in the fractions analysed (G=Gustav; S=SLU 7; N=NGB 114602; K=Karmosè; KV=KVL 301; SW=SW 28708; WE= water-extractable fraction; NaE= alkali-extractable fractions; Res= Residual fractions; DP 3-9= normalised values of β-glucan fragments occurrence with DP 3-9; DP5+DP6= sums of fragments with DP5 and DP6; DP3/DP4= ratio of trisaccharides to tetrasaccharides).*

β-Glucan structure of fractions released after incubation of β-glucan isolates with wheat extract

Effects of wheat β-glucanase were studied only in water-extractable and water-unextractable β-glucan fractions since previous results in this study showed minor differences between alkali-extractable and residual isolates. These fractions were incubated with wheat extract at 37 °C resembling the fermentation step of the baking process, which is critical for β-glucan molecular weight reduction (Åman *et al*., 2004; Andersson *et al*., 2004; Trogh *et al*., 2004). After centrifugation, the fraction named WU-WhE-Ins contained the β-glucan that remained insoluble. From the supernatant two fractions were obtained. The WE-WhE-Sol fraction is the soluble fraction, while the WU-WhE-Sol is the fraction containing the β-glucan released from the WU fraction, so complementary to the WU-WhE-Ins. β-Glucanase quantification in the wheat extract indicated an average value of 2.34 U/Kg dw (dry weight).

The study of oligosaccharides distribution (Table 3.3) after lichenase treatment and analysis with HPAEC-PAD revealed the structure of β-glucan in these fractions. Barley variety SLU 7 showed higher DP3/DP4 ratios in all the fractions compared to SW 28708 and Gustav. In the WE-WhE-Sol fractions, DP3/DP4 ratio is the highest compared to the other two β-glucan isolates. When the structure of β-glucan in the six barley varieties was studied, the ratio resulted the lowest in the water-soluble fraction (Table 3.2). These results showed that the effects of wheat β-glucanase on the soluble β-glucan involve the release of β-glucan with a more tighten structure and interestingly, this behaviour was in place for all the three cultivars analysed.

Table 3.3. *Oligosaccharides distribution in β-glucan isolates incubated with wheat extract (mean values of normalised data: Area DPx/ Area(DP3+DP4)). WU-WhE-Ins = insoluble fraction obtained after incubation of water unextractable β-glucan with wheat extract; WU-WhE-Sol= soluble fraction obtained after incubation of water unextractable β-glucan with wheat extract; WE-WhE-Sol= soluble fraction obtained after incubation of water extractable β-glucan with wheat extract.*

Sample		DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP3/DP4	DP5+DP6
Gustav	WU-WhE-Ins	0.71	0.29	0.03	0.01	0.003	0.004	0.012	2.49	0.05
	WU-WhE-Sol	0.71	0.29	0.03	0.01	0.003	0.004	0.009	2.42	0.05
	WE-WhE-Sol	0.73	0.27	0.03	0.01	0.002	0.002	0.006	2.69	0.04
SLU 7	WU-WhE-Ins	0.76	0.24	0.04	0.01	0.003	0.003	0.006	3.13	0.05
	WU-WhE-Sol	0.75	0.26	0.04	0.02	0.002	0.003	0.006	2.92	0.06
	WE-WhE-Sol	0.77	0.23	0.03	0.01	0.002	0.002	0.004	3.31	0.04
SW 28708	WU-WhE-Ins	0.76	0.24	0.03	0.01	0.002	0.002	0.008	3.12	0.04
	WU-WhE-Sol	0.75	0.25	0.03	0.01	0.002	0.002	0.007	3.06	0.04
	WE-WhE-Sol	0.76	0.24	0.03	0.02	0.002	0.002	0.005	3.19	0.04

When analysing data of fractions WU-Whe-Ins and WU-WhE-Sol, values are very similar in cultivar SW 28708 and Gustav leading to the conclusion that wheat β-glucanase acted releasing β-glucan from the insoluble fraction which kept the same DP3/DP4 ratio and hence the same structure.

On the other hand, in variety SLU 7 a more marked difference between WU-Whe-Ins and WU-WhE-Sol can be noted. It can be speculated that in this variety the wheat β-glucanase released a group of polysaccharides with a different structure.

These results can lead us to argue that, since the incubation conditions were the same for all the varieties, it seemed that wheat enzymes display a selective behaviour of hydrolysis towards specific β-glucan

population, possibly with lower DP3/DP4 ratio and so with a more accessible structure to enzymic action. Barley varieties SLU 7 results to be different specifically in the insoluble fractions as, after incubation with wheat extract, a population of β-glucan with a higher ratio was recovered, so the occurrence of β-glucan usable for hydrolysis by wheat enzymes results to be lower compared to the other cultivars

In figure 3.6 an overview of the variation between replicates is given. In SW 28708, the chromatographic data was less repeatable than for the other two varieties. Percent difference in WU-WhE-Ins, WE-WhE-Sol and WU-WhE-Sol were 10.3%, 8.5% and 7.5%, respectively, for cultivar SW 28708, higher than SLU 7 (3.8%, 0.3% and 1.7%) and Gustav (2.4%, 6.7% and 1.2%).

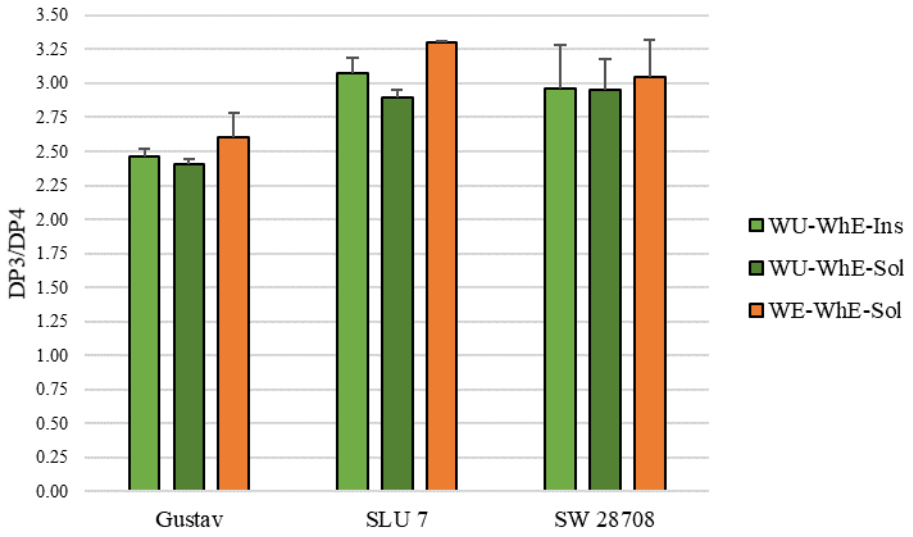

Figure 3.6. *Variation of DP3/DP4 ratio among cultivars in the fractions WU-WhE-Ins, WU-WhE-Sol and WE-WhE-Sol. Error bars refer to differences between replicates.*

β-Glucan quantification in the obtained fractions

In Table 3.4, β-glucan levels in fractions WE, NaE and Res are reported, together with the sum of β-glucan in the three fractions and the relative proportion. In addition, the percentage of recovery was calculated considering the total amount of β-glucan obtained from the extraction process and β-glucan level in the flour samples according to Djurle *et al*. (2016). WE extractable β-glucan had the highest proportion in all the cultivar considered, ranging between 51.7% and 82.2%. SLU 7 and SW 28708 displayed the highest proportion in WE fraction with 76.9% and 82.2% respectively. In Res fractions, β-glucan concentration was higher compared to the alkali extractable fractions for all cultivars. Overall, the recovery rate through the extraction process is 90.8% on average. It is noteworthy that this value in SLU 7 definetely does not stand out compared to the other cultivars. This may highlight that the structure rather than content or extractability of β-glucan is crucial for β-glucanase access and hydrolysis.

Table 3.4. *β-Glucan concentration (% of dw of barley flour, mean value ± sd) in fractions WE (water extractable), NaE (alkali-extractable) and Res (residual).*

Sample		β-glucan	Proportion[2](%)	Recover[3](%)
Gustav	WE	2.3 ± 0.08	69.7	91.7
	NaE	0.4 ± 0.00	12.1	
	Res	0.7 ± 0.03	21.2	
	Total[1]	*3.3*		
NGB 114602	WE	2.2 ± 0.03	66.7	89.2
	NaE	0.5 ± 0.03	15.2	
	Res	0.6 ± 0.01	18.2	
	Total	*3.3*		
SLU 7	WE	5.0 ± 0.12	76.9	86.7
	NaE	0.6 ± 0.01	9.3	
	Res	0.9 ± 0.02	13.8	
	Total	*6.5*		
Karmosè	WE	3.1 ± 0.18	68.9	84.9
	NaE	0.6 ± 0.06	13.3	
	Res	0.8 ± 0.01	17.8	
	Total	*4.5*		
KVL 301	WE	1.5 ± 0.02	51.7	96.7
	NaE	0.3 ± 0.01	10.4	
	Res	1.1 ± 0.07	37.9	
	Total	*2.9*		
SW 28708	WE	3.7 ± 0.13	82.2	95.7
	NaE	0.3 ± 0.00	6.7	
	Res	0.5 ± 0.04	11.1	
	Total	*4.5*		
Average	WE	3.0	69.4	90.8
	NaE	0.4	11.1	
	Res	0.8	20.0	

[1]Sum of β-glucan obtained in the three fractions.
[2]Value calculated on the total amount of β-glucan in the three fractions.
[3]Value calculated as the percentage on the total amount (%) of β-glucan in the flour according to Djurle *et al*. (2016).

β-Glucan quantification was performed on the water insoluble fraction (WU) and on the fraction "WU-WhE-Ins" with the aim to highlight the proportion of β-glucan released in the supernatant by the wheat enzymes, given by the difference calculated between the amount of β-glucan in the two fractions (Table 3.5). The results showed that from the insoluble population of β-glucan 52.3% was released in cultivar SLU 7, 45.3% in Gustav and 44.1% in SW 28708.

Table 3.5. *β-Glucan concentration (% of dw of flour, mean value ± sd) and solubilised fraction (% of the amount of β-glucan in WU isolates).*

Sample		β-glucan	Solubilised fraction (%)
Gustav	WU	1.0 ± 0.05	45.3
	WU-WhE-Ins	0.6 ± 0.04	
SLU 7	WU	1.4 ± 0.06	52.3
	WU-WhE-Ins	0.7 ± 0.01	
SW 28708	WU	0.7 ± 0.02	44.1
	WU-WhE-Ins	0.4 ± 0.02	

3.4. Conclusion

Analysis of extracted β-glucan with HPAEC-PAD in the six barley varieties showed a higher DP3/DP4 ratio in variety SLU 7 in all the three fractions obtained (water and alkali-extractable and residuals) compared to the other varieties. A higher DP3/DP4 ratio is related to a higher occurrence of (1→3)-

linkages and a higher number of interchain interactions and thus a more compact structure. There were also higher levels of fragments with DP5-DP6 in the water-soluble fractions of β-glucan.

Incubation of water-extractable and water-unextractable β-glucan with wheat extract and analysis with HPAEC-PAD after lichenase digestion, allowed evaluation of effects of wheat β-glucanase. β-Glucan quantification of water-unextractable fractions before and after incubation with wheat extract showed that a considerable proportion of β-glucan (on average 47%) was released by β-glucanase. Wheat β-glucanase activity on water unextractable β-glucan in SLU7 released polysaccharides with a more marked difference in DP3/DP4 ratio compared to the other analysed cultivars. From these results we argue that, while in variety SW 28708 and Gustav the released fragments preserved the same structure of the remaining β-glucan, in SLU 7 it seems that the enzyme acted more specifically on a selected β-glucan population probably more accessible to its action.

Taken together these findings may partially explain the reduced breakdown of β-glucan in SLU 7 during the baking process reported in Djurle *et al*. (2018). In fact, it can be speculated that not all the β-glucan in this variety is available to be hydrolysed by wheat enzymes, leading to a more conserved structure in the final product.

Further research is needed to confirm these data on a wider population of shrunken endosperm barleys. It is possible that this feature may, in fact, be correlated to a more unreachable structure of β-glucan by wheat enzymes, and thus being of great interest for the production of functional bread with a high content of β-glucan, that at the same time more efficiently preserves its molecular weight during processing.

3.5. References

AbuMweis S, Thandapilly SJ, Storsley J, & Ames N (2016). Effect of barley β-glucan on postprandial glycaemic response in the healthy human population: a meta-analysis of randomized controlled trials. *Journal of Functional Foods*, **27**: 329–342.

Ajithkumar A, Andersson R, Christerson T, & Åman P (2005). Amylose and β-glucan content of new waxy barleys. *Starch/Staerke*, **57**(6): 235–239.

Åman P, Rimsten L, & Andersson R (2004). Molecular weight distribution of β-glucan oat-based foods. *Cereal Chemistry*, **81**(3): 356–360.

Ames N, Blewett H, Storsley J, Thandapilly SJ, Zahradka P, & Taylor C (2015). A double-blind randomised controlled trial testing the effect of a barley product containing varying amounts and types of fibre on the postprandial glucose response of healthy volunteers. *British Journal of Nutrition*, **113**(9): 1373–1383.

Andersson AAM, Armö E, Grangeon E, Fredriksson H, Andersson R, & Åman P (2004). Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*, **40**(3): 195–204.

Andersson AAM, Lampi, AM, Nyström L, Piironen V, Ward J, Li L, Gebruers K, Courtin CM, Delcour JA, Boros D, Fras A, Dynkowska W, Rakszegi M, Bedő Z, Shewry PR, & Åman P (2008).

Phytochemical and fiber components in barley varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, **56**(21): 9777–9784.

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.

Böhm N, & Kulicke W (1999). Rheological studies of barley (1-3)(1-4)-β-glucan concentrated solution: mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research*, **315**: 302–311.

Clarke B, Liang R, Morell MK, & Bird AR (2008). Gene expression in a starch synthase IIa mutant of barley: changes in the level of gene transcription and grain composition. *Functional and Integrative Genomics*, **8**: 211–221.

Cui W, Wood PJ, Blackwell B, & Nikiforuk J (2000). Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan-comparison with other cereal β-D-glucans. *Carbohydrate Polymers*, **41**(3): 249–258.

De Paula R, Rabalski I, Messia MC, Abdel-Aal E-SM, & Marconi E (2017a). Effect of processing on phenolic acids composition and radical scavenging capacity of barley pasta. *Food Research International*, **102**: 136-143.

De Paula R, Abdel-Aal E-SM, Messia MC, Rabalski I, & Marconi E (2017b). Effect of processing on the β-glucan physicochemical properties in barley and semolina pasta. *Journal of Cereal Science*, **75**: 124-131.

Djurle S, Andersson AAM, & Andersson R (2016). Milling and extrusion of six barley varieties, effects on dietary fibre and starch content and composition. *Journal of Cereal Science*, **72**: 146–152.

Djurle S, Andersson AAM, & Andersson, R (2018). Effects of baking on dietary fibre, with emphasis on β-glucan and resistant starch, in barley breads. *Journal of Cereal Science*, **79**: 449-455.

EFSA (European Food Safety Authority) (2011). Scientific opinion on the substantiation of health claims related to β-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236,1299), increase in satiety leading to a reduction in energy intake (ID 851,852), reduction of post-prandial glycemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *Efsa Journal*, **9**(6): 2207.

FDA (Food and Drug Administration) (2017) Food labeling. Health claims: soluble dietary fiber from certain foods and risk of coronary heart disease (CHD). Code of Federal Regulation. Title 21, Chapter I. Sec. 101.81.

Fincher GB (1975). Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brewing*, **81**: 116–122.

Flander L, Salmenkallio-Marttila M, Suortti T, & Autio K (2007). Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT - Food Science and Technology*, **40**(5): 860–870.

Izydorczyk MS, & Dexter JE (2008). Barley β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products-a review. *Food Research International*, **41**(9): 850–868.

Izydorczyk MS, Macri LJ, & MacGregor AW (1998a). Structure and physicochemical properties of barley non-starch polysaccharides – I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, **35**: 249–258.

Izydorczyk MS, Macri LJ, & MacGregor AW (1998b). Structure and physicochemical properties of barley non-starch polysaccharides – II. Alkali extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, **35**: 259-269.

Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, & Rossnagel BG (2000). Variation in total and soluble β-glucan content in hulless barley: effects of thermal, physical, and enzymic treatments. *Journal of Agricultural and Food Chemistry*, **48**(4): 982–989.

Källman A (2013). *Barley starch: molecular structure and properties*. Acta Universitatis Agriculturae Sueciae. Doctoral thesis No. 2013:73. Retrieved from: http://pub.epsilon.slu.se/10845/.

Keogh GF, Cooper GJS, Mulvey TB, McArdle BH, Coles GD, Monro JA, & Poppitt SD (2003). Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *American Journal of Clinical Nutrition*, **78**(4): 711–718.

Lazaridou A, & Biliaderis CG (2007). Molecular aspects of cereal β-glucan functionality: physical properties, technological applications and physiological effects. *Journal of Cereal Science*, **46**(2): 101–118.

Lazaridou A, Chornick T, Biliaderis CG, & Izydorczyk MS (2008a). Composition and molecular structure of polysaccharides released from barley endosperm cell walls by sequential extraction with water, malt enzymes, and alkali. *Journal of Cereal Science*, **48**(2): 304–318.

Lazaridou A, Chornick T, Biliaderis CG, & Izydorczyk MS (2008b). Sequential solvent extraction and structural characterization of polysaccharides from the endosperm cell walls of barley grown in different environments. *Carbohydrate Polymers*, **73**(4): 621–639.

Ma J, Jiang Q, Wei L, Wang J, Chen G, Liu Y, Li W, Wei, Y, Liu C, & Zheng Y (2014). Characterization of shrunken endosperm mutants in barley. *Gene*, **539**(1): 15–20.

Messia MC, Candigliota T, De Arcangelis E, & Marconi E (2017). Arabinoxylan and β-glucan assessment in cereals. *Italian Journal of Food Science*, **29**: 112–122.

Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, Rahman S, Buleon A, Batey IL, & Li Z (2003). Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *The Plant Journal*, **34**: 173–185

Morrison WR, Milligan TP, & Azudin MN (1984). A relationship between the amylose and lipid contents of starches from diploid cereals. *Journal of Cereal Science*, **14**(3): 257–271.

Oscarsson M, Parkkonen T, Autio K, & Åman P (1997). Composition and microstructure of waxy, normal and high amylose barley samples. *Journal of Cereal Science*, **26**(2): 259–264.

Papageorgiou M, Lakhdara N, Lazaridou A, Biliaderis CG, & Izydorczyk MS (2005). Water extractable (1→3,1→4)-β-D-glucans from barley and oats: an intervarietal study on their structural features and rheological behaviour. *Journal of Cereal Science*, **42**(2): 213–224.

Rieder A, Balance S, & Knutsen SH (2015). Viscosity based quantification of endogenous β-glucanase activity in flour. *Carbohydrate Polymers*, **115**: 104–111.

Schulman AH, & Ahokas H (1990). A novel shrunken endosperm mutant of barley. *Physiologia Plantarum*, **78**: 583–589.

Schulman AH, Tomooka S, Suzuki A, Mylliirinen P, & Hizukuri S (1995). Structural analysis of starch from normal and shx (shrunken endosperm) barley (*Hordeum vulgare* L.). *Carbohydrate Research*, **275**: 361–369.

Tester RF, & Morrison WR (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, **67**(6): 551–557.

Tiwari U, & Cummins E (2011). Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels. *Nutrition*, **27**(10): 1008–1016.

Tiwari U, Cummins E, Sullivan P, Flaherty JO, Brunton N, & Gallagher E (2011). Probabilistic methodology for assessing changes in the level and molecular weight of barley β-glucan during bread baking. *Food Chemistry*, **124**(4): 1567–1576.

Tosh SM (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition*, **67**(4): 310–317.

Trogh I, Courtin CM, Andersson AAM, Aman P, Sorensen JF, & Delcour JA (2004). The combined use of hull-less barley flour and xylanase as a strategy for wheat / hull-less barley flour breads with increased arabinoxylan. *Journal of Cereal Science*, **40**: 257–267.

Varavinit S, Shobsngob S, Varanyanond W, Chinachoti P, & Naivikul O (2003). Effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of thai rice. *Starch/Staerke*, **55**(9): 410–415.

Wang Y, Ames NP, Tun HM, Tosh SM, Jones PJ, & Khafipour E (2016a). High molecular weight barley β-glucan alters gut microbiota toward reduced cardiovascular disease risk. *Frontiers in Microbiology*, **7**(Feb): 1–15.

Wang Y, Harding SV, Eck P, Thandapilly SJ, Gamel TH, Abdel-Aal, ESM, Crow GH, Tosh SM, Jones, PJ, & Ames NP (2016b). High-molecular-weight β-glucan decreases serum cholesterol differentially based on the CYP7A1 rs3808607 polymorphism in mildly hypercholesterolemic adults. *The Journal of Nutrition*, **146**(4): 720–727.

Wolever T, Susan TM, Alison GL, Jennie BM, Alison DM, Valerie H, Lamarche B, Thomson B, Duss, R, & Peter WJ (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans randomised clinical control trial. *The American Journal of Clinical Nutrition*, **92**(4): 723–732.

Wood PJ (2002). Relationships between solution properties of cereal β-glucans and physiological effects – a review. *Trends in Food Science and Technology*, **15**(6): 313–320.

Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, & Collins MW (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *The British Journal of Nutrition*, **72**(5): 731–743.

Xue Q, Wang L, Newman RK, Newman CW, & Graham H (1997). Influence of the hulless, waxy starch and short-awn genes on the composition of barleys. *Journal of Cereal Science*, **26**(2): 251–257.

Yanagisawa T, Domon E, Fujita M, Kiribuchi-Otobe C, & Takayama T (2006). Starch pasting properties and amylose content from 17 waxy barley lines amylose effect on starch pasting properties in waxy barley. *Cereal Chemistry*, **83**(4): 354–357.

4. Air classification as a valuable technique to obtain barley flours with a high nutritional value

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Abstract

Air classification is a dry technology that can be applied on previously micronized flours to obtained fractions enriched in bioactive compounds, specifically β-glucan. The aim of this study was to apply air classification to micronized barley flours derived specifically from waxy cultivars, in order to assess the efficiency of the process and obtain barley by-products of interest for the production of functional foods. Four waxy lines and one normal starch line underwent micronization and air classification obtaining a coarse and a fine fraction. Proximate composition, β-glucan and dietary fibre were assessed in each fraction. In cultivar CDC Alamo (waxy) and Shangrila (non-waxy) an enrichment of +84% in β-glucan content in the coarse fraction was registered compared to the micronized flour. Despite this, waxy cultivar CDC Fibar displayed the highest accumulation (13.5% fw) of these compounds in the coarse fraction due to the higher content in the starting material. In all the coarse fractions enrichment in total dietary fibre was also achieved. Results of this study highlight the potential of the air classification technology to be applied to previously micronised flours obtained from barley genotypes tha accumulate a higher quantity of β-glucan (high amylose and shrunken endosperm barleys). Moreover, the obtained barley by-products meet the requirements fixed by both the EFSA and FDA to bear a health claim on β-glucan.

Keywords: Air classification, barley, dietary fibre, β-glucan,

4.1. Introduction

Barley (*Hordeum vulgare* L.) is a cereal with valuable nutritional properties especially as it is an excellent source of (1→3)(1→4) mixed linkage β-glucan (hereafter referred as β-glucan). Positive impact of β-glucan on human health include the reduction of blood cholesterol and postprandial glycaemia (EFSA, 2011). Among the different genotypes of barleys, waxy barleys display a higher accumulation compared to normal starch barleys (Andersson *et al*., 2008; Izydorczyk *et al*., 2000), while hull-less barley contains a higher level of β-glucan compared to hulled barley (Holtekjølen *et al*., 2006; Izydorczyk *et al*., 2000).

For the formulation of functional food products bearing nutritional or health claims on β-glucan, it is therefore of interest for food industry to have ingredients enriched in these polysaccharides. Dry and wet technologies can be used to concentrate β-glucan. Wet processing can be applied to meal or flour and usually involve concentration of β-glucan using aqueous or semi-alcoholic solvent (Vasanthan & Temelli, 2008). On the other hand, dry fractionation techniques use separation of previously milled flour, according to particle size and density with the aim to obtain fractions enriched in bioactive compounds. In general, the use of dry techniques has advantages over wet fractionation. Firstly, there are risks of contamination of food products with residues of non-edible extractants. Secondly, avoiding chemicals use minimises the effects on physicochemical and functional properties of the compounds. Moreover, environmental impact of wet processes is notable due to the large amount of water consumption and energy for drying step (Berghout *et al*., 2015).

Air classification is a technological method of separation of particles according to their size and density. The initial material is divided in two products: coarse and fine. A classifying device is equipped with an interior space (Figure 4.1) where particles interact with air stream and separation occurs (Shapiro & Galperin 2005). For flours particles classification is achieved by the action of two opposite drag forces: air traction and centrifugal. By changing air-flow it is possible to obtain different yields of coarse and fine fraction.

For its properties air classification is used for a wide range of application with the aim to concentrate valuable compounds (Boye *et al*., 2010; Pelgrom *et al*., 2015a; Pelgrom *et al*., 2013; Pelgrom *et al*., 2015b; Schutyser *et al*., 2015; Wu & Doehlert, 2002). Application of air classification to barley flour yields fine fractions rich in starch and proteins (Vasanthan & Bhatty, 1995; Wu *et al*., 1994). On the other hand, bioactive compounds like β-glucan and phenolic compounds can be concentrated in the coarse fraction (Ferrante *et al*., 2001; Ferrari *et al*., 2009; Verardo *et al*, 2011a; Verardo *et al*., 2011b). The objective of this study was to evaluate efficiency of air classification specifically on waxy barley cultivars to produce β-glucan enriched fractions to emply for the production of functional foods.

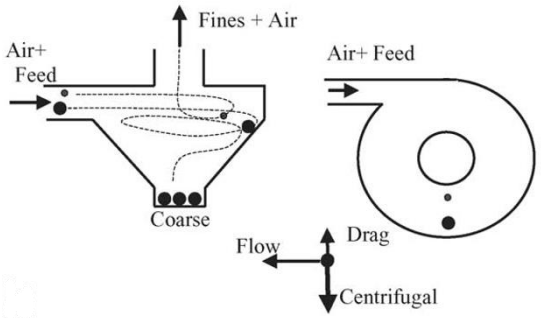

Figure 4.1. *Centrifugal-counterflow zone of air classification device (Shapiro & Galperin, 2005).*

4.2. Materials and methods

Samples

Thirteen barley cultivars were grown in experimental fields in Perugia (Italy) in randomised blocks in year 2013-2014 and 2014-2015. Five cultivars were selected for air classification process. Four of these cultivars are waxy, hulless (CDC Alamo, CDC Candle, CDC Fibar, CDC Rattan), while cultivar Shangrila was selected as a non-waxy, hulled variety. Samples belonging to each year were pooled together. Covered barley Shangrila was dehulled while waxy cultivars were slightly pearled since part of the kernels had still hulls attached. Samples were hammer milled and micronized (Separ microsystem mod. KMX-300). After that, micronized flour were air-classified into coarse fraction (CF) and fine fraction (FF) according to Verardo *et al*. (2011a). Samples were stored at +4 °C before analysis.

Analytical methods

Moisture determination. Samples were dried in a drying oven at 130 °C until constant weight was achieved.

Total ash content determination. Samples (1 g) were weighed in porcelain crucible. The sample was burnt with 100% ethanol and put in muffle at 525 °C until the residue resulted white. The crucibles were then weighed and total ash content calculated.

Lipid extraction. Samples were weighed in a thimble and fats are extracted with boiling petroleum ether for at least 6 hours in a Soxhlet apparatus. Dry fat remaining in previously dried and tared fat flask was weighed.

Protein content. Protein content (N x 6.25) was determined by combustion nitrogen analysis with Leco FP-528.

β-Glucan content. Samples were analysed with the Megazyme kit (Megazyme International Ireland Ltd) AACC method 32-23. Samples are incubated with lichenase, that released β-gluco-oligosaccharides, and centrifuged. An aliquot of supernatant is then incubated with β-glucosidase to release D-glucose. Colorimetric measurement was performed with glucose oxidase/peroxidase reagent against blank.

Soluble and insoluble dietary fibre content. Samples were analysed with Megazyme kit (AACC method 32-05.1). Dried samples underwent enzymatic digestion by α-amylase, protease and amyloglucosidase. Insoluble dietary fibre (IDF) was obtained by filtering samples and washing the residue with warm distilled water. Filtrate is precipitated with ethanol 95% to obtain soluble dietary fibre (SDF). Precipitate is filtered and then dried. Both SDF and IDF residues were corrected for protein, ash and blank. Total dietary fibre (TDF) was calculated as the sum of soluble and insoluble dietary fibre.

Statistical analysis. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test was performed using IBM SPSS Statistics Base (Version 23) and significant differences set at $p<0.05$.

4.3. Results and discussion

Results of β-glucan quantification in barley varieties is reported in Table 4.1. On average, β-glucan content (% as is basis) was 5.1% in year 2013-14 and 5.7% in year 2014-15. The average β-glucan level in non-waxy barleys is 4.8% in year 2013-14 and 4.9% in year 2014-15. The average β-glucan content in waxy varieties showed differences between the two years. In year 2013-14 is 5.9% while in year 2014-15 is 7.7%. Discrepancies found in β-glucan level in waxy varieties in the years considered, are mainly due to cultivar CDC Alamo which displayed a β-glucan content of 3.1% in the first year and then 8.1% in the following year. Overall, these results are in agreement with previous works (Baik & Ullrich, 2008; Holtekjølen *et al*., 2006; Izydorczyk *et al*., 2000).

Table 4.1. β-*Glucan concentration (% w/w, as is basis) in barley cultivars grown in year 2013-14 (A) and 2014-15 (B) (mean values ± sd).*

Cultivar	% β-glucan (A)	% β-glucan (B)
Alimini	5.2±0.01	5.9±0.11
Atomo	4.2±0.13	4.6±0.07
Calanque	3.6±0.13	3.2±0.04
Cometa	5.5±0.17	5.8±0.03
Ketos	4.1±0.04	4.2±0.16
Lutece	5.3±0.09	5.4±0.01
Martino	5.0±0.04	5.1±0.01
Mattina	4.6±0.00	5.1±0.23
CDC Alamo	3.1±0.06	8.1±0.23
CDC Candle	5.0±0.21	6.8±0.03
CDC Fibar	8.3±0.00	9.3±0.12
CDC Rattan	7.0±0.10	6.6±0.09
Shangrila	5.8±0.11	4.9±0.04

Air classification of previously micronized barley flour yielded a coarse fraction and a fine fraction corresponding, respectively, at the 40% and 60% of the weight of the flour, which was reported as a good combination between yield and β-glucan content (Ferrante *et al*., 2001). Proximate composition of micronized flour, coarse fraction and fine fraction is reported in Table 4.2.

As expected, β-glucan content increased in the coarse fraction of all the cultivar analysed (Figure 4.2). Highest increase resulted in cultivar CDC Alamo and Shangrila (+84%) while the lowest in cultivar

CDC Fibar (+48%). These results are not completely in agreement with Gómez-Caravaca *et al*. (2015) where enrichment of β-glucan was between 74% and 108% more than in whole meal. In coarse fraction β-glucan level reached values between 9% (Shangrila) and 13.5% "as is" basis (CDC Fibar) (Figure 4.3).

SDF resulted more concentrated in the coarse fractions varying between 8.4% fw (CDC candle) and 13.5% fw (Shangrila). With these regards, cultivars can be distinguished in two groups with different trends. In cultivar CDC Alamo, CDC Fibar and Shangrila coarse fraction registered +160% of soluble dietary fibre on average compared to the micronized flour, while in the remaining cultivars CDC Candle and CDC Rattan the increase was much more moderate (+50%). ANOVA reported no statistically significant differences among cultivars ($p>0.05$).

Enrichment in insoluble dietary fibre of the coarse fraction displayed lower values compared to SDF, on average between +40.7% and +106.4%. In cultivar CDC Fibar and Shangrila insoluble dietary fibre accumulated also in the fine fraction with +25.4% and +27.9% respectively. No statistically significant differences were found ($p>0.05$).

Taken all these data together, enhancement in total dietary fibre occurred in all the coarse fractions, with more important results obtained in CDC Fibar in which the content was more than doubled, but at the same time occurring also the fine fractions of cultivar CDC Fibar and Shangrila. In the coarse fractions the proportion between soluble and insoluble dietary fibre is modified, rendering the soluble fraction the major part of the total dietary fibre in cultivar CDC Fibar and Shangrila (Figure 4.4).

Protein content in the micronized fraction varied between 8.5% and 11.8%. Protein enrichment (% increase) in the coarse fraction of waxy barleys, varied between 6.8 and 16.7% compared to the micronized flour. These results are not in agreement with previous works, but it may depend on particle size, since in oat Wu & Doehlert (2002) found a trend of decrease in protein content from the particle size of 15 μm and then an increase from the particle size of 24 μm. On the other hand, in cultivar Shangrila, a depletion of protein resulted in the coarse fraction.

Table 4.2. *Proximate composition (% w/w, as is basis) and dietary fibre content of micronized flour (M), coarse fraction (CF) and fine fraction (FF,) mean values ± sd. Different letters in the same column and fraction indicate statistically significant differences (p<0.05)*

Cultivar		Moisture	Ash	Fats	Protein[1]	β-glucan	SDF*	IDF**	TDF***
CDC Alamo	M	9.7±0.01	1.97±0.057	2.6±0.02	10.9±0.01^{a}	7.0±0.05^{a}	4.2±0.1^{ab}	8.1±0.21^{a}	12.4±0.14^{a}
	CF	10.2±0.01	2.11±0.042	3.0±0.01	12.5±0.59^{A}	12.9±0.28^{A}	10.7±1.5^{A}	12.2±0.24^{A}	22.9±1.27^{A}
	FF	10.7±0.06	1.51±0.007	2.5±0.10	9.0±0.18$^{\alpha}$	2.2±0.08$^{\alpha}$	2.7±0.1$^{\alpha}$	3.8±0.15$^{\alpha}$	6.5±0.09$^{\alpha}$
CDC Candle	M	10.2±0.06	2.02±0.064	2.4±0.03	10.8±0.04^{a}	6.9±0.06^{a}	6.0±0.21^{ac}	7.7±0.15^{a}	13.7±0.36^{a}
	CF	10.4±0.15	2.89±0.021	2.9±0.08	12.6±0.03^{A}	11.8±0.13^{B}	8.4±0.11^{A}	13.7±0.01^{A}	22.1±0.11^{A}
	FF	10.6±0.09	1.53±0.049	2.3±0.05	8.7±0.17$^{\alpha}$	2.7±0.02$^{\alpha}$	3.1±0.07$^{\alpha\beta}$	3.7±0.04$^{\alpha}$	6.8±0.11$^{\alpha}$
CDC Fibar	M	9.7±0.08	2.03±0.028	2.9±0.20	11.8±0.09^{b}	9.1±0.16^{b}	5.1±0.6^{a}	6.7±0.42^{a}	11.8±0.22^{a}
	CF	10.0±0.12	2.14±0.030	2.8±0.08	12.6±0.17^{A}	13.5±0.16^{A}	13.3±2.3^{A}	12.1±0.70^{A}	25.4±1.65^{A}
	FF	9.8±0.12	1.78±0.040	3.0±0.01	10.5±0.04$^{\beta}$	4.8±0.01$^{\beta}$	4.4±0.1$^{\delta}$	8.4±0.14$^{\beta}$	12.8±0.06$^{\beta}$
CDC Rattan	M	9.7±0.08	1.96±0.035	2.5±0.06	10.6±0.07^{a}	7.2±0.14^{a}	5.5±0.39^{a}	7.8±0.81^{a}	13.3±1.20^{a}
	CF	10.3±0.05	2.83±0.064	2.9±0.04	12.3±0.25^{A}	11.5±0.07^{B}	8.8±0.01^{A}	16.1±2.47^{A}	25±2.48^{A}
	FF	10.5±0.09	1.43±0.042	3.2±0.07	8.4±0.04$^{\alpha}$	3.6±0.01$^{\gamma}$	2.1±0.17$^{\alpha\gamma}$	4.2±0.28$^{\alpha}$	6.3±0.45$^{\alpha}$
Shangrila	M	10.3±0.11	1.47±0.009	1.8±0.12	8.5±0.08^{c}	4.9±0.018^{c}	5.1±0.3^{a}	8.6±0.23^{b}	13.7±0.5^{a}
	CF	10.2±0.00	2.19±0.057	2.2±0.03	8.2±0.06^{B}	9.0±0.01^{C}	13.5±0.5^{A}	12.1±0.78^{A}	25.5±1.32^{A}
	FF	10.2±0.11	0.99±0.021	1.5±0.01	7.5±0.01$^{\gamma}$	2.8±0.10$^{\alpha}$	7.1±0.1$^{\varepsilon}$	11.0±0.04$^{\gamma}$	18.1±0.05$^{\gamma}$

[1]N *x* 6.25

*Soluble Dietary Fibre **Insoluble Dietary Fibre ***Total Dietary Fibre

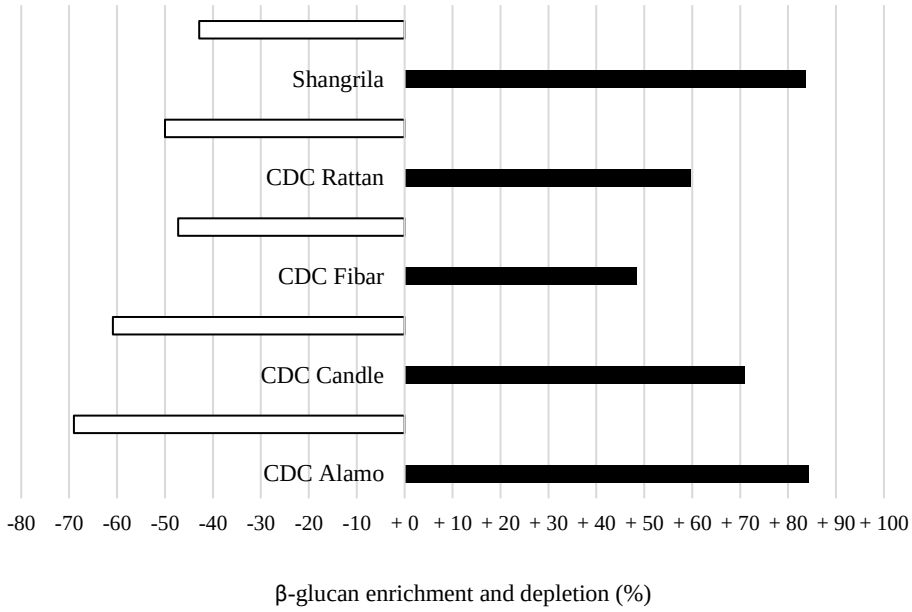

Figure 4.2. *β-Glucan enrichment and depletion in CF (black bars) and FF (white bars) respectively*.

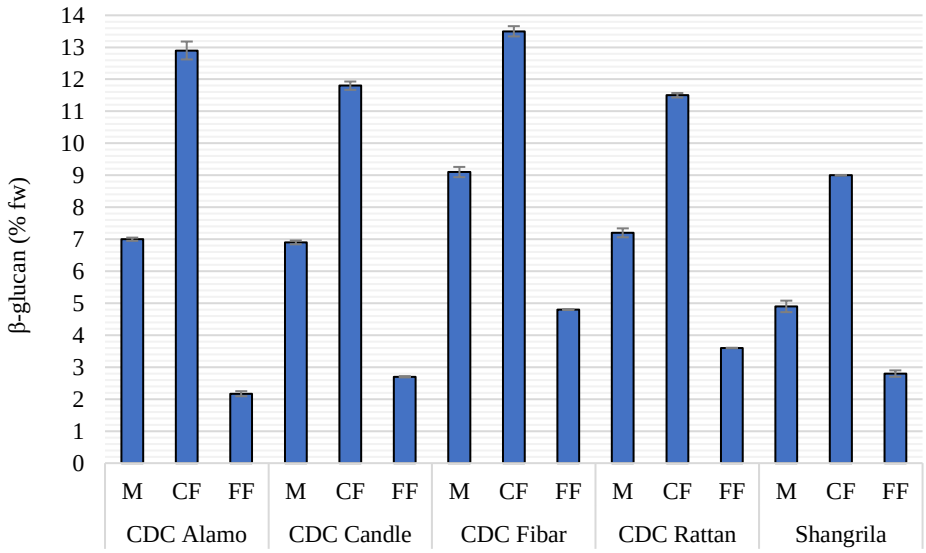

Figure 4.3. *β-Glucan distribution in the three fractions: micronized (M), coarse fraction (CF) and fine fraction (FF). Error bars refer to standard deviation*.

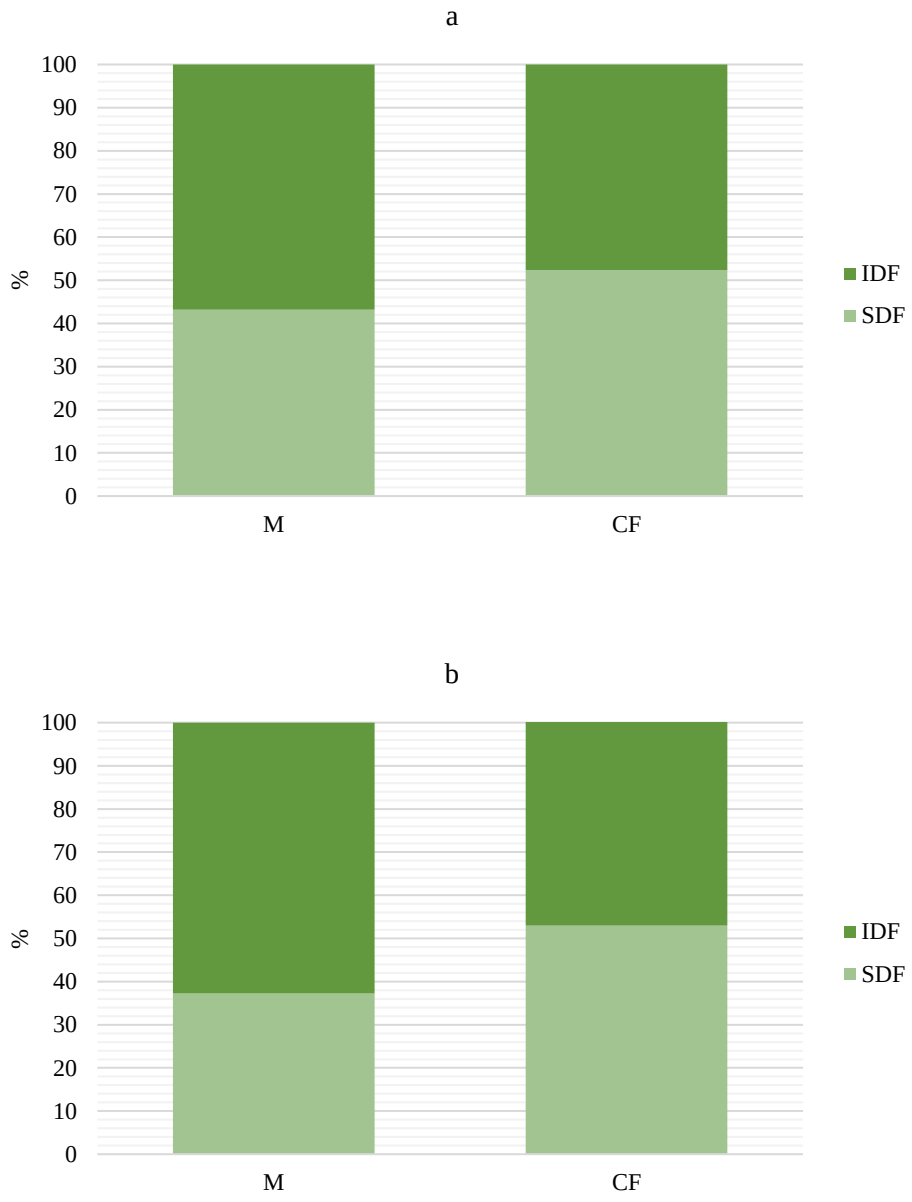

Figure 4.4. *Variation in soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) in micronized flour (M) and coarse fraction (CF) of cultivar CDC Fibar (a) and Shangrila* (b)

4.4. Conclusion

Results of this trial highlighted that air classification of waxy barley cultivars is a technique that can provide barley by-products with a consistent increase in β-glucan content. This technology can be successfully applied to other barley genotypes that display a high accumulation of β-glucan (high amylose and shrunken endosperm). In this study, enrichment in the coarse fraction compare to the micronised flour was the highest in the waxy barley CDC Alamo and in non-waxy barley Shangrila (+ 84%). Despite this, the starting high content of β-glucan in cultivar CDC Fibar lead to obtain a coarse

fraction with the highest content (13.5% fw) compared to the other cultivars. In all the coarse fractions obtained a concomitant increase in both soluble and insoluble dietary fibre was registered.

The waxy barley coarse fractions obtained represent valuable ingredients to be incorporated in cereal-based products (bread, pasta, breakfast cereals etc.) to enhance their nutritional value. This can lead the products to meet requirements given by Regulation 2006/1924/EC on nutritional claims on dietary fibre (3 g/100 g or 1.5 g/100 kcal of fibre, claim "*Source of fibres*"; 6 g/100 g or 3 g/100 kcal, claim "*High fibers*"). At the same time a health claim on β-glucan can be provided according to Regulation 2012/432/EU (1 g/quantified portion for the claim on cholesterol management and 4 g/ 30 g carbohydrates for the claim on postprandial glycaemia reduction). Enriched barley by-products obtained in this study also meet requirements given by the US Food and drug administration (0.75 g/portion).

4.5. References

Andersson AAM, Lampi, AM, Nyström L, Piironen V, Ward J, Li L, Gebruers K, Courtin CM, Delcour JA, Boros D, Fras A, Dynkowska W, Rakszegi M, Bedő Z, Shewry PR, & Åman P (2008). Phytochemical and fiber components in barley varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, **56**(21): 9777–9784.

Baik BK, & Ullrich SE (2008). Barley for food: characteristics, improvement, and renewed interest. *Journal of Cereal Science*, **48**(2): 233–242.

Berghout JAM, Pelgrom PJM, Schutyser MAI, Boom RM, & van der Goot AJ (2015). Sustainability assessment of oilseed fractionation processes: a case study on lupin seeds. *Journal of Food Engineering*, **150**: 117–124.

Boye J, Zare F, & Pletch A (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Research International*, **43**(2): 414–431.

EFSA (2011). Scientific opinion on the substantiation of health claims related to β-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236,1299), increase in satiety leading to a reduction in energy intake (ID 851,852), reduction of post-prandial glycemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *Efsa Journal* **9**(6): 2207

Ferrante MP, Falasca L, Irano M, Panfili G, Marconi E, & Cubadda E (2001). Produzione di sfarinati d'orzo arricchiti in β-glucan e tococromanoli mediante classificazione ad aria. Production of barley flours enriched in β-glucans and tocols by air classification. *Tecnica Molitoria*, Agosto: 876–883.

Ferrari B, Finocchiaro F, Stanca AM, & Gianinetti A (2009). Optimization of air classification for the production of β-glucan-enriched barley flours. *Journal of Cereal Science*, **50**(2): 152–158.

Gómez-Caravaca AM, Verardo V, Candigliota T, Marconi E, Segura-Carretero A, Fernandez-Gutierrez A, & Caboni MF (2015). Use of air classification technology as green process to produce functional barley flours naturally enriched of alkylresorcinols, β-glucans and phenolic compounds. *Food Research International*, **73**: 88–96.

Holtekjølen AK, Uhlen AK, Bråthen E, Sahlstrøm S, & Knutsen SH (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. *Food Chemistry*, **94**(3): 348–358.

Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, & Rossnagel BG (2000). Variation in total and soluble β-glucan content in hulless barley: effects of thermal, physical, and enzymic treatments. *Journal of Agricultural and Food Chemistry*, **48**(4): 982–989.

Pelgrom PJM, Boom RM, & Schutyser MAI (2015a). Method development to increase protein enrichment during dry fractionation of starch-rich legumes. *Food Bioprocessing Technology*, **8**: 1495–1502.

Pelgrom PJM, Vissers AM, Boom RM, & Schutyser MAI (2013). Dry fractionation for production of functional pea protein concentrates. *Food Research International*, **53**(1): 232–239.

Pelgrom PJM, Wang J, Boom RM, & Schutyser MAI (2015). Pre- and post-treatment enhance the protein enrichment from milling and air classification of legumes. *Journal of Food Engineering*, **155**: 53–61.

Regulation 2006/1924/EC of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.

Regulation 2012/432/EU of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

Schutyser MAI, Pelgrom PJM, van der Goot AJ, & Boom RM (2015). Dry fractionation for sustainable production of functional legume protein concentrates. *Trends in Food Science & Technology*, **45**(2): 327–335.

Shapiro M, & Galperin V (2005). Air classification of solid particles: a review. *Chemical Engineering and Processing*, **44**: 279–285.

Vasanthan T, & Bhatty RS (1995). Starch purification after pin milling and air classification of waxy, normal and high amylose barleys. *Cereal Chemistry*, **72**(4): 379–384.

Vasanthan T, & Temelli F (2008). Grain fractionation technologies for cereal β-glucan concentration. *Food Research International*, **41**(9): 876–881.

Verardo V, Gómez-Caravaca AM, Marconi E, & Caboni MF (2011a). Air classification of barley flours to produce phenolic enriched ingredients: comparative study among MEKC-UV, RP-HPLC-DAD-MS and spectrophotometric determinations. *LWT - Food Science and Technology*, **44**(7): 1555–1561.

Verardo V, Gomez-Caravaca AM, Messia MC, Marconi E, & Caboni MF (2011b). Development of functional spaghetti enriched in bioactive compounds using barley coarse fraction obtained by air classification. *Journal of Agricultural and Food Chemistry*, **59**: 9127–9134.

Wu YV, Stringfellow AC, & Inglett GE (1994). Protein- and β-glucan enriched fractions from high-protein, high β-glucan barleys by sieving and air classification. *Cereal Chemistry*, **71**(3): 220–223.

Wu YV, & Doehlert DC (2002). Enrichment of β-glucan in oat bran by fine grinding and air classification. *Lebensmittel-Wissenschaft & Technologie*, **33**: 30–33.

5. Conclusions and future perspectives

Over the last years food manufactures have taken action to supply food market with healthier choices mirroring increasing interest of consumers in products contributing to physical and mental well-being. In this context, the objective of current research on food science includes broadening the knowledge on positive impacts of compounds on human health and, at the same time, supporting industry to fulfil the aforementioned objectives.

Barley (*Hordeum vulgare* L.) is the most important crop grown worldwide after maize, wheat and rice with a total production of 144.3 million tonnes (year 2014-15). Barley is a crop that displays a great adaptability to very diverse environmental conditions. It matures earlier than wheat thus requiring a lower amount of water, while requiring less nitrogen fertilizers to reach the highest yeld compared to wheat. The larger amount of barley production worldwide is employed for feed use and malt production while only 4% is consumed "as is" in the diet of the population. Nevertheless, over the last decades this cereal has gained attention for its dietary fiber composition and phytochemicals content, beyond its higher environmental sustainability, leading to a growing interest in its use for food production to achieve important health outcomes, like lowering LDL cholesterol and regulating postprandial glycaemia.

The overall aim of this doctoral thesis was to characterise different barley cultivars focusing on bioactive compounds fructan and β-glucan to contribute to the fully characterization of this cereal and enhance its inclusion in cereal-based functional foods production.

The study of the evolution of the content of fructan and β-glucan in seven developing barley cultivars was performed in order to evaluate immature barley flour as an innovative functional ingredient. The peak of fructan concentration expressed on a fw basis was recorded between 6 and 17 DAA. Barley cultivars Scarlett and Dingo showed the highest fructan accumulation being 9.5 g/100 g flour (fw) and 6.6 g/100 g flour respectively. At maturation, fructan content varies between 1.1 and 1.6 g/100 g flour. Data expressed on a per kernel basis showed a peak between 10 and 17 DAA with cultivars Scarlett and Dingo reporting values of 3.1 and 2.3 mg. At the peak of fructan content β-glucan content is below 1 g/100 g flour for all the cultivars analysed. Total starch tends to increase linearly in all the cultivars. Linear accumulation of proteins occurred when considering data on a per kernel basis. Results of this study highlight the potential of immature barley flour to produce prebiotic cereal-based foods. Among the cultivars considered Scarlett stands out for the notable content of fructan in immature kernels.

The second section of this PhD thesis investigated molecular features of β-glucan in six barley varieties with different starch and dietary fibre composition and effects of wheat β-glucanase correlated to baking process. Analysis of oligosaccharide distribution in β-glucan isolates (water-extractable, alkali extractable and residual) with HPAEC-PAD (high performance anion exchange chromatography-pulsed amperometric detection) after lichenase digestion in barley varieties revealed a higher DP3/DP4 ratio in cultivar SLU 7 (shrunken endosperm barley) compared to the other varieties. A higher ratio trisaccharides to tetrasaccharides is related to a higher number of interchain interactions and thus a more

tighten structure of β-glucan chain. Incubation of water-extractable and water unextractable β-glucan fractions with wheat extract was followed by lichenase digestion of pellets obtained and analysis with HPAEC-PAD to evaluate effects of wheat β-glucanase on the structure. On average, 47% of β-glucan was released from the water-unextractable fraction. Activity of wheat enzymes on water unextractable fractions in SLU 7 lead to release a β-glucan population with a more defined difference in DP3/DP4 ratio compared to the other cultivars. From these results, we argue that while in cultivar Gustav and SW 28708 the remaining and released fragments after β-glucanase action maintained the same structure, in SLU 7 the enzymes acted only on a specific β-glucan population with a lower DP3/DP4 ratio and thus more accessible to hydrolysis by wheat enzymes. These data need to be confirmed on other shrunken endosperm varieties in order to evaluate if this characteristic may be correlated to a more resistant structure of β-glucan degradation by wheat β-glucanase. In fact, these findings may be of great interest for the production of functional bread in which β-glucan molecular weight is more preserved over the baking process.

The last part of this work concerned the application of air classification technology on previously micronized waxy barley flours to evaluate enrichment in β-glucan in coarse fractions. The highest efficiency of the process was obtained in cultivar CDC Alamo (waxy) and Shangrila (non-waxy) (+84% enrichment) but the highest content of β-glucan resulted in the coarse fraction of waxy cultivar CDC Fibar (13.5% fw) due to the normal higher accumulation of these compounds in the grain. Air classification is thus a technology that can be applied to other barley genotypes that accumulate a high quantity of β-glucan (high amylose and shrunken endosperm) to produce flour with a notable content of these bioactive compounds. Overall, the obtained fractions represent ingredients with a high nutritional value that can be incorporated in cereal-based products (pasta, bread, breakfast cereals etc.) in order to bear nutritional claims on dietary fibre (according to Regulation 2006/1924/EC) and health claims on β-glucan according to Regulation 2012/432/EU and according to parameters given by the US Food and Drug Administration.

Acknowledgements

I would like to thank Prof. Emanuele Marconi for being my supervisor, for having guided me through the work of these three years. Being part of his research team at University of Molise has been a great self-development experience. Thanks to Prof. Mauro Moresi for being my Tutor and co-supervising my work. Thanks to Prof. Maria Cristina Messia for supporting and helping me during these years. Your advice and suggestions have been precious to improve and develop my skills in the research field. Thanks to Dr. Luisa Falasca for your help and for your constant supervision at the beginning of the lab work that allowed me to learn very quickly.

I would like to thank the research team at the Department of Molecular Sciences of the Swedish University of Agricultural Sciences in Uppsala (Sweden) for the opportunity to develop part of my doctoral thesis there. In particular, thanks to Prof. Roger Andersson; I have learnt so much under your guidance, thanks for teaching me new methods and technique for β-glucan analysis and being co-author of the manuscript of chapter 3 of this thesis. Thanks also to Susanne Djurle and Annica Andersson for contributing to the development of the work.

Thanks to my wonderful colleagues Manuela Oriente, Serena Niro and Martina Angelicola for the great support, for making the days in lab also fun and enjoyable.

Thanks to all my friends in Campobasso, Rome and Uppsala that filled my free time with joy and pleasant time.

Finally, thanks to my Family for everything.

List of publications

De Arcangelis E (2017) Fructan and β-glucan accumulation in barley kernel and development of flours enriched in bioactive compounds. In Proceedings Book. *XXIInd Workshop on the developments of the Italian PhD research on food science, technology and biotechnology*. *Bolzano, September 20th-22nd, 2017,* pp 273-277.

Messia MC, Candigliota T, De Arcangelis E, Marconi E (2017) Arabinoxylans and β-glucan assessment in cereals. *Italian Journal of Food Science*, **29:** 112-122.

De Arcangelis E (2016) Assessment of fructan and β-glucan during barley kernel maturation and development of flours enriched in bioactive compounds. In Proceedings book. 21st Workshop on the *Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Naples Federico II, Portici, September 14th-16th, 2016*, pp 139-140.

De Arcangelis E (2015) Mature and immature barley kernels: characterization of bioactive compounds and development of innovative functional foods. In Proceedings book. *XXth Workshop on the developments of the Italian PhD research on food science, technology and biotechnology*. *Perugia, September 23rd-25th*, 2015, pp 44-45.