Sveriges lantbruksuniversitet
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Abstract

In cereal industry, when cereal grains are milled to extract the starchy endosperm for white flour, large quantities of bran are classed as by-products and end up in animal feed. Bran, depending on the extraction rate, comprises of small amount of germ and endosperm along with aleurone layer, nucellar epidermis, seed coat and fruit coat. Bran is rich in dietary fibre, phytochemicals like phytic acid, phenolic components, lignans and flavonoids which is important in healthy human diet. To make use of all the nutrients present in this fraction of the cereal by-product could be a new value added product.

This thesis aims at investigating if twin screw extrusion cooking of wheat (n=18) and rye bran (n=18) has an effect on β-glucan and fructan content. The total β-glucan content and fructan content was analysed using enzymatic assay kits available from Megazyme ltd. Then using HPSEC and Calcoflour detection method, molecular weight analysis using area under the curve of the peaks was done along with extractable β-glucan content analysis. Un-extruded reference for each cereal grain was used in all the analyses. The results from the different analyses were tested for significance with every varying extrusion parameter. The three varying extrusion parameters were temperature, moisture content and screw speed.

Total β-glucan content in both extruded wheat and rye bran samples had an slight increase. In case of extruded wheat, the average overall increase was about 17% compared to un-extruded wheat bran. In extruded rye bran, total β-glucan content was 4% higher than its un-extruded reference. Of all extrusion parameters, temperature had the most influence on total β-glucan content in extruded wheat and rye. Fructan content in both extruded wheat and rye samples showed an increase compared to their references. In case of extruded wheat, there was a 9% increase from reference and 8% in case of extruded rye. Except for varying moisture in rye samples, no extrusion parameter had a statistical difference in the fructan content in both cereals.

Molecular weight analysis of β-glucan in extruded wheat revealed that there was a 2.8 fold increase in extruded samples compared to the reference with statistical differences recorded with varying temperature and screw speeds (90% confidence interval). In extruded rye bran samples, the average molecular weight decreased by 7% with no statistical difference among varying extrusion parameters.

The extractable β-glucan content in extruded wheat bran samples showed an average increase of 16% from reference and an 8% decrease in case of extruded rye samples. While there was a significant difference in extractable β-glucan content with varying moisture content for extruded wheat samples, changing speeds seemed to influence the extractable β-glucan content in extruded rye samples.

While individual extrusion parameters seemed to have an effect on β-glucan and fructan content, it is still unclear so as to what combination is best for improving the functionality of β-glucan (molecular weight) and fructan.

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1.0 INTRODUCTION

Profound changes are visible in modern day diet that came with introduction of agriculture and animal husbandry since the past 10,000 years. These changes, when seen on the evolutionary timescale are what humans are still getting adjusted to. In particular, changes in staple food and food processing techniques introduced during the industrial periods have fundamentally altered many nutritional characteristics such as glycemic load, macronutrient composition, micronutrient density, acid-base balance, sodium potassium ratio and dietary fibre content (Cordain et al., 2005). Healthy lifestyle is a combination of diet, genetic predisposition and exercising regime in an individual. Nutrition comes from macronutrients and micronutrients (DellaPenna, 1999, Johnstone et al., 1996). Great importance is now focussed on dietary fibre as a method to improve healthy eating. Most food processing methods aim at extracting the most of macronutrients from the endosperm of cereal grains which is the primary site for storage of carbohydrates (Lopes and Larkins, 1993). However, the bran in cereals consists of dietary fibre and phytochemicals like phytic acid, phenolic components, lignans and flavonoids. These components have shown to possibly protect against cancers in the colon and breast (Ferguson and Harris, 1999).

When cereal grains are milled into white flour, large quantities of bran is produced which is classified as a by-product and used in animal feed rather than for human consumption (Van Craeyveld et al., 2009, Hemery et al., 2007). Taste could be a reason hindering the use of bran in human consumption as the there are bitter tasting compounds present (Heiniö et al., 2008, Jensen et al., 2011). Of the dietary fibre components, β-glucan and fructan are of great interest due to their health promoting effects. Mixed linkage(1-3)(1-4)-β-D-glucan give viscosity to oat and barley products and helps in reducing the amplitude of postprandial glycemic and insulinemic response (Wood, 2007). Other components of dietary fibre are polymers of fructose called fructan and fructo-oligosaccharides that are potential “functional food ingredients” which target colonic microflora, the physiology of the gastrointestines, immunity, bioavailability of minerals, metabolism of lipids and carcinogenesis of the colonic tissue. Positive health implications also include better gut health, reducing the risk of non insulin dependent diabetes, obesity and osteoporosis (Roberfroid, 1999, Kaur and Gupta, 2002).

While food processing such as milling may decrease the grain layers rich in dietary fibre, especially while producing white flour. This fraction of bran gets classed as animal feed. Lantmannen aims at developing new products with bran that can be incorporated in a healthy diet through extrusion. Extrusion is also used to improve the eating quality of the fraction. Extrusion cooking of Barley flour has shown to increase both soluble dietary fibre and Total dietary fibre while increase in insoluble dietary fibre was variety dependent (Vasanthan et al., 2002). Bran can be used as a replacement for flour, fat and sugar and can also serve as emulsifiers in food retaining oil and water (Elleuch et al., 2011). From the industrial perspective, for their additional treatment and resources put into this by-product, products with higher commercial value have to be obtained(Rose and Inglett, 2010).

2.0 OBJECTIVE

This project work is a part of a conglomerate of projects organised by Lantmannen and the department of Food science at the Swedish University of Agricultural Sciences (SLU) under the name- KLIFUNK ("New technologies to improve the properties of bran"). The idea behind this is to increase the use of wheat and rye bran in food products by use of processing techniques and product development. This thesis aims at finding how extrusion cooking affects the β-glucan content, fructan content and molecular weight of β-glucan and its extractability in 18 different sample of wheat and rye bran. The effect of different extrusion parameters such as moisture, temperature and screw speed will also be studied.

3.0 LITERATURE REVIEW

3.1 Wheat and rye

Both cereals in this project belong to the grass family, *Gramineae* with the fruit being commonly referred to as "kernel" or "grain" which is the *caryopsis* (Buttrose, 1963). Both *caryopsis* consists of the same parts- Fruit coat and seed consisting the germ, endosperm, nucellar epidermis and seed coat. Fruit coat (*pericarp*) surrounding the seed is made up of many layers and is connected to the seed coat (*testa*) which is conjoined to the nucellar epidermis. This nucellar epidermis if bound to the aleurone layer that completely surrounds the endosperm and germ (*kernel*). Botanical classifications associates aleurone layer to endosperm but while milling, it is removed with the bran fraction. Bran is made of man layers that makes it a complex in both structure and composition (Hemery et al., 2011). The fraction of the grain that actually constitutes bran is about 11% (Rose and Inglett, 2010).

3.2 β-Glucan

While the content of β-glucan ranges close to 3 % in dry matter (DM) in wheat bran, it is about 5% (DM) in rye bran. The cellulose content however ranges close to 12% (DM) in wheat bran in comparison to 6% (DM) in rye bran (Kamal-Eldin et al., 2009, Van Craeyveld et al., 2009).
The chemical structure of cereal β-glucan is a linear homopolymer of β-D-glucopyranosyl residues linked mostly by 2-3 consecutive (1-4) linkages (approx. 70%) with interruption by a single (1-3) linkage (approx. 30%) as shown in Figure 1

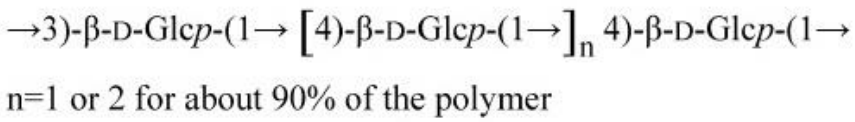

Figure 1. General structure of cereal mixed linkage (1-3)(1-4)-β-D-glucan (Rakha, 2011)

The evidence so far suggests that the chain has no consequtive (1-3) linkages in the linear structure (Izydorczyk, 2010). Structure of β-glucan after enzymatic hydrolysis revealed that over 90% of the molecule consists of 3-O-β-D- cellobiosyl-D- glucose (trisaccharide unit) and 3-O-β-D-cellotriosyl-D-glucose (tetrasaccharide unit) (Cui and Wang, 2009). The remainder of the molecule contains longer sequences with 5-20 consecutive (1-4)- linked β-D-glucopyranosyl residues (Izydorczyk et al., 1998b, Izydorczyk et al., 1998a). Different cereal grains have different oligomers . The water soluble β-glucan in barley has oligomers with degree of polymerisation (DP) upto 13 with DP5, 6 and 9 being predominant among those larger than DP4. (Izydorczyk et al., 1998b). For its alkali-extractable counterpart, the largest oligomer is DP 20, while DP9 is the most predominant among those greater than DP4 (Izydorczyk et al., 1998a). The low solubility of long chain cellulose-like sequences of (1-4)-linked β-D-glucan has to do with the strong internal and external hydrogen bonding of the structure (Lazaridou and Biliaderis, 2007, Izydorczyk et al., 1998b). What gives the grain its characteristic is the ratio of trisaccharides to tetrasaccharides. The highest ratio is found in wheat (3.7-4.8), second highest is in barley and rye (2.7-3.6) and lowest in oats (1.7-2.4)(Wood, 2010). The functionality of β-glucan, including its solubility, is determined by the molar ratio of DP3 to DP4 (Cui et al., 2000). Higher proportions of DP3 oligomers implies higher consecutive cellotriosyl units resulting in a more regular, less soluble structure(Wood, 2010, Izydorczyk and Dexter, 2008). This molar ratio is also dependent on the tissue the β-glucan is sourced from. For example, β-glucan from outer

grain layers like pericarp and aleurone show greater DP3 to DP4 ratio when compared to that from starchy endosperm cell walls (Izydorczyk and Dexter, 2008). This property of β-glucan makes the extractability very method dependent. About 70-75% of β-glucan in oats is extractable by using hot water but only 10-20% in case of rye (Wood, 2010). Due the same reason, we see a wide array in molecular weights for different cereal grains, with the trend being oats>barley>rye>wheat (Cui and Wang, 2009). Reported molecular weights of these cereals were in range of 0.65-31.0 x 10^5 g/mol in oats, 0.31-27.0 x 10^5 g/mol in barley, 0.21-11.0 x10^5 g/mol in rye and 2.1-4.9 x10^5 g/mol in wheat (Lazaridou and Biliaderis, 2007). The variation in the molecular weights of β-glucan amongst different cultivars of the same cereal is also governed by environmental factors (Ajithkumar et al., 2005). Result of these molecular weights in scientific work is dependent upon extraction method and also the determination method used. In some cases, due to lack of inactivation of endogenous β-glucanases or high pH at high temperature, β-glucan has been significantly degraded during extraction (Wood, 2010, Lazaridou and Biliaderis, 2007).

3.3 Fructan

The role of fructan and fructo-oligosaccharides in plants is to mainly reduce the negative impact of physical and chemical factors in a specific environment or the phenomenon commonly referred to as abiotic stress (Livingston et al., 2009, Valluru and Van den Ende, 2008, Vijn and Smeekens, 1999). Certain abiotic stresses that fructan provides protection against are freeze injury and drought. Specifically in dicots, fructan serves as a long term energy reserve of carbohydrates in underground organs, whereas, in monocots, its stored in roots, stems and leaves (Valluru and Van den Ende, 2008). Fructan is found in many species of bacteria and flowering plants and in certain algae and liverworts. About 15% of the total angiosperm flora contain fructan; being distributed in roots, seeds, stems and leaves as a carbohydrate reserve. Members of the grass family *Poaceae*, which includes cereals are of major economic importance (Hendry, 1993). Rye has fructan content of up to 6.4% which is highest amongst all other cereals. Industrial sources of fructan are tubers of Jerusalem artichoke (*Helianthus tuberosus*) and roots of chicory plant (*Chicorium intybus*) (Boskov Hansen et al., 2002).
Chemically, plant fructan is a polymer of β-D-fructofuranosyl residues with or without a terminal glucose residue (Valluru and Van den Ende, 2008, Vijn and Smeekens, 1999). Figure 2 shows the general structure of fructan.

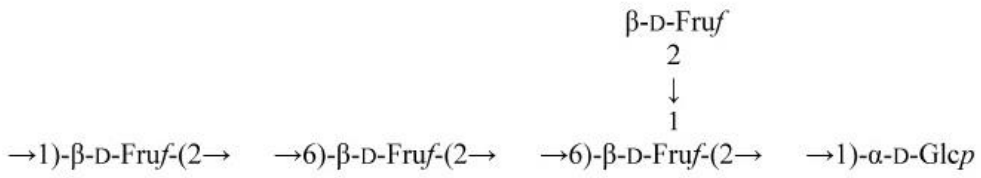

Figure 2. General structure of fructan (Rakha, 2011)

There is great structural diversity with varying chain lengths with degree of polymerisation (DP) ranging anywhere from three to hundreds of fructose residues (Ritsema and Smeekens, 2003). Generally, fructan including fructo-oligosaccharides in plants has DP in range of 30-50 and is divided to 5 categories (Vijn and Smeekens, 1999).

- Inulin- type fructan with a linear (2-1)-linked β-D-fructosyl residues attached to fructosyl part of sucrose. Commonly seen in dicots like *Chicorium intybus* and *Helianthus tuberosus.* The shortest of them is a trisaccharide called isokestose (1-kestose)
- Levan or Phlein has the short 6-Kestose molecule. These have a linear structure made of (2-6)-linked β-D-fructosyl residues. Commonly seen in grasses like *Dactylis glomerata* and *Poa secunda (Bonnett et al., 1997, Wei et al., 2002)*
- Mixed levan or graminan-type fructan is made up of (2-6)-linked β-D-fructosyl residues chain with (2-1)-linked branches attached to the fructosyl portion of sucrose. Mixed levan is mostly found in the plant species belonging to the order Poales, whereas, graminan type is most typical to member of the Poaceae family (e.g. wheat and barley) (Bonnett et al., 1997)
- Levan neoseries fructan is a polymer of (2-6)-linked β-D-fructosyl chains attached to first and sixth carbon atom of the glucose residues. This structure has fructose chains on either sides of the glucose residue. This is found commonly in plants species belonging to the order Poales, like oats (Livingston et al., 1993)
- Inulin neoseries have (2-1)-linked β-D-fructosyl chains attached to first and sixth carbon atom of the glucose portion of sucrose. In this category, neoketose is the smallest molecule. Plants

belonging to the family *Liliaceae* contain this type of fructan, e.g. onion (*Allium cepa*) and asparagus (*Asparagus officinalis*) (Shiomi, 1989)

Fructo-oligosaccharides (DP3-9) and fructan are soluble dietary fibre with prebiotic properties (Gibson et al., 2004). Fructan is known to stimulate the growth of probiotics as it is resistant to digestion and absorption in the upper gut and is fermentable by the microflora in the intestines. It also has a positive influence over the immune system by altering the structure and composition of mucosa and microflora, raising guard against infection (Lomax and Calder, 2009, Roberfroid, 2007). Fructans also have a role in enhancing the absorption of calcium, reducing the risk of osteoporosis (Abrams et al., 2005). Also, fructans have been associated in the reduction of risk factor of colonic cancer in animal models (Roberfroid, 2007, Hughes and Rowland, 2001, Alexiou and Franck, 2008)

3.4 Extrusion cooking and dietary fibre

Extrusion cooking was first introduced in the food and feed processing during the 1950s and since then, the systems have evolved and grown in popularity that are now most efficient and flexible. Extrusion cooking method is mostly used in cereal and protein processing industry when developing new flavour generation, encapsulation and sterilisation (Hernandez-Izquierdo and Krochta, 2008). Thermoplastic extrusion is considered a high temperature, short time (HTST) process which permits production of a wide array of food and feed products (Camire et al., 1990, Chang et al., 2001, El-Dash et al., 1983).

Depending on the raw materials and the desired characteristics of the final product, extruders operate with low, medium or high shear; but, in the case of high shear, thermoplastic extruders are used. For example, processed meat products and pasta are produced with low shear (cold extrusion); imitation meat and pet foods are produced with medium shear; and expanded snack products, breakfast cereals and textured vegetable proteins are made with high shear (thermoplastic extrusion)(Akdogan, 1999).

Two governing factors influencing the characteristics of extruded products are raw material characteristics and operational conditions of the extruder. The characteristics of the raw material can be type of material, moisture content, physical state, chemical composition (type of starch, proteins, fats and sugars) and pH of the material. Operational factors are temperature, pressure, die diameter and shear force (Tolstoguzov, 1993, Harper and Clark, 1979). Research has shown that extrusion cooking has considerable influence on structural characteristics and physicochemical properties of dietary fibre. The main effect is redistribution of insoluble fibre to soluble fibre (Camire et al., 1990, Guillon et al., 1992, Larrea et al., 2005). The reason could be due to rupture of covalent and non-covalent bonds between carbohydrates and proteins associated with the fibre, leading to smaller molecular fragments that are more soluble (Fornal et al., 1987, Lai and Kokini, 1991).

Milling improves extractability of β-glucan by reducing particle size but, food processing methods in general has been shown to affect β-glucan content (Tosh et al., 2010, Andersson et al., 2004). Hydrothermal treatments may also change their capacity to form viscous solutions, thereby affecting its extractability (Zhang et al., 1998). β-glucan is also prone to enzymatic depolymerisation and chemical hydrolysis. Their molecular weight ranges from 3×10^6 and higher in certain oat variety (Wood et al., 1991). For a range of processed products, molecular weights have been seen to fluctuate from 0.6×10^6 to 2.9×10^6 (Beer et al., 1997, Wood et al., 1991).

4.0 MATERIAL AND METHODS

4.1 Material

Table 1. Extrusion parameters of wheat and rye

Variable	Level
Bran type	Wheat (n=18) Rye (n=18)
Moisture content	24% (For wheat only) (n=9) 30% (For rye and wheat) (n=9 for each) 36% (For rye only) (n=9)
Temperature (Celsius)	90 110 130
Screw speed (rpm)	200 300 400

Eighteen samples of extruded wheat and extruded rye and two of un-extruded wheat and rye bran were used in this study (n=38). The samples were obtained in pelletized form and were milled to obtain a homogenous hygroscopic mix. The samples had been extruded in combinations of varying moisture content (two variations), screw speed (three variations) and temperature (three variations) as shown in Table 1. A representative sample of each of the milled fraction sample *(Retsch, Haan, Germany)* was taken in duplicates for this analysis. The results are determined on a dry matter basis, determined by oven drying the samples at 105 celsius for 16 hours (*AACC method 44-15A, 2000*).
All samples were analysed in duplicates for β-glucan content, fructan content and molecular weight analysis of water extractable β-glucans in specific. All data was compared to un-extruded wheat and rye as a reference.

4.2 Methods

4.2.1 Mixed linkage β-glucan analysis

The mixed linkage β-glucan analysis was done using an enzymatic method (McCleary and Codd, 1991) available in kits from Megazyme limited (*AACC Method 32-23*). The results were adjusted for the sugar added prior to extrusion to facilitate gelling of pellets.

Hydrated samples were suspended in an alkaline buffer solution (pH 6.5), incubated with purified lichenase enzyme and then filtered. Then, an aliquot of the filtrate was completely hydrolysed using β-glucosidase. The D-glucose produced was assayed using a glucose oxidase/peroxidise reagent. Absorbance measured at 410nm using a spectrophotometer.

As seen in the figure 3, lichenase acts only on alternating β-(1-3) and β-(1-4) linkages in the β-glucan molecule but never only on individual β-(1-3) or β-(1-4) glucosidic bonds. These smaller molecules are then broken down to monomers of D-glucose by β-glucosidase activity. This is because β-glucosidase exhibits exoglucanase activity and hydrolyzes (1→3) and (1→6)-β-glucosidic linkages most effectively.

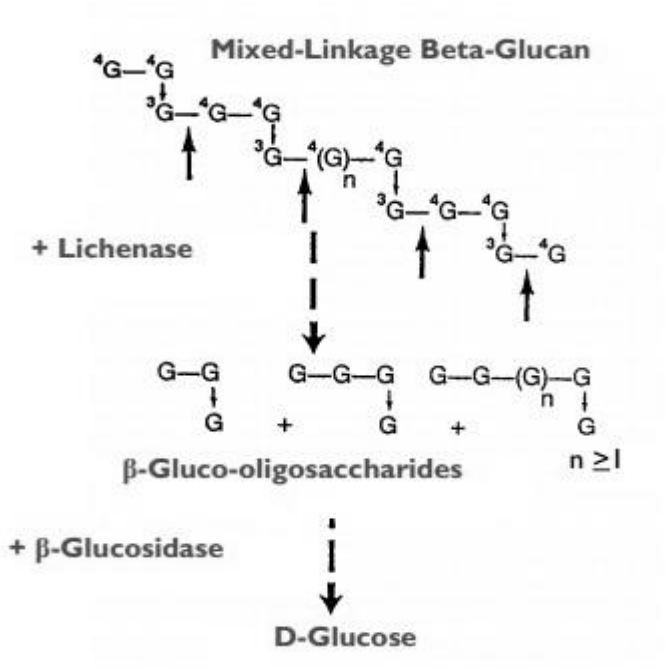

Figure 3. Enzymatic cleaving of β-glucan (Megazyme, Bray, Ireland)

4.2.2 Fructan analysis

Fructan was analysed according to Mc Cleary et. al (1997) by a spectrophotometric method using enzymatic assay kit (Megazyme, Bray, Ireland). In this method, sucrose was hydrolysed to D-fructose and D-glucose using sucrose specific enzyme. The samples were treated with α-galactosidase (Megazyme, Bray, Ireland) to remove raffinose-type oligosaccharides. Concurrently, using highly purified β-amylase, pullulanase and maltase, all soluble starch and maltosaccharides were hydrolysed to D-glucose. These reducing sugars were then reduced to sugar alcohols using alkaline borohydride. Excess borohydride was then removed from the solution using dilute acetic acid. Using purified fructanase (exo-inulinase), the fructan was then hydrolysed to D-fructose and D-glucose and then these reducing sugars were measured with PAHBAH reducing sugar method.

4.2.3 Molecular weight analysis of β-glucan using HPSEC

High performance size exclusion chromatography (HPSEC) was used to quantify the molecular weights of β-glucan in all 38 samples of wheat and rye. For extraction of β-glucan from samples, boiling water with added α-amylase was used. Complete inactivation of endogenous enzymes was done using 50% ethanol solution in boiling water for 15 minutes, initially. The detection is based on the principle of specific binding of Calcoflour to β-glucan in samples (Rimsten et al., 2003). A fluorescent detector (1100 series G1321A, Agilent Technologies, Waldbroon, Germany) was used with wavelengths λ_{ex} =415nm and λ_{em}=445nm at a gain setting of 8. These samples were run in advance against purified fractionated β-glucan in narrow molecular weight ranges (calibration) (Rimsten et al., 2003).

4.2.4 Statistical analysis

Statistical analyses to study the effect of moisture, temperature and screw speed during on fructan content, β-glucan content and molecular weight of β-glucan was done by analysis of variance (ANOVA, general linear model) using Minitab statistical software. P values of <0.05 were considered significant.

5.0 RESULTS

5.1 β-Glucan

Table 2 shows the content of β-glucan in different samples of wheat and rye bran

Table 2. β-glucan content in wheat and rye samples

Wheat bran- Extrusion variables	β-glucan (% w/w)	Rye bran- Extrusion variables	β-glucan (% w/w)
Un-extruded bran	1.93	Un-extruded rye	5.80
24% H2O, 90°C 200 rpm	2.34	30% H2O, 90°C 200 rpm	6.04
24% H2O, 90°C 300 rpm	2.24	30% H2O, 90°C 300 rpm	6.41
24% H2O, 90°C 400 rpm	2.35	30% H2O, 90°C 400 rpm	6.46
30% H2O, 90°C 200 rpm	2.28	36% H2O, 90°C 200 rpm	6.43
30% H2O, 90°C 300 rpm	2.33	36% H2O, 90°C 300 rpm	6.41
30% H2O, 90°C 400 rpm	2.28	36% H2O, 90°C 400 rpm	6.59
24% H2O, 110°C 200rpm	2.31	30% H2O, 110°C 200 rpm	5.94
24% H2O, 110°C 300 rpm	2.32	30% H2O, 110°C 300 rpm	5.80
24% H2O, 110°C 400 rpm	2.15	30% H2O, 110°C 400 rpm	5.88
30% H2O, 110°C 200 rpm	2.26	36% H2O, 110°C 200 rpm	5.76
30% H2O, 110°C 300 rpm	2.29	36% H2O, 110°C 300 rpm	5.92
30% H2O, 110°C 400 rpm	2.27	36% H2O, 110°C 400 rpm	5.13
24% H2O, 130°C 200 rpm	2.17	30% H2O, 130°C 200 rpm	5.90
24% H2O, 130°C 300 rpm	2.18	30% H2O, 130°C 300 rpm	5.90
24% H2O, 130°C 400 rpm	2.28	30% H2O, 130°C 400 rpm	5.90
30% H2O, 130°C 200 rpm	2.18	36% H2O, 130°C 200 rpm	5.92
30% H2O, 130°C 300 rpm	2.30	36% H2O, 130°C 300 rpm	5.89
30% H2O, 130°C 400 rpm	2.30	36% H2O, 130°C 400 rpm	6.01

In wheat, the content of β-glucan ranged from 2.15 to 2.35% in extruded samples which was higher than in un-extruded wheat bran with 1.93%. The average content of β-glucan with moisture content of 30% (n=9) was 17.96% higher than the un-extruded bran, whereas, the group with the 24% moisture content (n=9) had an average value which was 17.10% higher than the un-extruded bran sample. If moisture content by itself was the governing parameter to enhance the β-glucan content, 30% moisture is preferred to 24%.
Among the groups of samples with different extrusion temperature, set of samples extruded at 90^0 celsius (n=6) had the highest average β-glucan content followed by set with 110^0 celsius (n=6) and 130^0 celsius (n=6). The averages were calculated to be 2.3, 2.26 and 2.24%.
Overall average of all wheat samples was calculated to be 2.26% marking a 17.1% increase from un-extruded bran.
With the trend seen in the average range of β-glucan content in wheat bran extruded with different combinations of extrusion parameters, there seems to be a general increase in the average value compared to that of the un-extruded wheat bran. The results do not show a trend line with any of the extrusion parameters, independently. For example, the β-glucan content does not increase with increase in extrusion temperature or screw speed. The averages amongst the different groups point out an ideal combination of the three parameters with higher average β-glucan content.

Table 3. Statistical significance of β-glucan in wheat with extrusion parameters

Parameter	p-value
Moisture content	0.6084
Temperature	0.05149
Screw speed	0.6628

As seen in table 3, no statistical significant difference was seen between varying moisture contents or screw speeds (p value>0.05). In case of the temperature gradient, statistical difference was seen with p-value of 0.05, which shows that temperature was the variable with most effect on β-glucan content.

For β-glucan content in extruded rye samples (n=18), the values ranged from 5.13 (36% moisture, 110 celsius and 400rpm) to 6.59 (36% moisture, 90^0 celsius and 400rpm), whereas its un-extruded counterpart had 5.8 (%w/w). One value out of the extruded samples seemed to have detrimental effect of extrusion as its β-glucan content was lower than the un-extruded bran.
Among samples with the same screw temperature, set with 90^0 celsius had an average β-glucan content of 6.39%, followed by the set with 130^0 celsius with a value of 5.92% and the group extruded at 110^0 celsius had a value of 5.74%. The set of samples extruded at 110^0 celsius seemed to have very little increase from the un-extruded bran value if not detrimental. It is unlikely that the extrusion temperature alone is the cause of negative effect, rather, in combination with the moisture content of the bran slurry.
The average content of all samples was found to be 6.01%, calculated to be 3.6% higher than its un-extruded sample.

Table 4. Statistical significance of β-glucan in rye with extrusion parameters

Parameter	p-value
Moisture content	0.9006
Temperature	0.01306
Screw speed	0.9951

Statistical analysis (Table 4) showed significant difference in β-glucan concentrations between the three temperatures (p value <0.05). No significant difference was seen for β-glucan content between varying moisture content and screw speed (p-value >0.05).

5.2 Fructan analysis

Table 5. Fructan content in wheat and rye samples

Wheat bran- Extrusion variables	Fructan (% w/w)	Rye bran- Extrusion variables	Fructan (% w/w)
Un-extruded bran	2.15	Un-extruded rye	6.12
24% H2O, 90°C 200 rpm	2.31	30% H2O, 90°C 200 rpm	6.53
24% H2O, 90°C 300 rpm	2.36	30% H2O, 90°C 300 rpm	6.45
24% H2O, 90°C 400 rpm	2.35	30% H2O, 90°C 400 rpm	6.45
30% H2O, 90°C 200 rpm	2.18	36% H2O, 90°C 200 rpm	6.37
30% H2O, 90°C 300 rpm	2.23	36% H2O, 90°C 300 rpm	6.77
30% H2O, 90°C 400 rpm	2.38	36% H2O, 90°C 400 rpm	6.56
24% H2O, 110°C 200rpm	2.37	30% H2O, 110°C 200 rpm	6.62
24% H2O, 110°C 300 rpm	2.39	30% H2O, 110°C 300 rpm	6.66
24% H2O, 110°C 400 rpm	2.27	30% H2O, 110°C 400 rpm	6.38
30% H2O, 110°C 200 rpm	2.67	36% H2O, 110°C 200 rpm	6.67
30% H2O, 110°C 300 rpm	2.63	36% H2O, 110°C 300 rpm	7.14
30% H2O, 110°C 400 rpm	2.69	36% H2O, 110°C 400 rpm	6.76
24% H2O, 130°C 200 rpm	2.18	30% H2O, 130°C 200 rpm	6.70
24% H2O, 130°C 300 rpm	2.12	30% H2O, 130°C 300 rpm	6.56
24% H2O, 130°C 400 rpm	2.26	30% H2O, 130°C 400 rpm	6.55
30% H2O, 130°C 200 rpm	2.27	36% H2O, 130°C 200 rpm	6.74
30% H2O, 130°C 300 rpm	2.30	36% H2O, 130°C 300 rpm	6.63
30% H2O, 130°C 400 rpm	2.31	36% H2O, 130°C 400 rpm	6.77

The result for fructan content (Table 5) in case of wheat bran ranged from 2.12 to 2.69%. The set of samples with moisture content 24% (n=9) had an average fructan content of 2.29% and 2.41% in case of 30% moisture (n=9). The marked increase from its un-extruded counterpart was 6.5% and 11.9%, respectively.

The set of samples extruded at 90, 110 and 130^0 celsius had an average fructan content of 2.3, 2.5 and 2.24% respectively, that gave an increase of 7.05, 16.41 and 4.2% compared to un-extruded wheat bran. However the

group extruded at 110^0 celsius with moisture content of 30% had an average fructan content of 2.66 % (23.9% more than the un-extruded wheat bran). The group extruded at 130 celsius with 24 % moisture showed the lowest average fructan content of 2.19% (1.7% more than the un-extruded wheat bran).

Statistical analysis (Table 6) showed no significant between the three temperatures, moisture content or screw speeds (p value > 0.05).

Table 6 Statistical significance of fructan in wheat bran with extrusion parameters

Parameter	p-value
Moisture content	0.1296
Temperature	0.5212
Screw speed	0.6311

In case of rye, fructan content ranged from 6.37 to 7.14% amongst the 18 extruded samples. An average increase of 8.3% was calculated compared to the un-extruded sample. For samples with varying moisture, samples with 30% (n=9) moisture had an average fructan content of 6.54% compared to 6.7% as seen with samples extruded at 36% (n=9) moisture content. The increase compared to un-extruded rye bran was calculated to be 6.9% and 9.6%, respectively. For samples with varying screw temperature, highest average was calculated for group with 110^0 celsius (n=6) where an increase by 10% compared to un-extruded rye bran was seen. Samples with 130^0 celsius (n=6) had the average increased by 8.82% compared to un-extruded bran and samples with 90 celsius (n=6) had the average increased by 6.54%. For samples with varying screw speed, samples extruded at 300 rpm (n=6) had the highest average fructan content of 6.7% followed by samples extruded at 200 and 400rpm (n=6) with an average content of 6.6%.

Statistical analysis of fructan content showed a significant difference for samples with varying moisture content ($p<0.05$). There were no significant differences for fructan content between different screw speeds or temperatures as shown in Table 7.

Table 7. Statistical significance of fructan in rye bran with extrusion parameters

Parameter	p-value
Moisture content	0.04384
Temperature	0.2036
Screw speed	0.8181

5.3 Analysis of molecular weights of β-glucan

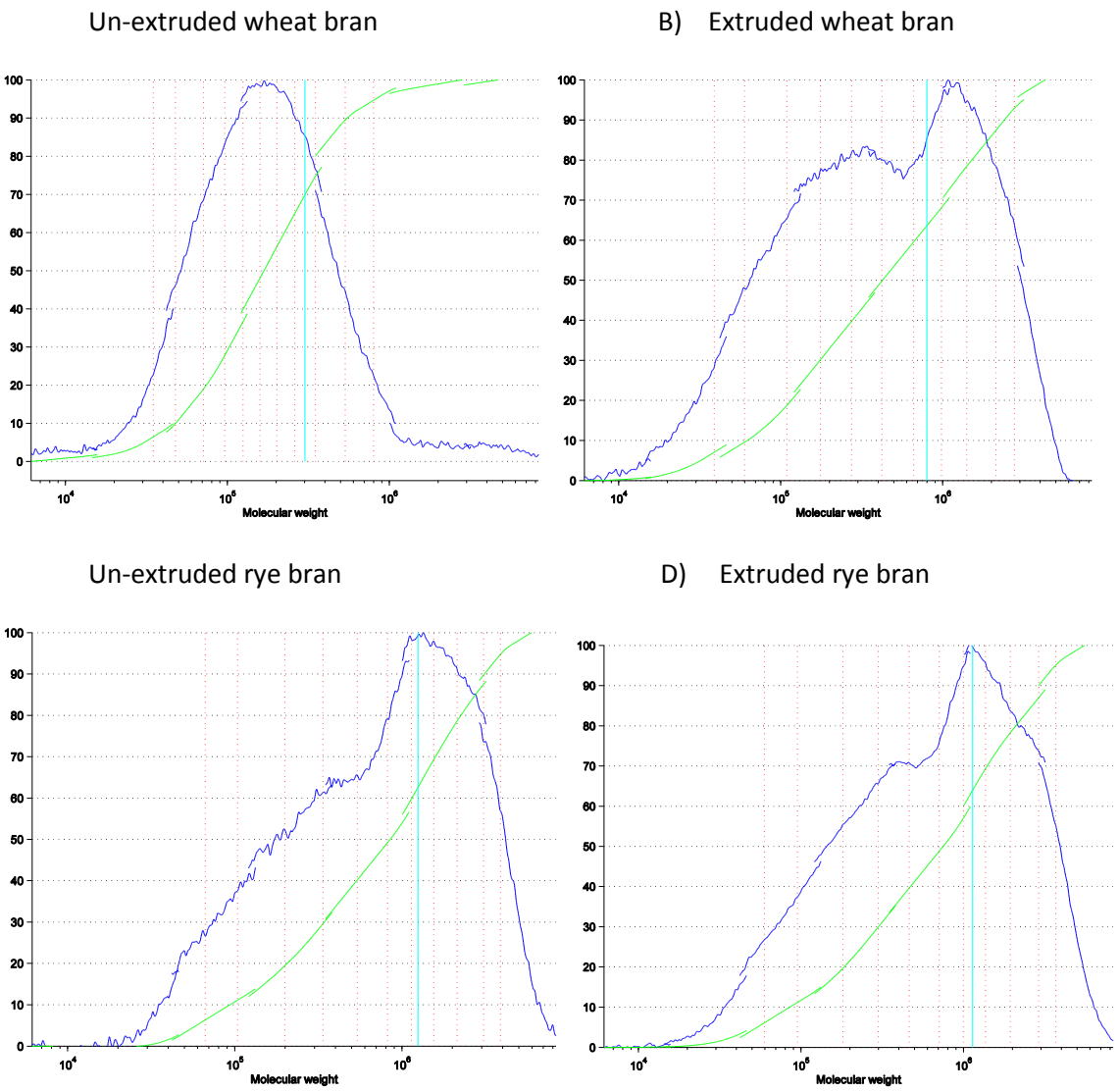

Figure 4. Molecular weight distribution of β-glucan in (A) un-extruded wheat bran, (B) extruded wheat bran (24% H2O, 90°C 200 rpm), (C) un-extruded rye bran and (D) extruded rye bran (30% H2O, 90°C 200 rpm)

Table 8. Average molecular weight distribution of β-glucan (g/mol) in wheat and percentiles (g/mol) describing the molecular weight ($\times 10^{-4}$) at which 10, 50 and 90 % of the distribution fall below that value

Wheat bran- Extrusion variables	Average MW ($x\ 10^4$ g/mol)	p10 ($x\ 10^4$ g/mol)	p50 ($x\ 10^4$ g/mol)	p90 ($x\ 10^4$ g/mol)
Un-extruded wheat bran	28.17	50.1	15.8	50.3
24% H2O, 90°C 200 rpm	104.0	65.5	50.7	286.3
24% H2O, 90°C 300 rpm	107.5	66.0	52.4	296.5
24% H2O, 90°C 400 rpm	105.9	65.8	52.2	289.6
30% H2O, 90°C 200 rpm	98.7	63.9	47.7	270.3
30% H2O, 90°C 300 rpm	101.2	66.6	49.9	275.4
30% H2O, 90°C 400 rpm	103.0	63.7	50.6	281.6
24% H2O, 110°C 200rpm	104.7	65.2	52.2	285.3
24% H2O, 110°C 300 rpm	105.3	66.1	53.3	286.1
24% H2O, 110°C 400 rpm	106.9	68.3	56.0	287.0
30% H2O, 110°C 200 rpm	102.4	67.9	51.7	277.1
30% H2O, 110°C 300 rpm	106.2	68.5	53.9	288.0
30% H2O, 110°C 400 rpm	113.0	71.5	58.6	305.5
24% H2O, 130°C 200 rpm	108.0	69.5	55.6	291.2
24% H2O, 130°C 300 rpm	108.0	71.0	57.7	288.2
24% H2O, 130°C 400 rpm	106.4	70.2	57.1	283.5
30% H2O, 130°C 200 rpm	103.3	67.8	51.8	279.9
30% H2O, 130°C 300 rpm	107.8	69.7	53.9	292.6
30% H2O, 130°C 400 rpm	110.3	71.2	56.3	299.1

Average molecular weight of β-glucan increased in extruded wheat samples by 2.75 folds compared to un-extruded wheat sample. The molecular weights in the extruded samples ranged from 98.7 x 10^4 (sample extruded with 30% moisture content at 90 celsius with 200 rpm) to 113 x 10^4 g/mol (30% moisture content, 110 celsius at 400 rpm).

Samples extruded with moisture content of 24% had an average molecular weight of 106.3 x 10^4 g/mol compared to 105.1 x 10^4 g/mol for moisture content of 30%. For samples with three different screw temperatures, 130^0 celsius had the highest average molecular weight followed by 110^0 celsius and then 90^0 celsius (107.3 x 10^4, 106.4 x 10^4 and 103.4 x 10^4 g/mol, respectively). With increasing screw speed, samples showed an increase in average molecular weight. Averages calculated to be 103.5 x 10^4 g/mol at 200 rpm, 106 x 10^4 g/mol at 300rpm and 107.6 x 10^4 g/mol at 400rpm.

The increase in average molecular weight in all extruded samples compared to un-extruded samples could be due to increased extractability of β-glucan during extrusion, leading to analysis of larger molecules.

Table 9. Statistical analysis (One way ANOVA) of average molecular weights of β-glucans in extruded wheat.

Parameter	p-value
Moisture content	0.466
Temperature	0.101
Screw speed	0.102

Table 9 shows that none of the parameters of extrusion had significant influence on the average molecular weight of β-glucans in wheat ($p>0.05$).

Table 10. Average molecular weight (g/mol) distribution of β-glucan in rye and percentiles (g/mol) describing the molecular weight (×10^{-4}) at which 10, 50 and 90 % of the distribution fall below that value

Rye bran- Extrusion variables	Average MW (x 10^4 g/mol)	p10 (x 10^4 g/mol)	p50 (x 10^4 g/mol)	p90 (x 10^4 g/mol)
Un-extruded rye bran	125.4	10.6	81.5	311.9
RB19-30% H2O, 90°C 200 rpm	114.4	9.7	70.9	291.0
RB20-30% H2O, 90°C 300 rpm	116.8	10.6	73.6	294.9
RB21-30% H2O, 90°C 400 rpm	114.7	11.4	73.9	286.5
RB22-36% H2O, 90°C 200 rpm	117.0	10.5	73.0	296.6
RB23-36% H2O, 90°C 300 rpm	118.0	11.2	76.0	295.2
RB24-36% H2O, 90°C 400 rpm	119.1	12.2	78.6	293.5
RB25-30% H2O, 110°C 200 rpm	121.1	11.7	78.9	297.3
RB26-30% H2O, 110°C 300 rpm	118.0	11.2	76.6	297.1
RB27-30% H2O, 110°C 400 rpm	116.9	11.2	77.2	287.8
RB28-36% H2O, 110°C 200 rpm	118.6	11.0	75.4	298.0
RB29-36% H2O, 110°C 300 rpm	116.6	11.0	75.4	290.0
RB30-36% H2O, 110°C 400 rpm	112.0	10.0	71.8	280.0
RB31-30% H2O, 130°C 200 rpm	119.8	12.8	79.1	294.0
RB32-30% H2O, 130°C 300 rpm	115.3	11.3	77.4	282.4
RB33-30% H2O, 130°C 400 rpm	118.2	11.4	79.5	289.7
RB34-36% H2O, 130°C 200 rpm	116.5	11.0	75.4	289.4
RB35-36% H2O, 130°C 300 rpm	109.0	7.6	63.4	283.7
RB36-36% H2O, 130°C 400 rpm	111.3	9.4	70.6	280.2

Unlike wheat samples, average molecular weights in extruded rye samples show a decrease when compared to the un-extruded rye sample. The average molecular weight of all extruded samples was 116.3 x10^4 g/mol compared to 125.4 x 10^4 g/mol in un-extruded rye bran. Their values ranged from 109 x 10^4 to 121.1 x 10^4 g/mol in extruded rye bran samples.

For samples extruded with moisture content of 30% (n=9), the average molecular weight was 117.2 x 10^4 g/mol and those with moisture content of 36% (n=9) had an average of 115.3 x 10^4 g/mol (marking reductions by 6.5 % and 8% from un-extruded rye). Samples extruded at 130^0 celsius (n=6) had average β-glucan molecular weight of 115 x 10^4 g/mol followed by 90^0 celsius (n=6) with average reduced to 116.67 x 10^4 g/mol and 110^0 celsius (n=6) reduced to average of 117.2 x 10^4 g/mol.

Table 11 Statistical analysis (One way ANOVA) of average molecular weights of β-glucans in extruded rye

Parameter	p-value
Moisture content	0.202
Temperature	0.471
Screw speed	0.313

The ANOVA results (Table 11) showed no statistical significance for each of the extrusion parameters with the average molecular weights recorded ($p>0.05$)

For molecular weight of β-glucan, if the confidence interval was set to be 90%, weak association is seen with parameters like screw temperature and screw speed. This leaves scope for further research in this area. Another reason could be that extrusion improves extractability of β-glucan. The molecules that are extracted have higher molecular weight as documented in the extruded wheat samples.

In extruded rye samples, the average decrease in molecular weight was 7.8% compared to the un-extruded rye. The decrease in average molecular weight would not necessarily mean enhanced solubility in solution. However, smaller chains have higher mobility due to lesser restriction to diffusion. This enables them to interact with each other forming a stable junction zones which on further association form three-dimensional gel networks. The other

reason could be that smaller molecules favour inter-molecular hydrogen bonding rather than intra molecular hydrogen bonds as seen in larger molecules (Cui and Wang, 2009).

Table 12. Extractable β-glucan content calculated using HPSEC peak analysis for extruded wheat and rye bran

Wheat bran- Extrusion variables	β-glucan (% w/w)	Rye bran- Extrusion variables	β-glucan (% w/w)
Un-extruded bran	0.19	Un-extruded rye	0.94
WB1- 24% H2O, 90°C 200 rpm	0.22	RB19-30% H2O, 90°C 200 rpm	0.88
WB2-24% H2O, 90°C 300 rpm	0.23	RB20-30% H2O, 90°C 300 rpm	0.87
WB3-24% H2O, 90°C 400 rpm	0.23	RB21-30% H2O, 90°C 400 rpm	0.92
WB4-30% H2O, 90°C 200 rpm	0.21	RB22-36% H2O, 90°C 200 rpm	0.80
WB5-30% H2O, 90°C 300 rpm	0.23	RB23-36% H2O, 90°C 300 rpm	0.88
WB6-30% H2O, 90°C 400 rpm	0.21	RB24-36% H2O, 90°C 400 rpm	0.86
WB7-24% H2O, 110°C 200rpm	0.20	RB25-30% H2O, 110°C 200 rpm	0.87
WB8-24% H2O, 110°C 300 rpm	0.22	RB26-30% H2O, 110°C 300 rpm	0.93
WB9-24% H2O, 110°C 400 rpm	0.25	RB27-30% H2O, 110°C 400 rpm	0.95
WB10-30% H2O, 110°C 200 rpm	0.21	RB28-36% H2O, 110°C 200 rpm	0.82
WB11-30% H2O, 110°C 300 rpm	0.21	RB29-36% H2O, 110°C 300 rpm	0.86
WB12-30% H2O, 110°C 400 rpm	0.20	RB30-36% H2O, 110°C 400 rpm	0.90
WB13-24% H2O, 130°C 200 rpm	0.23	RB31-30% H2O, 130°C 200 rpm	0.77
WB14-24% H2O, 130°C 300 rpm	0.23	RB32-30% H2O, 130°C 300 rpm	0.81
WB15-24% H2O, 130°C 400 rpm	0.22	RB33-30% H2O, 130°C 400 rpm	0.92
WB16-30% H2O, 130°C 200 rpm	0.21	RB34-36% H2O, 130°C 200 rpm	0.87
WB17-30% H2O, 130°C 300 rpm	0.17	RB35-36% H2O, 130°C 300 rpm	0.88
WB18-30% H2O, 130°C 400 rpm	0.20	RB36-36% H2O, 130°C 400 rpm	0.91

For extruded samples of wheat, the extractable β-glucan content ranged from 0.17 to 0.25. The overall average increase in β-glucan content for all extruded samples was about 16% higher than for the un-extruded sample. For the two different moisture contents, there was an average increase in extractable β-glucan content by 21% and 10.5% (moisture content 24% and 30 %, respectively). Extrusion temperatures of 90^0 and 110^0 Celsius, the average increase compared to un-extruded wheat sample was 15.8%. For samples extruded at 130^0 Celsius, the average extractable β-glucan content increased by only 10.5% compared to the un-extruded wheat bran. Samples extruded at 300 and 400 rpm the average increase was 15.8% and for 200rpm, it was 10.5%.

Average extractable β-glucan content in extruded rye samples ranged from 0.80 to 0.95. The overall average extractable β-glucan content in extruded rye samples decreased by 8% compared to the un-extruded rye bran. Samples extruded at moisture content of 30 % had an average decrease in extractable β-glucan content by 6.8% compared to 8.8% in samples with 36% moisture content. For groups with varying screw speeds, the average extractable β-glucan content increased with increasing speed. Samples extruded at 200 rpm had 11.9% less extractable β-glucan than un-extruded rye sample, whereas, samples extruded at 300 rpm had 8% lesser and samples at 400 rpm had 3% less extractable β-glucan.

Table 13. Statistical analysis (ANOVA) on β-glucan content and extrusion parameters in wheat and rye samples

Parameter	Wheat bran samples p-value	Rye bran samples p-value
Moisture content	0.028	0.488
Temperature	0.611	0.604
Screw speed	0.823	0.011

ANOVA on β-glucan content showed statistical significance ($p<0.05$) with of moisture content in case of wheat samples and screw speed in case of rye samples. The difference in the results of β-glucan content in both sets of samples of wheat and rye by the two quantification method reveal that result is method dependent.

6.0 DISCUSSION

Twin screw extrudates of wheat and rye bran seem to have a slight increase in their β-glucan and fructan content when compared to un-extruded reference sample. It is not only one extrusion parameter that governs the enhancement of these dietary fibre components, but in combination of moisture content, temperature and screw speed. While the enzymatic assay that determined the total β-glucan content, the HPSEC method calculated extractable β-glucan content only. For β-glucan content in extruded wheat samples calculated using the Megazyme kit, significant difference was only seen between samples with different temperature. For extractable β-glucan content in wheat samples, only varying moisture contents seemed to cause a significant difference. For extruded rye samples, total β-glucan content had significant difference between varying moisture contents. The extractable β-glucan analysis showed that extractable β-glucan content had slightly decreased in the extruded samples compared to un-extruded bran. There was a strong significant difference in samples with varying screw speeds. With these results it is unclear to find an optimum combination of extrusion parameters that ensure most increase in either total β-glucan content or extractable β-glucan content.

Fructan content in both extruded wheat bran and rye bran had increased slightly compared to their un-extruded counterpart. For fructan content, the only significant difference found was between different moisture contents in extruded rye. This result can be interpreted to be confounding as they suggest formation of β-glucan. This could mean that the number of reference samples could be increased.

The 2.8 fold increase measured in molecular weight analysis of β-glucan in extruded wheat samples could very well be due to the effect of extrusion, which may have enabled the extraction of molecules with much longer chain lengths. No statistical difference was recorded for any of the three extrusion parameters, however, if the confidence intervals were set to 90%, significant difference was seen with varying temperature and screw speeds with molecular weight of β-glucan. Another observation in wheat bran is that the molecular weight has been retained in the extraction process used in the HPSEC method. Molecules detected in all of the wheat samples consistently had an average increase which goes to show the stability of the β-glucan molecule. In case of extruded rye samples, there was a decrease in average molecular weight compared to the reference. This condition could favour the visco-elastic property of β-glucan in solution as smaller molecules diffuse better and form three dimensional aggregates through inter molecular bonding. Along with molecular structure, its chemical structure is also important in regard to its solubility.

For the increase measured in the analysis of β-glucan and molecular weights, it is important from productions point of view in a food company to see if adding value through use of energy actually improves the functionality of the product.

7.0 CONCLUSION

Extrusion cooking enhanced the total β-glucan content in wheat and rye. Total β-glucan content and its relation to individual extrusion parameters revealed that in extruded wheat, the only considerable effect was that of varying temperature. In extruded rye, the total β-glucan content increased in extruded samples compared to the un-extruded sample and significant difference was seen in samples with varying temperature. Molecular weight analysis of β-glucan in extruded wheat bran showed a 2.8 fold increase with significant differences in samples with varying temperatures and screw speeds at 90% confidence interval. In extruded rye samples, the molecular weight decreased compared to un-extruded sample. No extrusion parameter affected the molecular weight, significantly. Extractable β-glucan increased in extruded wheat bran and decreased in extruded rye bran compared to their un-extruded references. Varying moisture content affected extractable β-glucan content in extruded wheat bran and varying screw speeds in case of extruded rye bran.

In comparison to un-extruded wheat and rye bran, extruded samples had an increase in fructan content. Varying moisture contents had a significant influence on fructan content in extruded rye bran and none in extruded wheat bran.

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