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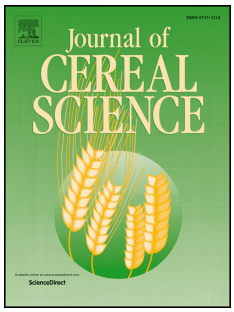

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Brans of the roller-milled barley fractions rich in polyphenols and health-promoting lipophilic molecules

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ABSTRACT

Three different roller-milled fractions namely bran, middlings, and flour of five commonly grown Irish barley varieties were investigated for the presence of β-glucan, polyphenols, and health-promoting lipophilic molecules. β-glucan was predominantly located in barley middlings. Polyphenols, as indicated by total phenolic content and the antioxidant activities, were abundant in the outermost bran fractions of barley. Similarly the health-promoting lipophilic molecules including phytosterols, unsaturated fatty acids, and tocols were most abundant in the barley bran fraction. However, the distribution of individual polyphenols and lipophilic compounds varied within the grain; for example ferulic acid and procyainidin C were not detected in flour fraction. Principal component analysis (PCA) clearly indicated a higher distribution of most bioactive molecules in bran as compared to middlings and flour fractions. The PCA also established possible correlations between the five barley varieties and their fractions based on their clustering in the plot.

KEYWORDS: roller milled barley; phytosterols; polyphenols; tocols.

1. Introduction

Barley is the fourth most produced cereal in the world after wheat, maize, and rice (http://faostat.fao.org/). While most of the produced barley is used as animal feed or in the brewing industry, only about 2% is used for human consumption.[1] Recently, there has been a considerable interest in the incorporation of barley and its components in food products.[1,2] This interest is due to the identification of a number of health-promoting bioactive compounds, besides nutrition, in barley.[2]

Barley has been heavily explored as a potential source of dietary fibre in the past two decades. The dietary fibre component of barley *β*-glucan has been approved by European Food Safety Authority (EFSA) and United States – Food and Drug Administration **(**US-FDA) for its ability to maintain normal LDL cholesterol levels in the body when consumed at the recommended levels.[3,4] The polyphenol content of barley has been well studied for its antioxidant properties.[5,6] The phenolic content of barley, and its associated antioxidant activity, is reportedly higher than that of wheat.[5] Flavanols have been identified as the most influential contributors to the antioxidant activity of barley.[6] Apart from its content of *β*-glucan and polyphenols, barley is also a source of functional oil, which has been studied for its cholesterol reducing properties.[7] Various components in the oil from barley, including phytosterols, polyunsaturated fatty acids (PUFA), tocopherols and tocotrienols (collectively known as tocols or Vitamin E), are likely to impart this hypocholesterolemic property to barley.[8,9] Barley oil is one of the richest sources of tocols among cereals with a favourable proportion of the biologically active homologues.[10] Human studies have indicated the absorption and bioavailability of tocotrienols in oil from barley to be higher as compared to palm oil, owing to the composition of the homologues in the barley oil.

All the health beneficial bioactive components in barley are non-uniformly distributed across the bran, endosperm and germ of the grain. While components such as β-glucan, phenolic compounds and tocotrienols are located in the outer parts of the grain, tocopherols are localised in the germ.[10] Thus, fractionation of barley by milling or abrasion can lead to concentration of its components leading to generation of bioactive-enriched fractions. Levels of β-glucan, tocols and phytosterols in scarification fractions and pearling by-products of barley have been determined in previous studies.[8,11] A several fold enrichment of these phytochemicals in the scarified/pearled fine fractions, compared to the whole grains was observed.

Roller milling has been mainly used by the baking industry for milling cereals such as wheat and oats. The flour and fibre-enriched milled fractions of these cereals are incorporated into baked and extruded snack products.[12] However, unlike wheat and oats, barley is not traditionally roller-milled on a commercial scale to obtain bran and flour. Pearling is a more commonly used fractionation technique for barley, and the pearled barley kernel is often used as the final product in food-related purposes. Roller-milling is a size-based fractionation technique and three main fractions are generated from the process; bran, middlings, and flour.[12] The bran is usually composed of the larger particles in the outer layers of the grain. The middlings consist of particles smaller than the bran and larger than the flour. The middlings of barley are often treated as a by-product of the milling process, and used as animal feed.[13] The endosperm, also known as the flour, includes small particles from the kernel/endosperm of the grain.

On an experimental level, barley has been roller-milled for determination of the physicochemical properties (water-holding capacity, hardness, fibre, viscosity) of its fractions.[14,15] Roller-milled and fibre-enriched fractions of barley have also been used to some extent for the fortification of products such as bread and noodles.[13,16] However, a

71 detailed evaluation of the major health-promoting components of the roller-milled fractions
72 of barley has not been undertaken to date. This study is aimed at filling the knowledge gaps
73 on the potential compositional and bio-functional attributes of the roller-milled barley
74 fractions, which would encourage the food industries to use roller-milling on a commercial
75 level for generation of barley fractions as functional food ingredients.

76 **2. Materials and methods**

77 **2.1 Materials**

78 Five cultivars of two-rowed, previously dehulled, Irish spring barley (Propino, Sanette,
79 Mickle, Taberna, Irina), grown in 2013 were provided by Seedtech (Waterford, Ireland). The
80 barley samples were tempered and dried to 16.5% moisture content prior to milling. The
81 barley grains were milled through the break side (over 3 grooved rollers) and sifted via
82 centrifugal sifting using a CD1 roller mill (Chopin Technologies, Villeneuve-la-Garenne,
83 France). Three barley fractions were generated from the grains: bran, middlings and the
84 endosperm. The bran fraction was further ground to a fine particle size using a Retsch
85 MM400 Mixer Mill (Haan, Germany). The mixed-linked β-glucan assay kit for β-glucan
86 determination was purchased from Megazyme International Ltd. Wicklow, Ireland. The
87 standards of polyphenols (catechin procyanidin B1 and ferulic acid), the reagents and
88 solvents for the assays including Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-
89 tetramethylchroman-2-carboxylic acid (Trolox), ferric chloride hexahydrate, 2,4,6-tri(2-
90 pyridyl)-s-triazine, gallic acid, sodium carbonate, sodium acetate anhydrous, 98% sulphuric
91 acid, sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH) and aluminium chloride ($AlCl_3$)
92 were purchased from Sigma Aldrich (Co. Wicklow, Ireland). For analysis of lipids, standards
93 of all the sterols, the fatty acid methyl esters (FAME) standard mix (Supelco 37 component
94 FAME mix, Bellefonte, PA, USA), tocopherol homologues, 5α-cholestan-3β-ol, tricosanoic

acid as well as the reagents bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane, potassium hydroxide, ascorbic acid, pyridine, acetyl chloride and the solvents, heptane, pentane, dichloromethane, methanol, hexane, were purchased from Sigma Aldrich (Wicklow, Ireland). The standards of *α*-, *γ*-, *δ*- tocotrienols were purchased from Chromadex, Inc (Irvine, CA, USA).

2.2 Determination of *β*-glucan content

β-glucan content of the fractions was quantified using the Megazyme mixed-linkage *β*-glucan kit. The concentration of *β*-glucan is reported as percentage (%) or g of *β*-glucan/100 g of sample.

2.3 Extraction of polyphenols and mass spectrometry analysis

The free and bound polyphenols were extracted from the roller-milled barley fractions as described previously.[6] Briefly, 1g sample was used for the extraction of the polyphenols. Free phenolics were extracted using 80% methanol, followed by extraction of the bound phenolics from the residual sample matrix using acid and enzymatic hydrolysis. The dried extracts of the free and bound polyphenols were dissolved in methanol, and combined to get a total polyphenol extract. The total polyphenol extract of the fractions were subsequently used for quantification studies using mass spectrometry analysis.

The polyphenols present in the extract were identified using the ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) (Waters Corporation, MA, USA), using the same protocol described in the previous paper.[6] Detection and quantification of the polyphenols were performed in the negative ion mode using multiple reaction monitoring method. Standards of polyphenols were prepared in 80% methanol in the concentration range of 0.5-10.0 μg/ml for catechin, and 1.0-10.0 μg/ml for

procyanidin B1 and ferulic acid, and the standard curve of procyanidin B1 was used for quantification of prodelphinidin B and procyanidin C.

2.4 Total phenol content (TPC) and Ferric reducing antioxidant power (FRAP)

The TPC and antioxidant capacity by FRAP assay of the methanolic extracts of fractions were determined in triplicates according to the methods previously outlined.[6] TPC of the fractions was expressed as μg gallic acid equivalent (μg GA eq.)/ml and the antioxidant capacity of the fractions was expressed as mM Trolox eq.

2.5 Total flavonoid content (TFC)

A method by Liu *et al*.[17] was used for the determination of TFC of the samples. For the assay, 125 μl of each extract was mixed with 625 μl of de-ionised water and 37.5 μl of 5% $NaNO_2$ solution. After 6 min, 75 μl of 10% $AlCl_3$ solution was added, and after another 5 min, 250 μl of 1M NaOH was added to the reaction mixture. Total volume of all the mixtures was made up to 1.25 ml. Following thorough mixing of the solutions, absorbance of the samples against the blank was determined at 510 nm. A standard curve of catechin in the range of 10-500 μg/ml was plotted and TFC was expressed as μg catechin eq./ml extract. All determinations were made in triplicate.

2.6 Fatty acids, sterols, and tocols

The previously described protocols were used for the extraction of lipophilic bioactive components from barley.[18] In brief, fatty acids were extracted using a microwave-assisted extraction and derivatised to fatty acid methyl esters (FAME). The FAME-derivatives were analysed and quantified using gas chromatography-flame ionisation detector (GC-FID). Each sample was extracted in duplicates for the FAME analysis. For the analysis and quantification of sterols, the samples were saponified before the sterols were silylated for their detection and

quantification using GC-FID. As for the tocols, hexane was used for their extraction. The tocols in the extract were then detected and quantified using reversed-phased HPLC analysis. Fatty acid samples were analysed in duplicates, whilst the sterols and tocols analysis were performed in triplicates.

2.7 Statistical analysis

For duplicate and triplicate determinations, data are reported as mean ± standard deviation. ANOVA was performed using IBM SPSS statistical software (v20, Chicago, IL, USA). To analyse the variation in the content of bioactives, principal component analysis (MATLAB R2009b, Mathworks Inc, Natick, MA, USA) was performed.

3. Results and discussion

3.1 *β*-glucan

The *β*-glucan content in the three milled fractions of barley varieties is illustrated in Figure 1. The highest amount of *β*-glucan was found in the barley middlings ranging between 5.2% and 6.2%. This was followed by the bran, which had between 3.9% and 4.7% *β*-glucan, while the flour fraction had the lowest *β*-glucan content ranging between 1.6% and 1.8%. The amount of *β*-glucan between the fractions varied significantly ($p<0.05$), and middlings of all the varieties had significantly higher *β*-glucan compared to the bran and flour. Middlings obtained from roller-milling barley could therefore serve as enriched sources of *β*-glucan. In general there was no significant difference in the *β*-glucan content between the same fraction-types of the five barley varieties. For example the middlings of Sanette, Taberna and Irina were not significantly different from each other in terms of their *β*-glucan content. The findings of high abundance of *β*-glucan in the middlings reflect the higher concentration of *β*-glucan in the inner cell wall of barley grain as compared to the outermost layers. The higher *β*-glucan content in the middlings is in contrast to a previous study where it was reported that

β-glucan is mostly located in the outer layers of the barley grain.[19] However in another study by Panfili *et al.*, the authors have shown increasing levels of β-glucan in the inner layers of the barley kernel with successive removal of the outer layers in the pearling process. The highest value of β-glucan (4.5%) was reported in the fraction VI of pearling, which is slightly lower than β-glucan in the middlings (5.2%-6.2%) of roller-milled barley. In the present study, the combined β-glucan content of the bran and middlings amounted to 9.5%-10.6%, which was about 2.5 times higher than the corresponding whole grain varieties that were in the range 2.6%-3.3% dry weight.

3.2 Phenolics, Flavonoids and Antioxidant capacity

The total phenolic content (TPC) of the roller-milled barley fractions measured as gallic acid equivalents (GA eq.), could be ranked in the following order: bran > middlings > flour (Figure 2a). The TPC levels of the bran were significantly higher ($p<0.05$) than the middlings and flour in all the five varieties. The TPC of the bran ranged between 125.9 and 152.9 µg GA eq./ml extract, and that in middlings and flour were between 63.3 to 71.1 and 12.9 to 20.1 µg GA eq./ml extracts, respectively. The phenolic content of the bran was as high as 12-fold more than that of the flour. This indicates that the phenolic compounds in barley are predominantly located in the outer layer of the barley grain, most likely occurring as bound compounds attached to the cell wall materials. The amount of phenolic compounds decreased towards the inner layers of the grain, and the endosperm had the least amount of phenolics. These findings are in good agreement with a previous report, where a decrease in TPC was observed when moving from the outermost layer to the centre of the grain in barley pearling fractions.[20]

The total flavonoid content (TFC) of barley fractions determined as µg epicatechin eq./ml extract were significantly ($p<0.05$) higher in the bran of the varieties, followed by the middlings and flour (Figure 2b). Flavonoid contents of the bran ranged from 85.8 to 105.4 µg

epicatechin eq./ml, while that of the middlings and flour ranged from 44.4 to 59.1 µg epicatechin eq./ml and 8.9 to 16.4 µg epicatechin eq./ml, respectively. Though the mean TFC values did not vary much between the varieties, the outer layers of the grain represented by bran and middlings contributed as much as 93% of the total flavonoids of the whole grain. This indicates the predominant location of flavonoids in the bran and outer layers of the grain compared to the endosperm. To our knowledge, no previous study has reported the TFC of milled barley fractions. Flavonoids are important phytochemical components of barley known to exhibit potent anti-oxidant activities,[6,21] and would potentially contribute to the overall health benefits of fractionated barley.

TPC and TFC have often been found to positively correlate with other antioxidant capacity assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and FRAP.[22] Therefore as expected, the bran of all the varieties were most efficient in reducing the ferric ion in the FRAP assay, followed by middlings, while the flour had the least antioxidant capacity (Figure 2b). In line with the TPC and TFC values, the bran extracts were the most efficient milled fractions in reducing the ferric ion. The FRAP of the samples further confirmed the marked localisation of phenolic compounds in the outermost layers of the barley grain. Antioxidant capacities measured by other methods like Trolox equivalent antioxidant capacity and DPPH radical have indicated similar results in a previous study.[20]

3.3 Distribution of flavanols and ferulic acid in roller-milled barley fractions

In our previous study,[6] we identified ‘flavanols’, a sub-group of polyphenols, as the strongest contributors to the observed antioxidant capacity of whole grain barley. The identified flavanols included procyanidin B, prodelphinidin B, procyanidin C and catechin. Along with these flavanols, ferulic acid - a phenolic acid was also identified as a potent contributor to the antioxidant capacity of barley. Therefore, in the present paper, levels of the above mentioned four flavanols and ferulic acid were determined in the roller-milled barley fractions.

Although TFC indicated barley bran to be the most abundant source of flavonoids, the distribution of individual flavanols varied within the grain (Table 1). The monomer catechin was mostly distributed uniformly between the three fractions with no significant difference ($p<0.05$) between the three fraction except in the variety Mickle and Irina. The flour fraction in Mickle had significantly higher catechin than the middlings, while in Irina the bran had significantly higher catechin than the flour. Thus catechin might not be more concentrated in one particular location within the grain, and depending on the genotype, any of the three roller-milled fractions of barley could serve as a potential source of catechin.

Unlike the monomeric catechin, the dimeric flavanols procyanidin B and prodelphinidin B were mostly located in the outer layer of the barley grain, and distributed in the following decreasing order: bran > middlings > flour. Procyanidin B and prodelphinidin B were the first and second most abundant flavanols in the bran of the samples that ranged from 376.6 to 503.4 µg/g and 182.5 to 244.9 µg/g, respectively. A wide variation in the distribution of these flavanols between the samples was observed. Like the dimers, trimeric flavanol procyanidin C was also most abundant in the bran followed by the middlings. This flavanol could not be detected in the endosperm of the samples represented by the flour. The procyanidin C in the bran ranged from 37.3 to 69.6 µg/g, which was 1.5 to 3 times higher than that in the middlings.

Ferulic acid in the bran ranged between 813.2 and 1510.1 µg/g, while that in the middlings ranged between 352.3 and 1232.5 µg/g. The wide variability in the amount of ferulic acid between the samples indicated a genotype-dependent availability of this phenolic acid in the grain of barley. Thus, ferulic acid was preferentially located in the outer layers of the barley grains. Ferulic acid could not be detected in the flour of barley, indicating its absence in the endosperm. This result is in agreement with previous studies, which also have indicated the

location of ferulic acid in the outer layers of the barley grain, most often as a bound cell wall component.[23, 24]

To the best of our knowledge, this is the first study to report the distribution of flavanols in the milled fractions of barley. In earlier studies,[20, 23] the distribution of the common phenolic acids in the pearled fractions of barley was reported, however the distribution of flavanols in the grain was not considered.

3.4 Sterols

The total phytosterol content differed significantly ($p<0.05$) between the bran, middlings and flour of the samples (Figure 3a). The highest concentration of phytosterols was found in the bran, ranging from 92.1 to 103.5 mg/100g, followed by the middlings (68.4 to 72.5 mg/100g), while the flour had the least amount of phytosterols between 29.7 and 34.9 mg/100g. This indicated that the phytosterols were largely concentrated in the outer layers of the grain, while their levels diminished towards the inner endosperm of the grain. This observation concurs with other studies,[8, 25] which reported the majority of phytosterols to be located in the outer pearling layers (pearling fines) of barley, which gradually decreased with each pearling step and were lowest in the abraded/pearled kernels. Lampi *et al.*[8] have reported phytosterols as high as 2.8 mg/g in pearling fines from the first pearling step and 1.5 mg/g in the fifth pearling fines of barley, which was much higher compared to 0.7 mg/g and 0.5 mg/g phytosterols in the whole grain and pearled grain of barley, respectively. Lampi *et al.*[8] also observed a similar trend for rye-sterols in their study.[11] In the present study, the bran and middlings in combination had a total of 1.60 to 1.74 mg/g phytosterols, a 2-3 fold higher than the corresponding whole grain barley varieties where the phytosterol ranged from 0.66 to 0.82 mg/g.[18] This emphasises the importance of roller-milling in concentrating the phytosterols into specific fractions.

The distribution of individual phytosterols in the milled fractions of barley has been provided in Supporting Information, Table 1. The most abundant sterol in all the barley fractions was *β*-sitosterol. The quantity of *β*-sitosterol in the bran ranged between 0.44 to 0.60 mg/g and was about 1.5 times higher than the middlings, and 3-4 times higher than the flour. Three other sterols including campesterol, stigmasterol and *β*-sitostanol were also present in significantly ($p<0.05$) higher amounts in the bran of the varieties, followed by the middlings and flour. This indicated a non-uniform distribution of these sterol types across the barley grain, highest at the grain's surface and gradually decreasing towards the endosperm. However, brassicasterol has shown to be uniformly distributed across the grain as no significant difference ($p<0.05$) between the bran, middlings and flour was observed.

3.5 Tocols

A previous study by Daneilson *et al.*[14] on the level of tocols in pearling by-products of barley reported the highest concentration of total tocols (247.4 μg/g) in the third pearled fractions of barley. The third pearled fraction of barley corresponded to its aleurone layers. In the present study, total tocols (∑T) were present in significantly ($p<0.05$) higher amounts in the bran of all samples in the range of 26.8-54.6 μg/g (Figure 3b). The bran of the variety Sanette had the highest ∑T (54.6 μg/g), which was almost twice that in the bran of Taberna (26.8 μg/g). The wide disparity in tocols in the bran of these two varieties was most likely due to variability in the level of one single predominant or multiple tocol homologues. Middlings represented the next abundant source of ∑T among the fractions ranging from 20.5 to 41.2 μg/g, while flour represented the least abundant source ranging from 1.2 to 9 μg/g. This indicated an abundance of tocols in the outer layers of the grain as also suggested by Panfili *et al.*[10] Bran and middlings in combination contained 52 – 89.8 μg/g total tocols which was about 1.5 times higher than the corresponding whole grain varieties.[18] The difference in ∑T between the bran and middlings varied in different varieties. For instance, in Propino and

Taberna, the amount of ∑T in the bran was slightly higher than middlings, indicating a very small difference between the two fractions (Figure 3b). However, in Sanette, Mickle and Irina, the ∑T in the bran was almost twice or more higher than the middlings, indicating a larger difference.

The most predominant tocol homologue in bran and middlings of all the samples was alpha-tocotreinol (*α*T3), while in the flour the predominant homologue varied between *α*T3 and alpha-tocopherol (*α*T) (Supporting Information, Table 2). In the bran, *α*T3 ranged between 16.3 µg/g in Taberna to almost twice the amount, 37.7 µg/g in Sanette. It therefore becomes clear that the difference in ∑T between the bran of Taberna and Sanette observed earlier predominantly arises from the higher level of the *α*T3 homologue in the latter variety. In Propino and Taberna, *α*T3 in the bran was slightly higher than the middlings, however the bran of the cultivars Sanette, Mickle and Irina had 2-3 times more *α*T3 than the middlings. This indicated that the homologue *α*T3 was highly concentrated in the outer layers of some varieties, while it was more evenly distributed in the others, indicating a lack of consistency in their distribution. The flour only contained minimum to no *α*T3.

The second and third abundant homologues in the bran and middlings of all but one variety were *α*T and γ-tocotrienol (*γ*T3), respectively. With regard to *α*T, the middlings of varieties Propino and Taberna had higher levels of this homologue as compared to the bran, while the vice-versa was observed for the other cultivars. This indicated probable genotype-dependant variability in distribution of *α*T across the grains, as also observed for *α*T3. The distribution pattern of *α*T and *α*T3 also suggests a genotypic similarity between the varieties Propino and Taberna with respect to the tocols. In terms of *γ*T3, the bran of all varieties had significantly ($p<0.05$) higher levels of this homologue as compared to the middlings, while flour had the lowest levels. Other tocol homologues that were detected in minimum amounts in the roller-

312 milled fractions of the varieties were, γ-tocopherol (γT), δ-tocotrienol (δT3), δ-tocopherol
313 (δT) and β-tocopherol (βT). The least abundant homologue detected in the fractions was βT.

314 The levels of phytochemicals presented in the aforementioned barley-cultivars hold true for
315 the harvest of 2013 only, as the concentration of phytochemicals can differ from one crop
316 year to another as their biosynthesis are influenced by climatic conditions amongst other
317 factors. The inter-sample variation within the cultivars was not tested, and the values are true
318 for the samples studied only.

319 **3.6 Fatty acids**

320 Barley is a rich source of unsaturated fatty acids (UFAs).[18] The total UFA content
321 ranged between 2033-2282 mg/100g in the bran, 1530.7-1866.8 mg/100g in the middlings
322 and 366.4-545.8 mg/100g in the flour (Figure 3c). Although the bran contained significantly
323 ($p<0.05$) more UFAs than the middlings, the difference in absolute values of UFAs between
324 these two fractions was negligible. Besides bran, middlings could also serve as a potential
325 source of UFAs. In case of the saturated fatty acids (SFAs), there was no significant
326 difference ($p<0.05$) at all between the bran and middlings (Figure 3d). The probable reason
327 for this is the inclusion of the germ portion of the grain in the middlings. The germ is the
328 primary lipid reserve of the grain, which explains the high levels of lipophilic components in
329 the middlings. The total UFAs in the combined bran and middlings of the samples were in the
330 range of 3681.6 and 4120.5 mg/100g, which were 2-3 times higher than their corresponding
331 whole grains (1200.2 and 1730.7 mg/100g).[18] Although wholegrain barley might not be
332 considered a rich source of UFAs as compared to cereals like buckwheat, or seeds and
333 legumes, the roller-milled bran or middlings could be deemed as an enriched source of these
334 fatty acids.

While the level of fatty acids in whole grain barley has been reported before,[9,26] their comprehensive distribution in the different fractions of the grain has never been explored. A total of 18 fatty acids (9 SFAs and 9 UFAs) were detected in the fractions (Data not shown), however for the sake of brevity only the seven most abundant fatty acids including 2 SFAs and 5 UFAs have been reported in Supporting Information, Table 3.

Similar to whole grain barley,[18] the most abundant fatty acid in all the milled fractions was the polyunsaturated fatty acid (PUFA) linoleic acid (C18:2n6). Linoleic acid was predominantly located in the bran (1452.9-1649.5 mg/100g). Palmitic acid (C16:0) was the second most abundant fatty acid in the fractions and its levels did not differ significantly ($p<0.05$) between the bran and the middlings. The third most abundant fatty acid in the fractions was the monounsaturated fatty acid (MUFA) oleic acid (C18:1n9), which was present in significantly higher amounts in the bran. The fractions of barley were also a reasonable source of the PUFA alpha-linoleic acid (C18:3n3). The amount of alpha-linoleic acid in the bran of the cultivars varied between 125 and 147 mg/100g sample, which was 1.2 to 1.5 times higher than the middlings. This fatty acid could not be detected in the flour of most of the cultivars except Irina. It has been reviewed and discussed the prospect of reduced risk of coronary heart diseases in adults, on regular consumption of omega-3 (n3) fatty acids.[27] EFSA has recommended a daily dose of omega-3 fatty acids between 250 and 500 mg, for maintenance of cardiovascular health.[28] The regular recommended dietary sources for omega-3 fatty acids are food items such as fish and nuts. Nonetheless, milled fractions of barley such as the bran or middlings or their mixture can serve as additional sources of the health-beneficial fatty acids since 100g bran and middlings mixture on an average would contain 230 mg of the omega-3 alpha-linoleic acid.

3.7 Principal component analysis (PCA)

PCA was conducted to get an overview of the correlation between the examined variables and their distribution within the grain (Figure 4). 74% of the variability in the data could be explained by the first component (PC1), while the second component (PC2) accounted for 9% of the variation in the data, thus cumulatively explaining 83% of the variation.

On the loading plot (Figure 4a), variables were separated by PC1 and PC2 based on their distribution in the grain. All the major variables including the dimeric and trimeric flavanols, ferulic acid, UFA, majority of sterols and tocols, were clustered on the right side of PC1 with loadings in the same direction. This indicated an abundance of these variables in the bran of the samples, followed closely by the middlings. There was a complete absence of loadings of the variables on the left side of PC1, indicating a lack of predominance of any of the variables in the flour. However, three variables including catechin, δT3, and brassicasterol were clustered close to the origin of component 1 with high loadings in the upward direction. This proposed no difference in the amounts of these variables between the bran, middlings and flour suggesting their uniform distribution across the three fractions.

The score plot (Figure 4b) separated the barley varieties along PC1 based on their fractions. In this plot, the bran of all the varieties were located to the extreme right of the plot indicating a similarity in their properties, as compared to the middlings which were concentrated near the origin of the plot. The flour of the samples were largely localised towards the extreme left of the plot. The samples were also separated along PC1 and PC2 based on the composition of phytochemicals in them. Samples with high scores in any given direction would be dominated by variables with large loadings in that direction. For example, the bran and middlings of Mickle represented by blue circles and crosses respectively would be high in the variables δT and γT. On the other hand, bran and flour of Irina represented by black circles and crosses, respectively would be high in the variables *β*-sitostanol and *β*-glucan. Thus, PCA

383 also indicated a variation in the composition of the barley fractions which most likely arises
384 from the difference in genotypes of the varieties.

385 **4. Conclusions**

386 Barley, a cereal that is currently not utilized substantially for human consumption,
387 contains a number of components which are potentially beneficial to human health. The
388 majority of these health-beneficial compounds are located in the outer layers of the barley
389 grain. *β*-glucan, associated with lowering LDL-cholesterol, is mostly located in the
390 middlings, while most polyphenols (flavanols, ferulic acid) are concentrated in the outermost
391 layers of the grain. The flavanol catechin is evenly distributed across the barley grain. Among
392 the lipophilic bioactives, most of the sterols, polyunsaturated fatty acids, and tocols are
393 located in the outermost layers. A few exceptions such as the brassicasterol, linoleic acid, and
394 *α*T are evenly distributed between the outer and inner endosperm cell walls of the grain.
395 Thus, the outer layers of the grain of barley, represented by the bran and middlings would
396 impart greater health benefits when consumed as a part of the diet. The endosperm portion of
397 the barley grain, represented by the flour fraction had the least amount of β-glucan, flavanols,
398 lipophilic components and antioxidant activity, among the three fractions. Some of the
399 phytochemicals like procyanidin C, ferulic acid, α linoleic acid, and *γ*T could either not be
400 detected at all or were detected in minimum amounts in the flour.

401 In summary, the findings here would encourage and promote the consumption of
402 whole barley flour as opposed to the refined barley flour among consumers. Physical
403 processes such as roller-milling allow a favourable distribution of these compounds, which
404 could be used to produce enriched fractions. The milled fractions of barley such as the bran
405 and middlings are currently treated as by-products of the roller-milling industry. Efficient
406 utilisation and increased incorporation of the bran and middlings in food products will help

redirect the use of these by-product fractions, and also improve the biological and health-promoting value of sustainable food products.

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489

FIGURE CAPTIONS

Figure 1: β-glucan content (%) of milled fractions: bran, middlings, flour of barley varieties (mean ± SD, n=4). Bars with no letter in common are significantly different from each other.

Figure 2: (a)Total phenol content of milled fractions (bran, middlings(semolina), flour) of barley varieties (mean ± SD, n=3), expressed as μg gallic acid eq./ml sample (b) Antioxidant capacity of milled fractions of barley varieties (mean ± SD, n=3), expressed as mM Trolox eq. (c) Total flavonoid content of milled fractions of barley varieties (mean ± SD, n=3), expressed as μg/ml epicatechin eq. Bars with no letter in common are significantly different from each other.

Figure 3. (a)Total sterols content (mg/ 100g) of milled fractions: middlings (semolina), bran, flour of barley varieties (mean ± SD, n=3). (b) Total tocols (μg/g) in milled fractions of barley varieties (mean ± SD, n=3). (c) Total unsaturated fatty acids (mg/ 100g) and (d) total saturated fatty acids (mg/ 100g) in milled fractions of barley varieties (mean ± SD, n=3). Bars with no letter in common are significantly different from each other.

Figure 4: a) Loading plot of the variables on PC1 and PC2 (b) Score plot of PC1 and PC2 from analysis of the fractions of five barley varieties. The bran fractions have been represented by circles, the middlings by crosses, and the flour by triangles. A colour code has been given for each variety.

Table 1: Levels of flavanols and ferulic acid (µg/g) in the three fractions: bran, middlings, and flour of roller-milled barley. The last column in the table provides the sum of the four flavanols and ferulic acid identified in various fractions of the varieties. In every column values followed by different alphabetical superscripts are significantly different from each other.

Cultivar/ Fraction	Catechin	Procyanidin B	Prodelphinidin B	Procyanidin C	Ferulic acid	Sum of individual phenols
Propino						
Brans	103.1±5.4^{a}	376.6±25.3^{a}	191.9±4.8^{a}	37.3±1.3^{a}	813.2±29.7^{a}	1522.1
Middling	66.1±4.1^{b}	231.6±39.1^{cd}	117.8±10.2^{cd}	19.4±4.5^{d}	489.6±50.5^{d}	924.5
Flour	99.2±7.9^{a}	43.9±7.7^{f}	22.2±4.7^{ef}	n.d.	n.d.	165.3
Sanette						
Brans	98.4±5.6^{ac}	426.4±57.9^{ab}	244.9±24.8^{b}	61.9±1.5^{b}	1062.7±44.0^{b}	1894.3
Middling	80.9±2.3^{c}	215.6±7.4^{d}	109.7±13.6^{cd}	20.4±4.0^{d}	865.9±15.8^{e}	1292.5
Flour	97.4±12.1^{ac}	19.6±1.7^{g}	10.3±3.3^{f}	n.d.	n.d.	127.3
Mickle						
Brans	155.2±17.2^{de}	400.0±23.1^{a}	197.9±22.2^{a}	49.4±5.6^{c}	1510.1±56.1^{c}	2312.6
Middling	133.0±22.6^{d}	189.7±4.4^{e}	91.7±12.7^{c}	24.3±5.7^{d}	1232.5±76.6^{f}	1671.2
Flour	188.5±5.5^{e}	16.6±1.2^{g}	13.7±3.7^{f}	n.d.	n.d.	218.8
Taberna						
Brans	97.6±3.2^{a}	376.9±17.3^{a}	182.5±10.2^{a}	43.4±4.4^{c}	902.4±45.4^{a}	1602.8
Middling	92.5±17.6^{a}	190.9±11.5^{e}	91.9±10.0^{c}	27.1±4.6^{d}	352.3±35.1^{g}	754.7
Flour	101.8±3.4^{a}	23.9±4.6^{g}	7.7±0.5^{h}	n.d.	n.d.	133.4
Irina						
Brans	58.1±10.7^{f}	503.4±18.8^{b}	191.7±18.5^{a}	69.6±10.7^{b}	1108±58.9^{b}	1988.9
Middling	46.4±2.1^{g}	254.8±25.2^{c}	132.9±12.4^{d}	46.4±4.9^{e}	827.4±32.3^{e}	1788.4
Flour	29.1±1.8^{h}	46.7±0.01^{f}	24.4±1.3^{e}	n.d.	n.d.	100.2

n.d. – not detected

Figures

Figure 1.

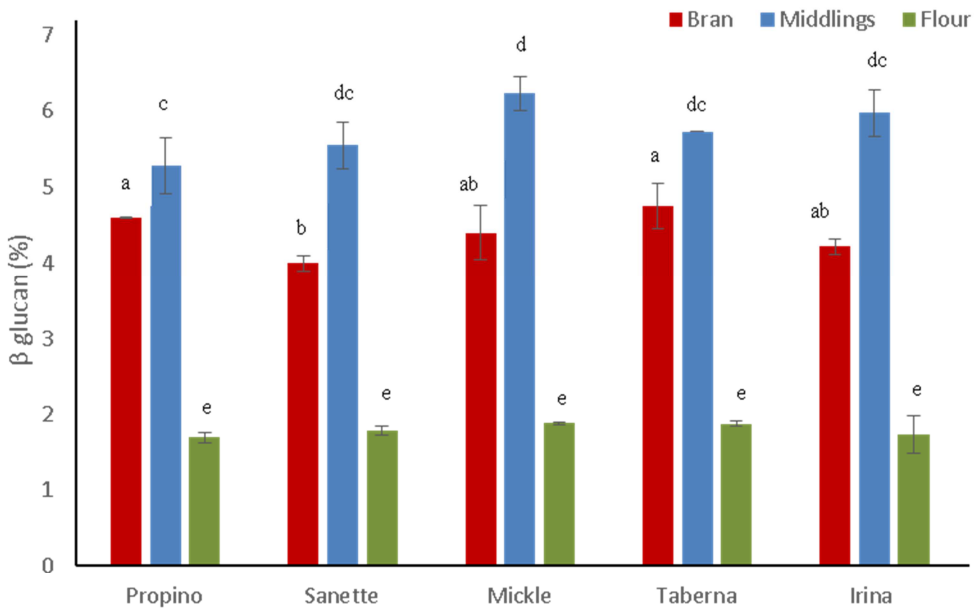


Figure 2.

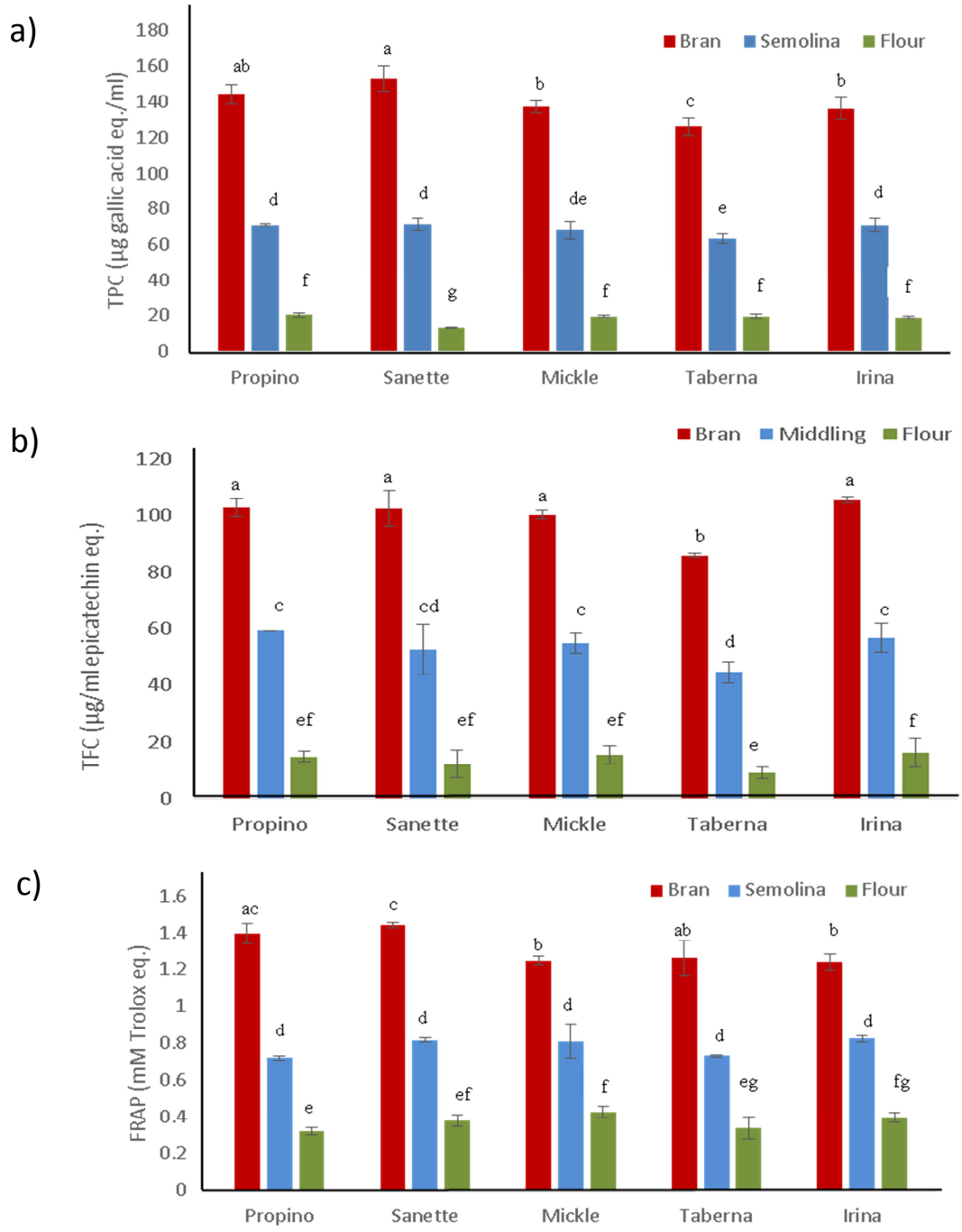

a)
TPC (µg gallic acid eq./ml)
180
160
140
120
100
80
60
40
20
0
Bran
Semolina
Flour
ab
d
f
a
d
g
b
de
f
c
e
f
b
d
f
Propino
Sanette
Mickle
Taberna
Irina
b)
TFC (µg/ml epicatechin eq.)
120
100
80
60
40
20
0
Bran
Middling
Flour
a
c
ef
a
cd
ef
a
c
ef
b
d
e
a
c
f
Propino
Sanette
Mickle
Taberna
Irina
c)
FRAP (mM Trolox eq.)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Bran
Semolina
Flour
ac
d
e
c
d
ef
b
d
f
ab
d
eg
b
d
fg
Propino
Sanette
Mickle
Taberna
Irina

Figure 3.

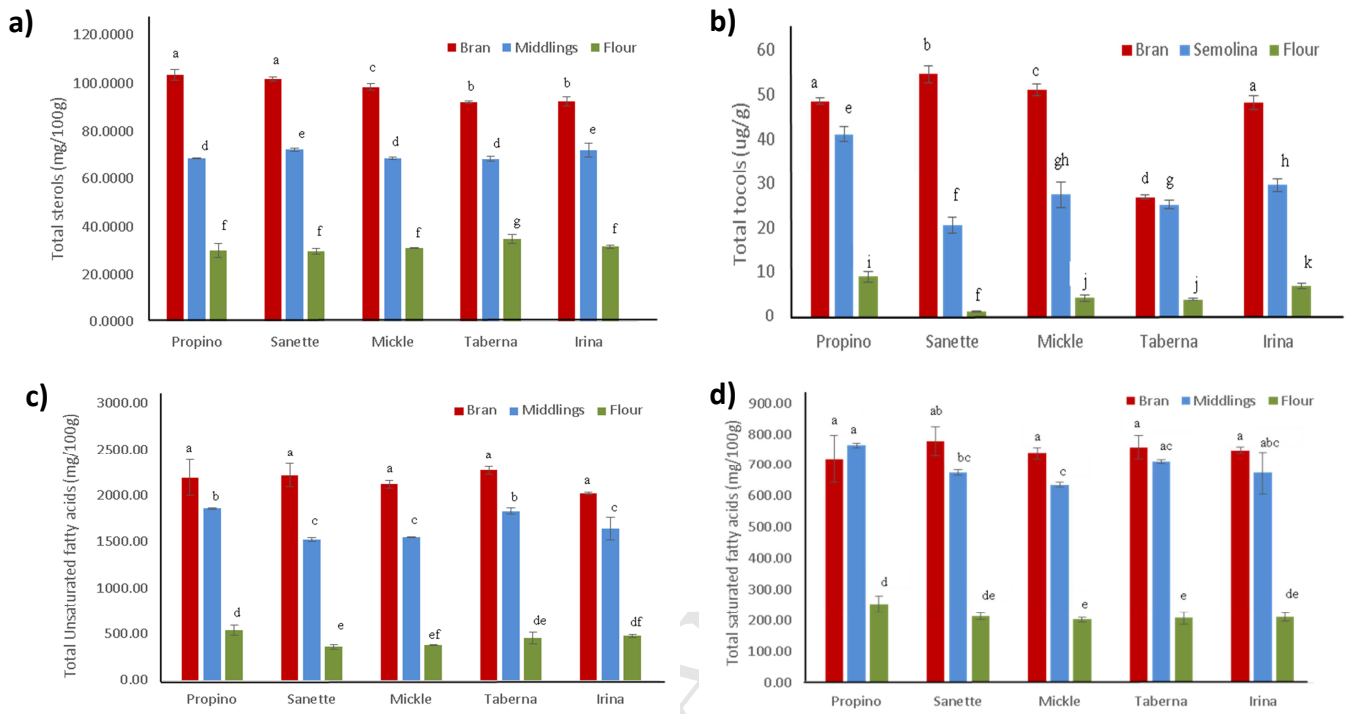
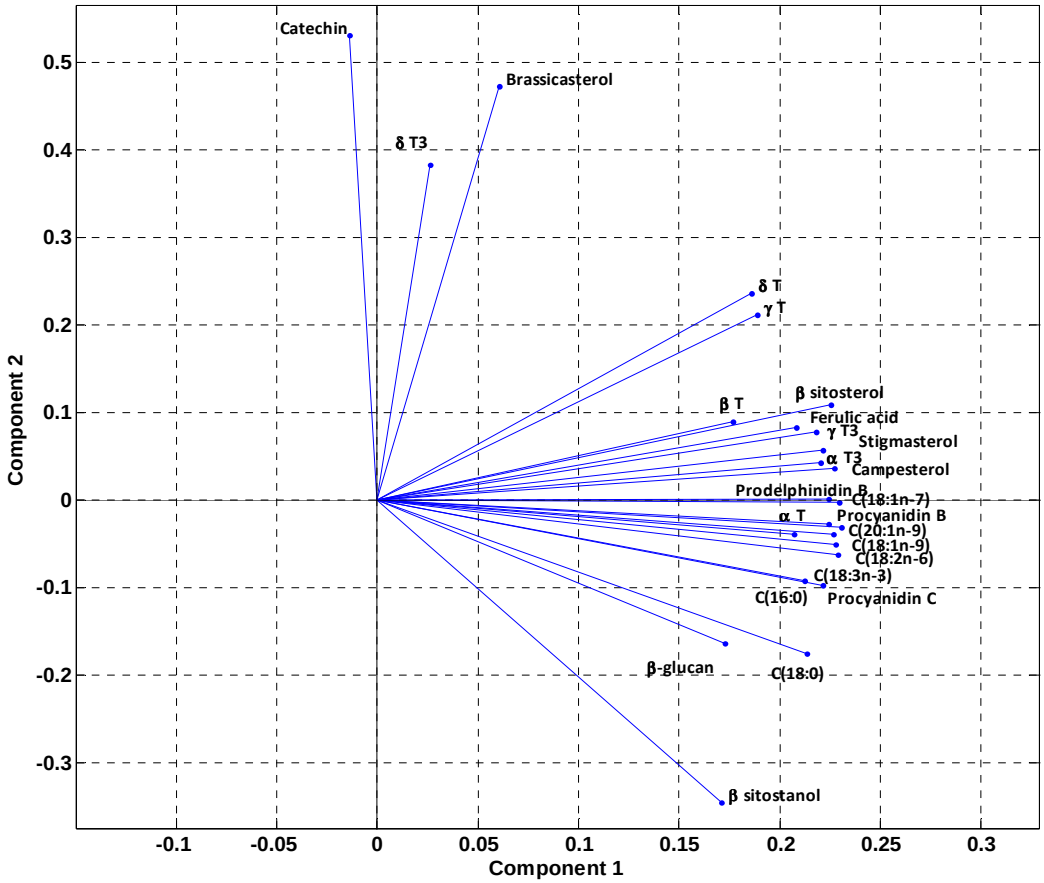
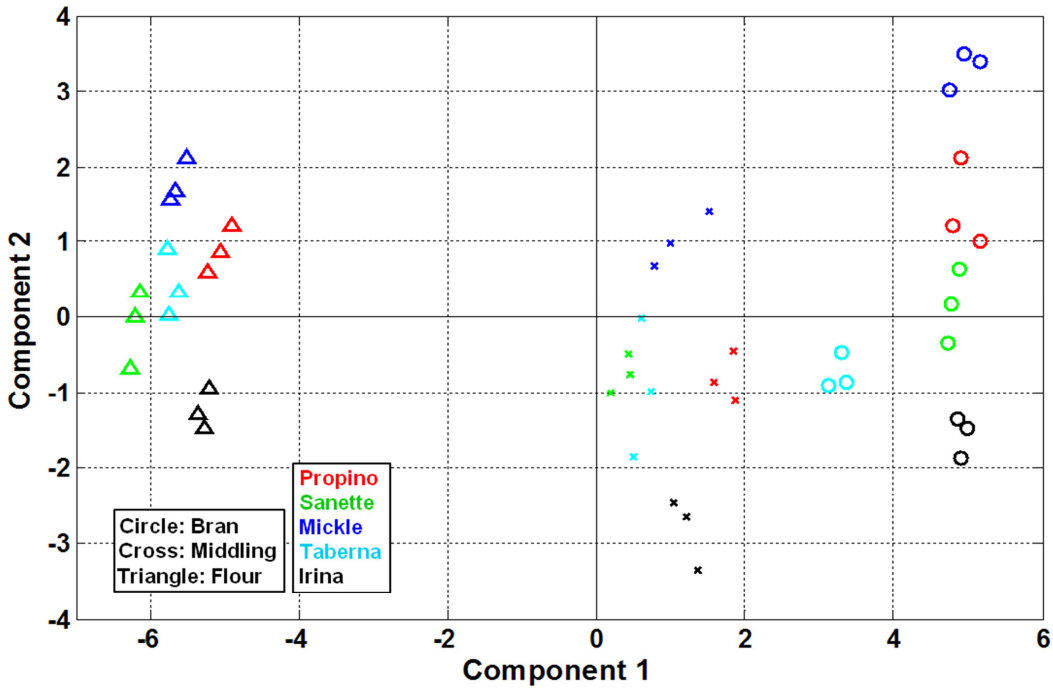


Figure 4.

a)


Catechin
Brassicasterol
δ T3
δ T
γ T
β T
β sitosterol
Ferulic acid
γ T3
Stigmasterol
α T3
Campesterol
Prodelphinidin B
C(18:1n-7)
α T
Procyanidin B
C(20:1n-9)
C(18:1n-9)
C(18:2n-6)
C(18:3n-3)
C(16:0)
Procyanidin C
β-glucan
C(18:0)
β sitostanol
Component 2
Component 1
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.1
-0.05
0
0.05
0.1
0.15
0.2
0.25
0.3

b)


Component 2
Component 1
4
3
2
1
0
-1
-2
-3
-4
-6
-4
-2
0
2
4
6
Propino
Sanette
Mickle
Taberna
Irina
Circle: Bran
Cross: Middling
Triangle: Flour

Supplementary Material

Table 1: Distribution of sterols (mg/100g) in the bran, middlings and flour fractions of roller-milled barley. In every column, values followed by different alphabetical superscripts are significantly different from each other.

Cultivar/Fraction	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	β-Sitostanol
Propino					
Bran	8.23±1.3^{a}	18.16±0.4^{a}	4.83±0.1^{a}	60.62±1.2^{a}	11.64±0.2^{a}
MIddlings	6.19±0.4^{d}	12.13±0.04^{d}	1.95±0.1^{c}	40.64±0.1^{f}	7.55±0.1^{e}
Flour	6.99±0.03^{ad}	4.17±0.01^{h}	0.44±0.01^{d}	15.7±2.9^{ijkl}	2.9±0.04^{j}
Sanette					
Bran	6.06±1.3^{bcd}	19.91±0.3^{b}	5.05±0.1^{a}	55.29±0.5^{b}	15.55±0.2^{b}
MIddlings	5.57±0.6^{d}	14.57±0.1^{e}	2.44±0.04^{d}	39.31±0.3^{g}	10.65±0.1^{f}
Flour	5.1±1.1^{d}	4.88±0.2^{i}	0.4±0.03^{d}	15.65±0.2^{i}	3.75±0.03^{k}
Mickle					
Bran	8.73±0.8^{a}	18.99±0.9^{ab}	4.38±0.02 ^{b}	57.14±0.8^{c}	9.27±0.1^{c}
MIddlings	6.47±0.1^{e}	12.96±0.1^{f}	2.02±0.03^{c}	40.81±0.4^{f}	6.16±0.01^{g}
Flour	6.03±0.3^{de}	4.47±0.1^{j}	0.35±0.01^{e}	17.87±0.3^{j}	2.4±0.04^{l}
Taberna					
Bran	7.06±0.3^{ab}	16.18±0.1^{c}	4.24±0.1^{b}	48.61±0.5^{d}	15.97±0.2^{b}
MIddlings	6.94±0.9^{ad}	11.89±0.2^{d}	1.97±0.1^{c}	35.97±0.6^{g}	11.56±0.2^{h}
Flour	7.55±1.2^{a}	4.74±0.1^{i}	0.41±0.1^{d}	16.87±0.3^{k}	5.34±0.1^{m}
Irina					
Bran	4.84±0.3^{c}	15.76±0.3^{c}	4.39±0.2^{b}	44.32±0.8^{e}	23.17±0.5^{d}
MIddlings	4.51±0.6^{a}	12.8±0.5^{g}	2.13±0.1^{c}	33.85±1.2^{h}	18.84±0.7^{i}
Flour	4.09±0.5^{a}	4.86±0.1^{i}	0.42±0.03^{d}	14.5±0.2^{l}	7.81±0.1^{n}

Supplementary Material

Table 2: Distribution of tocols (µg/g) in the bran, middlings and flour fractions of roller-milled barley. In every column, values followed by different alphabetical superscripts are significantly different from each other.

Cultivar/ Fraction	δ T3	γ T3	α T3	δ T	β T	γ T	α T
Propino							
Bran	1.15±0.08^{a}	5.49±0.08^{a}	30.39±0.3^{a}	0.91±0.1^{a}	0.40±0.04^{a}	2.28±0.1^{a}	7.93±0.3^{a}
Middlings	1.02±0.1^{a}	2.99±0.1^{f}	26.32±0.7^{e}	0.63±0.1^{e}	0.31±0.03^{d}	0.88±0.2^{d}	9.02±0.4^{d}
Flour	1.22±0.1^{a}	0.47±0.01^{h}	4.51±0.6^{i}	0.29±0.03^{h}	0.18±0.01^{e}	0.25±0.02^{g}	2.06±0.2^{f}
Sanette							
Bran	1.45±0.2^{b}	7.37±0.2^{b}	37.71±1.2^{b}	0.42±0.02^{b}	0.27±0.01^{b}	0.78±0.1^{b}	6.62±0.4^{b}
Middlings	1.25±0.1^{ab}	2.39±0.1^{g}	11.93±0.9^{f}	0.35±0.03^{f}	0.23±0.04^{d}	0.23±0.01^{e}	4.08±0.5^{e}
Flour	1.16±0.1^{a}	n.d.	n.d.	0.12±0.01^{i}	n.d.	n.d.	n.d.
Mickle							
Bran	1.67±0.3^{b}	6.12±0.3^{c}	33.61±0.7^{c}	0.90±0.02^{a}	0.39±0.1^{a}	1.51±0.1^{c}	6.95±0.3^{b}
Middlings	1.20±0.1^{a}	2.09±0.2^{g}	15.71±1.1^{g}	0.83±0.1^{g}	0.32±0.1^{d}	0.87±0.1^{d}	6.34±0.6^{b}
Flour	1.17±0.1^{a}	0.27±0.01^{i}	0.73±0.2^{j}	0.29±0.04^{h}	0.19±0.03^{e}	0.15±0.01^{h}	1.43±0.2^{g}
Taberna							
Bran	0.96±0.3^{a}	4.0±0.3^{d}	16.28±0.3^{d}	0.50±0.03^{c}	0.18±0.01^{c}	0.82±0.1^{b}	4.06±0.3^{c}
Middlings	1.0±0.2^{a}	2.36±0.1^{g}	14.53±0.5^{g}	0.35±0.1^{f}	0.21±0.03^{d}	0.46±0.02^{f}	6.23±0.4^{b}
Flour	1.03±0.02^{a}	0.33±0.02^{j}	1.08±0.02^{k}	0.17±0.02^{j}	n.d.	0.08±0.02^{i}	1.24±0.2^{g}
Irina							
Bran	1.05±0.1^{a}	5.14±0.1^{e}	31.39±1.1^{a}	0.79±0.03^{d}	0.47±0.1^{a}	1.48±0.1^{c}	7.90±0.3^{a}
Middlings	0.99±0.1^{c}	2.83±0.1^{f}	19.0±0.8^{h}	0.35±0.1^{f}	0.21±0.01^{d}	0.28±0.04^{e}	5.84±0.5^{b}
Flour	1.33±0.1^{a}	0.46±0.03^{h}	2.95±0.3^{l}	0.14±0.01^{j}	0.20±0.01^{e}	n.d.	1.85±0.2^{f}

n.d. – not detected

Supplementary Material

Table 3: Distribution of fatty acids (mg/100g) in the bran, middlings and flour fractions of roller-milled barley. For every variety, values followed by different alphabetical superscripts are significantly different from each other.

Cultivar/ Fraction	C16:0	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C20:1n9
Propino							
Bran	634.85±68.1^{a}	43.75±4.5^{ab}	430.5±33.4^{ad}	26.25±2.3^{a}	1573.4±142.6^{abcd}	131.8±11.9^{a}	22.25±1.7^{a}
Middlings	686.6±6.9^{a}	45.95±0.2^{a}	323.35±0.2^{e}	21.65±0.03^{b}	1386.4±4.8^{d}	108.45±1.6^{b}	16.35±0.07^{b}
Flour	227.8±23.2^{d}	16.65±1.7^{g}	88.55±9.1^{i}	6.3±0.7^{f}	445.95±43.5^{f}	n.d.	4.35±0.5^{d}
Sanette							
Bran	694.9±42.1^{a}	37.2±0.8^{a}	363.3±19.1^{b}	25.4±1.6^{a}	1649.5±101.2^{b}	147.3±1.6^{b}	24.3±1.5^{a}
Middlings	608.25±7.6^{b}	33.8±0.3^{d}	244.95±3.1^{f}	18.35±0.3^{c}	1146.3±17.3^{e}	96.2±1.5^{c}	16.25±0.2^{b}
Flour	192.1±9.6^{def}	12±0.6^{h}	46.1±3.4^{j}	4.2±0.3^{gh}	312.75±21.3^{g}	n.d.	2.7±0.2^{e}
Mickle							
Bran	663.7±14.7^{a}	35.3±1.8^{ae}	396.5±9.6^{abc}	26.5±0.5^{a}	1537.15±29.5^{a}	128.55±2.6^{a}	23.2± 0.6^{a}
Middlings	576.4±6.1^{c}	32.05±0.5^{de}	258.85±1.2^{g}	19.25±0.05^{d}	1158.75 ±2.4^{e}	96.5 ±0.5^{c}	15.5± 0.1^{c}
Flour	185.85±8.4^{ef}	11.35±0.04^{i}	53.55±0.2^{k}	4.7±0.1^{gh}	327±5.1^{g}	n.d.	3.0 ±0.005^{e}
Taberna							
Bran	673±33.9^{a}	43.65±1.3^{bc}	455.85±3.8^{d}	26.3±0.6^{a}	1633.15±35.7^{b}	127.1 ±0.2^{a}	24.05± 1.2^{a}
Middlings	643.3±8.2^{a}	38.95 ±0.6^{f}	324.8±2.1^{e}	20.25 ±0.2^{e}	1364.5 ±24.2^{d}	102.7 ±4.6^{b}	16.55 ±0.04^{b}
Flour	187.25±15.6^{f}	13.4 ±2.0^{hi}	74.75±12.4^{i}	5.05 ±0.7^{g}	375.6 ±45.8^{fg}	n.d.	3.8±0.5^{f}
Irina							
Bran	656.2±9.6^{a}	42.15±0.2^{c}	393.6±2.4^{c}	23.9 ±0.6^{a}	1452.95±8.5^{c}	125 ± 0.6^{a}	23.05 ±0.2^{a}
Middlings	597.5±64.4^{a}	41.65±4.2^{be}	289.15±20.4^{h}	17.95 ±1.5^{d}	1218.15±92.1^{e}	96.25±6.1^{c}	16.4 ±1.1^{b}
Flour	187.3±10.9^{f}	14.3±1.7^{gh}	76.75 ±2.1^{i}	4.3 ±0.1^{h}	377.3±13.9^{f}	22.35 ±1.3^{d}	4.05 ±0.2^{d}

n.d. – not detected

Highlights

1. First report on distribution of health-salutary molecules in roller-milled fractions in barley cultivars.
2. Polyphenols, tocols and phytosterols were abundant bran fraction.
3. β-glucan was predominant in the middlings.
4. Bran and middling fractions, generally considered as by-products, are good functional food ingredients.