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Fate of beta-glucan, polyphenols and lipophilic compounds in baked crackers fortified with different barley-milled fractions

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Abstract

Four types of crackers were prepared, whereby wheat flour was substituted with different percentages of barley flour and bran. These formulations were compared to a 100% wheat flour (control) cracker with respect to β-glucan, polyphenols and lipophilic bioactives. Incorporation of barley fractions enriched the β-glucan, and phenolic content, as well as *in vitro* antioxidant capacities of the crackers. However, some polyphenols including procyanidin C and ferulic acid could not be detected in the crackers owing to the probable degradation of these compounds during baking. The β-glucan, flavanols (catechin and procyanidin B), as well as fatty acids and sterols were least affected; while the α-tocotrienols showed degradation following the baking process. Overall, barley fractions can serve as valued ingredients for enhancing the health-salutary components of fortified crackers or the products thereof.

Keywords: barley bran; barley flour; baking; fortified crackers

1. Introduction

Processing is a common pre-requisite for the consumption of whole grains as it transforms them into an organoleptically suitable form for inclusion into other foods. Indeed, knowledge of the effects of food processes, such as baking or extrusion, on the amount and availability of bioactive compounds in the grain is important when considering the development of nutritionally enriched wholegrain food products. Although the incorporation of bioactive-enriched barley fractions in food products such as noodles (Izydorczyk, Lagassé, Hatcher, Dexter, & Rossnagel, 2005), breads (Holtekjølen, Bævre, Rødbotten, Berg, & Knutsen, 2008), cakes (Gupta, Bawa, & Semwal, 2009), and extruded snacks (Altan, McCarthy, & Maskan, 2009) has been discussed, key knowledge gaps exist in the area of evaluation of the effect of these food processes on bioactive compounds in the grain. A number of studies have reported the effect of extrusion cooking and baking on the total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity of barley flour (Sharma, Gujral, & Singh, 2012; Sharma & Gujral, 2014), the effect of food processing on the most abundant polyphenols or the major lipophilic components in barley has never been investigated.

Processing by sourdough fermentation is widely used for baking breads and crackers, which is known to improve the flavour and structure of wheat and rye breads (Lorenz & Brummer, 2003). Sourdough fermentation has been shown to modify, and enhance the levels of bioactive compounds in wholemeal rye and wheat breads (Liukkonen et al., 2003; Kariluoto et al., 2004; Katina et al., 2005; Katina et al., 2007), however its effect on the bioactives in barley fractions has not been explored to date.

The variability in distribution of bioactive compounds within the grain of barley was discussed in the previous work (Gangopadhyay et al., 2018), which indicated that the incorporation of barley fractions in food products could provide potential health and nutritional benefits. Therefore, the objectives of this study were: 1) to analyse the level of bioactive compounds, including β-glucan, polyphenols, lipophilic bioactives, and antioxidant capacities in crackers substituted with barley fractions, and 2) to evaluate the impact of sourdough fermentation and baking on these compounds.

2. Materials and Methods

2.1. Chemicals

The mixed-linked β-glucan assay kit for *β*-glucan determination was purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). Polyphenol standards catechin and

ferulic acid, the reagents and solvents for the assays including Folin-Ciocalteau reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferric chloride hexahydrate, 2,4,6-tri(2-pyridyl)-s-triazine, gallic acid, sodium carbonate, sodium acetate anhydrous, 98% sulphuric acid, sodium nitrite, sodium hydroxide and aluminium chloride were purchased from Sigma Aldrich (Now Merck, Co. Wicklow, Ireland). For analysis of lipids, standards of all the sterols, the fatty acid methyl ester (FAME) standard mix (Supelco 37 component FAME mix, Bellefonte, PA, USA), *α*-, *β*-, *γ*-, *δ*- tocopherol (T) homologues, reagents and solvents including 5*α*-cholestan-3*β*-ol, tricosanoic acid, bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (TMCS), potassium hydroxide, ascorbic acid, pyridine, acetyl chloride, heptane, pentane, dichloromethane, methanol, hexane, were purchased from Sigma Aldrich (Now Merck, Co. Wicklow, Ireland). The standards of *α*-, *γ*-, *δ*- tocotrienols (T3) were purchased from Chromadex, Inc (Irvine, CA, USA).

2.2. Plant material

The Irish spring barley variety Mickle, used for this study, was provided by Seedtech (Waterford, Ireland). The barley grains were milled through the break side (over 3 grooved rollers), and sifted via centrifugal sifting using a CD1 roller mill (Chopin Technologies, Villeneuve-la-Garenne, France). The milled fractions, i.e. barley bran (BB) and barley flour (BF), were used for incorporation in the cracker formulations.

2.3. Preparation of the cracker formulations

Five formulations of crackers were prepared at Teagasc Ashtown based on preliminary optimisation studies. The aromatic composition and physicochemical characteristics of these crackers have been comprehensively performed as a separate set of experiments and published previously as a part of the NutriCerealIreland project (O'Shea, Kilcawley, & Gallagher, 2017). These five formulations included a control cracker formulated using 100% plain white wheat flour, and four test crackers with the wheat flour partially substituted with varying percentages of barley bran (BB) or flour (BF) as outlined in Table 1.The chemical compositions (β-glucan, polyphenols, lipophilic bioactives) of the raw materials (plain white flour, barley bran, barley flour) have been shown in the Supplementary Material (Table S1).

All crackers were prepared in an identical manner, beginning with the preparation of the sponge using wheat flour (with or without barley bran/flour), yeast, starter cultures and water. After proofing for 18 h at room temperature the sponge was made into a dough by further addition of wheat flour, salt, soda and shortening (vegetable fat). After proofing for another

1.5 h, the dough was sheeted and baked into crackers. The baked crackers were cooled for 2 hours and stored in polyethylene bags at room temperature until further analysis.

2.4. Determination of β-glucan content

β-glucan content in the crackers was determined using the Megazyme mixed-linkage β-glucan kit (Megazyme Ltd, Co. Wicklow, Ireland). The concentration of β-glucan has been reported as percentage (%) or g of β-glucan/100 g of sample. Molecular weight (MW) determination of β-glucan in the crackers by size exclusion chromatography was completed using the protocol outlined in a previous study published by the authors (Gangopadhyay, Hossain, Rai, & Brunton, 2015).

2.5. Determination of total phenol content (TPC), total flavonoid content (TFC), and ferric reducing antioxidant power (FRAP)

Polyphenols in the crackers were extracted in three sequential steps using 80% methanol at a solid to solvent ratio of 1:10 for 40 min at 60 ^{O}C (Gangopadhyay, Rai, Brunton, Gallagher, & Hossain, 2016a). TPC, TFC, and antioxidant capacity of the polyphenolic extracts of crackers were determined by adapting previous methods (Lin & Tang 2007; Stratil, Klejdus, and Kubáň, 2006). Each sample was analysed in triplicate for the assays; TPC of the crackers was expressed as mg gallic acid (GA) equivalent (eq.)/ 100g cracker, TFC was expressed as mg epicatechin eq./ 100g cracker, while antioxidant capacity by FRAP was expressed as μg Trolox eq./ 100g cracker.

2.6. Determination of flavanols and ferulic acid

Three flavanols (catechin, procyanidin B, procyanidin C) and ferulic acid were detected in the methanolic extracts of the crackers using ultra-high performance liquid chromatography coupled with tandem mass spectrometry using in-house protocol described before (Gangopadhyay, Rai, Brunton, Gallagher, & Hossain, 2016a). Detection and quantification of these polyphenols were performed in the negative ion mode using multiple reaction monitoring experiments. 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. The standard curve for procyanidin B1 was used for quantification of procyanidin B and procyanidin C.

2.7. Determination of lipophilic bioactives

Lipophilic bioactives namely unsaturated fatty acids, sterols and tocols (Vitamin E) were extracted and analysed in the crackers. Composition of fatty acids, sterols, and tocol

homologues in the crackers was determined as per the protocol outlined in our previous paper (Gangopadhyay, Rai, Brunton, Gallagher, & Harrison, 2016b).

The fatty acids in the crackers were extracted using a microwave-assisted extraction, and derivatised to fatty acid methyl esters (FAMEs), which were analysed and quantified using a gas chromatography-flame ionisation detector (GC-FID). Each sample was tested in duplicates for FAME analysis.

For determination of sterols, the samples were extracted by saponification, and further silylated for their detection and quantification using GC-FID. The samples were tested in triplicates for the analysis of sterols.

For analysis of tocols, the samples were mixed with hexane for extraction of the tocols, which were then analysed for detection and quantification of tocols using reversed-phase HPLC analysis.

2.9. Statistical analysis

For duplicate and triplicate determinations, data has been reported as mean ± standard deviation. ANOVA was performed using IBM SPSS statistical software (v20, Chicago, IL, USA).

3. Results and Discussion

The conventional recipe for making crackers involves the use of 100% plain white wheat flour. The barley bran (BB) is an important source of many health-salutary compounds (Gangopadhyay et al., 2018). However, the BB is an underutilised product of milling, and hence its incorporation into food products could be beneficial in enhancing the nutritional and biofunctional value of the products. Therefore, wheat flour was substituted with barley bran at two addition levels (10% and 15%) in the crackers. Two other cracker formulations wherein the wheat flour was substituted with barley flour, at levels of 15% and 35% substitution) were also prepared. The presence of health-salutary compounds and the impact of sourdough fermentation and baking on these compounds in the final products (crackers) was evaluated.

3.1. β-glucan content of crackers

β-glucan is an important dietary fibre component of barley, and therefore the effect of fermentation and baking on the β-glucan content and its MW was determined. The β-glucan

contents of the cracker formulations are presented in Table 2. Among the crackers, the control cracker with 100% WF had the lowest β-glucan content of 0.06 g/100g sample. All the test crackers in which the wheat flour was partly substituted with BB or BF had significantly ($p<0.05$) higher β-glucan than the control cracker. This indicated enrichment in β-glucan content of the crackers due to the incorporation of barley fractions. The highest β-glucan content (1.06 g/100g sample) was determined in the cracker containing 35% BF. However the low β-glucans in BB incorporated crackers despite the fact BB contained ~2.4 fold higher β-glucan content than BF (Gangopadhyay et al., 2018) could be a result of the action of the endogenous enzyme β-glucanase located in the bran of barley grain (Litts, Simmons, Karrer, Huang, & Rodriguez, 1990). During the process of sourdough fermentation, hydration of the flour mixture catalyses partial degradation of the β-glucan by β-glucanase, which results in decreased retention of β-glucan in crackers containing BB. On the other hand, β-glucanase would be little or absent in samples containing BF.

MW determination studies indicated low MW of β-glucan in the range of 10 to 15 kDa in all the examined test crackers (data not shown). Size-exclusion chromatograms (Fig. 1) showing a bimodal and late eluting peak of β-glucan at 21.8 min in the cracker substituted with 15% BB (Fig. 1b), as opposed to a unimodal peak at 11.9 min in the wholegrain barley extract (Fig. 1a) have been provided. The late peak in the 15% BB cracker corresponded to a low MW β-glucan (12 kDa) formed possibly as a degradation product following baking. A similar decrease in MW of a high MW β-glucan in barley during baking was reported by Cleary, Anderson, & Brennan, 2007.

3.2. TPC, TFC and antioxidant capacity (FRAP analysis) of crackers

The TPC, TFC and antioxidant capacity (measured by FRAP) of the control and test crackers are illustrated in Fig. 2. The control cracker containing 100% WF had significantly lower TPC as compared to all the other test crackers. The TPC in all the samples were significantly different from each other. The test cracker substituted with 15% BB had the highest TPC followed by 10% BB and then by 35% BF, while the least in the 15% BF containing crackers. This arises due to the inherently high TPC of BB, which imparts higher TPC values to the crackers containing BB. A similar trend was observed in the FRAP assay indicating a high correlation between the two assays. In case of flavonoids (TFC) as well, the cracker with 15% BB had significantly higher TFC than all the other test crackers. The

following decreasing order of TFC was observed in the test crackers: 15% BB > 35% BF > 10% BB > 15% BF > 100% WF. All the assays indicated an enrichment of polyphenols in the crackers fortified with different types of barley fractions.

3.3. Flavanols and phenolic acids in crackers

Although the effect of food processes like extrusion cooking and roasting on the TPC and antioxidant capacity of barley has been evaluated to some extent (Altan, McCarthy, & Maskan, 2009; Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, & Pulido-Alonso, 2010; Sharma & Gujral, 2011; Sharma, Gujral, & Singh, 2012), their effect on specific classes of polyphenols in barley has never been reported before. We previously identified phenolic compounds belonging to the classes of flavanols and phenolic acids as the major contributors to the antioxidant capacity of barley (Gangopadhyay, Rai, Brunton, Gallagher, & Hossain, 2016a). Therefore, in the present research paper, the effect of fermentation and baking on three major barley-flavanols, namely catechin, procyanidin B, procyanidin C, along with ferulic acid was evaluated (Table 2).

Catechin and procyanidin B were detected in all the BB and BF containing test crackers but not in the control (Table 2). The absence of polyphenols in control cracker (100% plain white wheat flour) may be a result of the absence of flavanols in the endosperm of wheat (Adom, Sorrells, & Liu, 2005). The highest amount of catechin was detected in the test cracker substituted with flour fractions (35% BF followed by 15% BF), whilst the highest procyanidins were in BB incorporated crackers correlating the phenolic profile recorded on this barley variety (i.e. Mickle) in our previous work (Gangopadhyay et al. 2018).

Procyanidin C and ferulic acid could not be detected in any of the five cracker types, possibly as a result of their degradation during the processes of fermentation and/or baking. Degradation of oligomeric flavanols and anthocyanins in grape and blueberry pomace due to extrusion processing has previously been reported (Khanal, Howard, & Prior, 2009a; Khanal, Howard, Brownmiller, & Prior, 2009b). It has been reported that the temperature sensitive polymeric flavanols degrade and convert to lower oligomers and monomers during heat treatment. Ferulic acid has been known to degrade to vinylguaiacols in presence of heat (Fiddler et al. 1967).

3.4. Lipophilic bioactive content of crackers

As reported in the previous paper (Gangopadhyay, Rai, Brunton, Gallagher, & Harrison, 2016b), barley is a source of the three main classes of lipophilic bioactives: fatty

acids, phytosterols, and tocols (Vitamin E), however no studies exist on the effect of processing on these lipid components, which was taken into consideration in the current study.

Fig. 3 gives an overview of the effect of fermentation and baking on the overall content of unsaturated fatty acids (UFA), sterols and tocols in barley. A sum total of 9 types of UFA (including monounsaturated and polyunsaturated fatty acids), 5 types of sterols, and 7 types of tocols (including 4 tocopherols (Ts) and 3 tocotrienols (T3s)) were considered for this study. No significant difference ($p<0.05$) was observed in the total UFA and sterols contents between the five types of crackers. In tocols, the highest level was detected in the crackers containing 15% BB, which did not differ significantly ($p<0.05$) from the control cracker. In general, no noticeable effect of the incorporation of barley fractions on the overall content of lipophilic bioactives in the crackers was observed.

A detailed analysis of the effect of processing on the individual fatty acids, sterols and tocols in the crackers was also undertaken in our study.

3.4.1. Fatty acids in crackers

A total of 18 saturated (SFA) and unsaturated fatty acids (UFA) were identified in the control and test crackers. However, only the two most abundant SFA namely palmitic acid (16:0) and stearic acid (18:0), and the three most abundant UFA, i.e. linoleic acid (18:2n6), oleic acid (18:1n9), and α-linolenic acid (18:3n3) were considered in this study (Table 3). No significant differences ($p<0.05$) were observed in the contents of the two SFA between the control and test crackers. Although linoleic acid is the most abundant UFA in barley and its fractions (Gangopadhyay, Rai, Brunton, Gallagher, & Harrison, 2016b), the most abundant UFA detected in the crackers was oleic acid, which was likely due to the high oleic acid content of the shortening used for dough making. With regard to UFA, none of the cracker formulations was particularly high in any of the three UFA, the probable reason being the high fatty acid content of the shortening used in the process of dough making (data not shown), which masked the small differences arising from the contribution of barley fractions in the crackers.

3.4.2. Sterol content of crackers

The most abundant sterol in all the crackers was β sitosterol (Table 3). The cracker containing 15% BB had the highest amounts of β-sitosterol, campesterol, stigmasterol and β-

sitostanol; however, their levels did not differ significantly ($p<0.05$) from the crackers containing BF. The wheat flour and shortening used for preparation of the crackers could be a good source of β-sitosterol (data not shown), contributing to most of the β-sitosterol in the crackers. The shortening also contained several times higher amounts of brassicasterol and campesterol, which masked the differences between the control and test crackers as in the case of fatty acids. In case of stigmasterol and β-sitostanol, the lowest amounts were detected in the control cracker indicating the contribution of barley fractions in enhancing their level in the test crackers.

3.4.3. Tocols in the crackers

As reported previously (Gangopadhyay, Rai, Brunton, Gallagher, & Harrison, 2016b), the most abundant tocol homologue in the barley fractions was α-tocotrienol (αT3). However, αT3 could not be detected in any of the five types of crackers (Table 3) indicating complete degradation. The abundant tocol homologues in the crackers were α-tocopherol (αT) and γ-tocotrienol (γT3). In the case of αT, the highest amount (~570 μg/100g) was detected in the control cracker, while the 15% BB containing test cracker had significantly ($p<0.05$) higher amount of the homologue γT3, as well as γ-tocopherol (γT), δ-tocopherol (δT), and δ tocotrienol (δT3) compared to the control and other test crackers (Table 3). Between the control and BF substituted test crackers, the control cracker had significantly ($p<0.05$) higher amounts of all the seven tocol homologues, except γT, as compared to the substituted BF crackers. Thus, substituting wheat flour with barley flour in the cracker formulations did not affect the levels of the tocols in the final cracker. In summary, although incorporation of the barley bran enhanced the levels of the tocol homologues (except αT and βT) in the test crackers, incorporation of barley flour diminished the levels of the tocols in the test crackers as compared to the 100% plain white wheat flour crackers. A 63 – 94% loss of tocols due to hydrothermal processing in cereals such as barley, rye, and oat (Zieliński, Kozlowska, & Lewczuk, 2001), and a 62% loss in buckwheat groats (Zieliński, Michalska, Piskula, & Kozlowska, 2006) has been reported previously, which indicated the susceptibility of tocols to heat degradation.

4. Conclusions

Barley is a source of several bioactive compounds including β-glucan, polyphenols, and lipophilic bioactives. Majority of these bioactive molecules are located in the bran of barley (BB), which is an under-utilised milling by-product. Test crackers incorporated with

barley bran (BB) or barley flour (BF) were richer in the β-glucan and polyphenol contents, and had higher antioxidant capacity compared to the control cracker (100% wheat). Specific polyphenols, namely catechin and procyanidin B, were high in barley-fraction (BB, BF) fortified crackers, whilst these compounds were absent in the control crackers. However the trimeric flavanol (procyanidin C) and ferulic acid were probably thermally degraded as they could not be detected in the barley-fortified crackers. The overall lipophilic (fatty acids, sterols, tocols) contents between the test and control crackers did not significantly differ, but the individual components (β-sitosterol, β-sitostanol, stigmasterol, βT, γT, δT, γT3, and δT3) were found in higher amount in the 15% BB crackers with respect to the control. On the other hand, the most abundant α-tocotrienol in barley could not be detected in the barley-incorporated crackers indicating its complete degradation upon baking. Nevertheless, the barley bran and flour would serve as valued ingredients for enhancing the levels of health-salutary compounds in prepared products such as crackers.

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Tables

Table 1: Formulations of crackers including a control cracker formulated using 100% plain white wheat flour, and four test crackers with the wheat flour partially substituted with varying percentages of barley bran or flour.

Cracker Type	% Wheat flour	% Barley flour	% Barley bran
Control	100	0	0
Test 1	90	0	10
Test 2	85	0	15
Test 3	85	15	0
Test 4	65	35	0

Table 2: The % β-glucan content (mean±SD, n=2) and detected polyphenols (catechin and procyanidin B) in µg/100g (mean±SD, n=2) in the crackers prepared by sourdough fermentation and baking. For all the crackers, the values followed by different alphabetical superscripts are significantly different from each other.

Cracker Type	100% wheat flour	10% barley bran	15% barley bran	15% barley flour	35% barley flour
β-glucan (%)	0.06±0.01^{a}	0.39±0.02^{b}	0.60±0.004^{c}	0.50±0.01^{d}	1.06±0.02^{e}
Catechin (µg/100g)	not detected	2137.55±7.25^{a}	3494.04±514.62^{b}	2988.34±74.89^{b}	6352.24±297.17^{c}
Procyanidin B (µg/100g)	not detected	5415.16±429.99^{a}	6593.78±65.22^{b}	1287.4±201.71^{c}	3507.2±211.73^{d}

Table 3: The detected amount of fatty acids (mg/100g), sterols (mg/100g) tocols (μg/100g) in the crackers prepared by sourdough fermentation and baking. In all the tables the values reported for a single lipophilic compound in a row followed by different alphabetical superscript are significantly different from each other.

Cracker Type	100% wheat flour	10% barley bran	15% barley bran	15% barley flour	35% barley flour
Fatty acids (mg/100g)					
C16:0	1303.54±45.69^{a}	1404.32±50.41^{a}	1331.85±89.99^{a}	1431.35±35.96^{a}	1348.49±21.30^{a}
C18:0	123.56±4.15^{a}	130.46±3.68^{a}	123.47±5.11^{a}	134.27±5.78^{a}	125.83±4.34^{a}
C18:1	1339.30±31.55^{a}	1418.46±42.71^{ab}	1352.15±42.57^{ab}	1455.4±41.01^{b}	1342.0±18.58^{ab}
C18:2	815.17±62.13^{a}	957.18±38.61^{b}	931.08±93.81^{ab}	900.0±22.21^{ab}	894.93±14.24^{ab}
C18:3	103.97±4.91^{a}	117.02±4.96^{b}	113.63±7.43^{ab}	114.17±3.17^{ab}	109.7±1.05^{ab}
Sterols (mg/100g)					
Brassicasterol	10.27±2.44^{a}	8.26±1.68 ^{a}	10.23±0.66 ^{a}	9.61±1.54 ^{a}	10.13±2.33^{a}
Campesterol	12.26±0.17^{ab}	12.08±0.82^{a}	13.84±0.81^{b}	12.92±0.62^{ab}	11.99±1.3^{a}
Stigmasterol	0.65±0.01^{a}	0.95±0.09^{b}	1.20±0.03^{c}	0.84±0.04^{d}	0.95±0.09^{b}
β-sitosterol	30.89±1.1^{a}	30.38±1.8^{a}	34.51±1.5^{b}	32.65±1.5^{ab}	31.34±2.6^{ab}
β-sitostanol	1.12±0.27^{a}	1.84±0.11^{b}	2.46±0.11^{c}	1.92±0.06^{ab}	2.6±0.23^{c}
Tocols (μg/100g)					
αT	569.66±18.44^{a}	259.84±10.3^{b}	378.19±39.84^{c}	315.92±9.28^{d}	186.73±10.98^{e}
βT	137.36±7.19^{a}	101.86±11.32^{b}	130.24±9.81^{a}	88.06±3.99^{c}	65.74±5.64^{d}
γT	70.32±13.97^{ac}	75.75±9.09^{a}	96.57±9.87^{b}	59.16±4.37^{c}	62.19±6.29^{c}
δT	57.36±3.85^{a}	47.98±3.11^{b}	67.62±6.46^{c}	38.33±1.42^{d}	38.89±2.11^{d}
γT3	351.07±31.59^{a}	368.05±40.99^{a}	454.19±51.47^{b}	222.07±6.83^{c}	224.94±30.08^{c}
δT3	239.45±14.22^{a}	194.95±13.71^{b}	256.53±26.84^{c}	189.06±11.59^{b}	174.15±11.53^{b}

Figure captions

Figure 1: HPLC-RI chromatogram showing the peak of β-glucan in the a) extract of raw wholegrain barley with molecular weight of 391 kDa eluting at 11.9 min, and b) extract of cracker containing 15% barley bran showing a peak of 12 kDa β-glucan eluting at 21.8 min.

Figure 2: (a) Detected TPC (mg GA eq./100g), FRAP (µg Trolox eq./100g), and TFC (mg epicatechin eq./100g) in the crackers (mean±SD, n=3). For each assay, the bars bearing different letters are significantly different ($p<0.05$) from each other.

100% wheat flour, 10% barley bran, 15% barley bran, 15% barley flour, 35% barley flour

Figure 3: (a) Detected concentrations of total unsaturated fatty acids (UFA) in mg/g, total sterols in mg/100g, and total tocols in µg/g in the crackers. For each class of lipophilic compounds, the bars bearing different letters are significantly different ($p<0.05$) from each other**.**

100% wheat flour, 10% barley bran, 15% barley bran, 15% barley flour, 35% barley flour

Figure 1

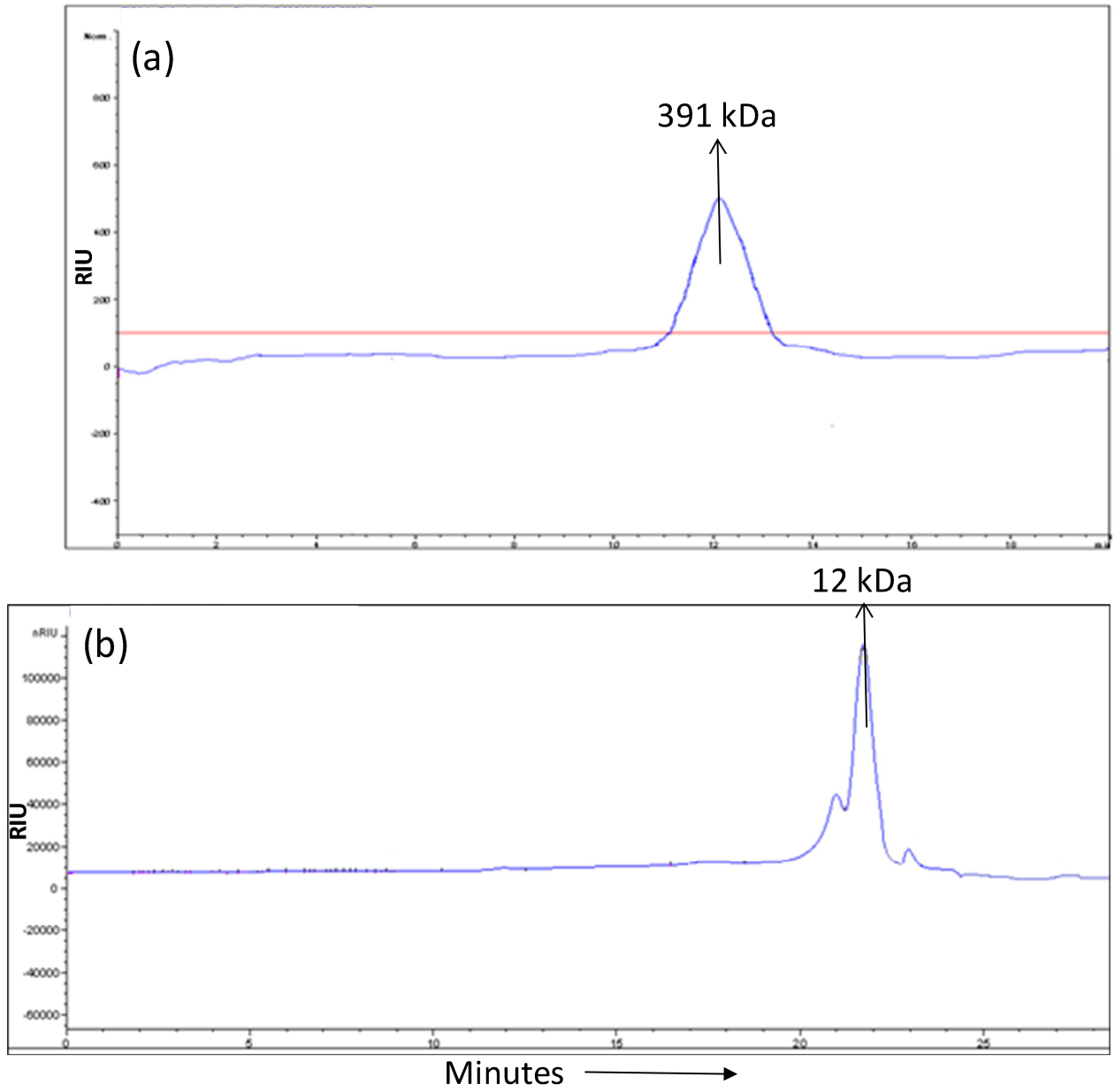


Figure 2

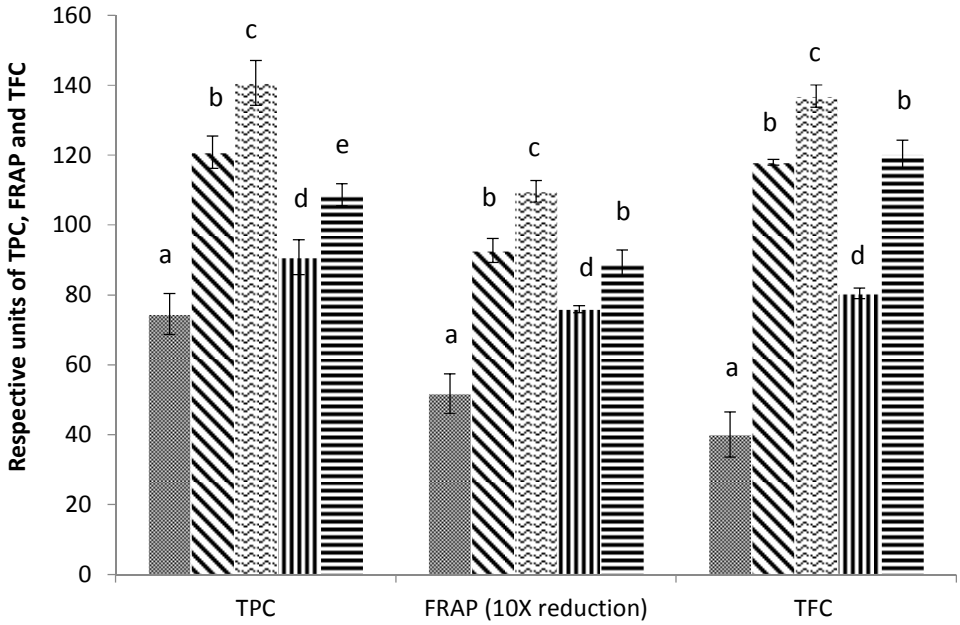
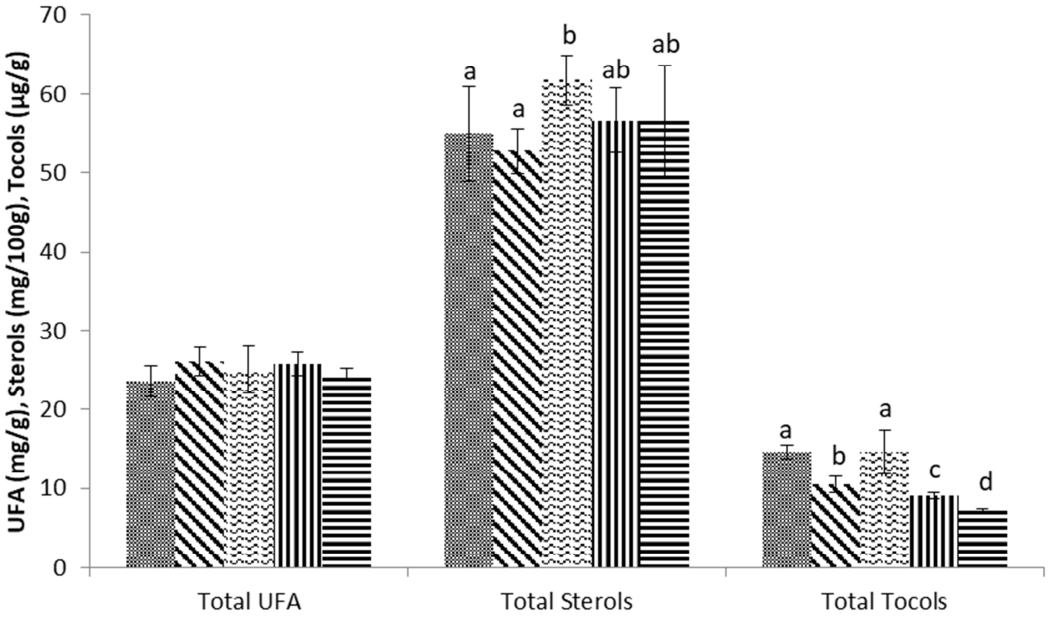


Figure 3



Title: Fate of beta-glucan, polyphenols and lipophilic compounds in baked crackers fortified with different barley-milled fractions

Highlights

- Incorporation of barley bran and flour fractions improved cracker's functional quality
- Barley bran enhanced β-glucan, polyphenols, and antioxidant ability of crackers
- The overall lipophilic content of barley substituted crackers remained unaffected
- Processing caused thermal degradation of procyanidin C, ferulic acid, and tocols

Conflicts of interest

The authors declare no conflict of interest.