*Research Article***IMPACT OF SELECTED INDUSTRIAL AND LABORATORY PROCESSING TECHNIQUES ON THE PHYTIC ACID CONTENT IN BARLEY FLOUR: A COMPARATIVE APPROACH****Sandaru Jayathissa Nuwara Pakshage^{1✉}, Anne Taylor-Vissers¹**¹*School of Health and Life Sciences, Teesside University, United Kingdom*[✉]*Corresponding author: Sandaru Jayathissa Nuwara Pakshage E mail: sandaru.food@outlook.com;**Mobile: +447900710853 ORCID:0000-0002-4369-217X*<https://doi.org/10.34302/crpjfst/EUUU2020>**Article history:****Received:**November 25th, 2025**Accepted:**August 25th, 2026**Published**September 20th, 2026**Keywords***Phytic acid;**Malting;**Roasting;**Megazyme assay kit.***Abstract**

Barley is mainly utilized in brewing, and for processing breakfast cereals and fermented bakery products. Phytic acid (PA) is the major storage form of phosphorus in barley. It is considered an antinutrient because it chelates polyvalent mineral cations, hereby reducing their bioavailability in humans and animals. Therefore, the main aim of the study was to apply malting and roasting as treatments in order to understand enzymatic and thermal effects on reducing PA level. Four industrially processed barley flour types—whole or raw barley (RB), malted barley (MB), and malted barley subjected to both mild (MB-l) and intense (MB-i) roasting—along with two raw flours subjected to mild (R-l) and intense (R-i) roasting in the laboratory, were analysed in quadruplicates for their PA contents using the Megazyme® phytic acid assay kit. Data were analysed by one way ANOVA and Tukey HSD tests using SPSS. The PA content of whole raw barley was 0.86 ± 0.03 g/100 g d.b. All malted flours—MB, MB-l, MB-i—contained significantly less ($P < 0.05$) PA than raw flour but treated flours did not differ significantly from one another ($P > 0.05$). Similarly, roasted barley in the laboratory—R-l and R-i—contained significantly ($P < 0.05$) less PA compared to RB. The free phosphorus content of industrial barley flour increased as follows ($P < 0.05$): RB < MB < MB-l < MB-i. Similarly, among the roasted flours, R-i contained the highest free phosphorus level. Endogenous phytase activity during malting was likely the primary factor responsible for phytate degradation. Additionally, it can be suggested that the reduction of PA during roasting in the laboratory, was due to thermal degradation of phytic acid. Among all treatments, intense roasting of raw barley decreased phytic acid content by 32.56% suggesting that as the most promising strategy for reducing PA levels in barley-based food ingredients.

1.Introduction

Phytic acid (PA), which is scientifically abbreviated as InsP6 or IP6, is chemically defined as *myo*-inositol-1,2,3,4,5,6-

hexakisphosphate (Chen and Xu, 2023; Marolt and Kolar, 2021). According to its molecular formula ($C_6H_{18}O_{24}P_6$), there is a *myo*-inositol ring that is bounded with six phosphate groups

(Bloot et al., 2023; Doung et al., 2017). This unique chemical structure allows phytic acid (PA) to chelate with mineral cations forming stable complexes at physiological pH levels (Chen and Xu, 2023). This chelation depicts antinutrient effects as it resulted in altering the solubility and digestibility of some essential minerals contained in food such as iron and zinc, ultimately obstructing their absorption to body (Farag, Xiao and Abdallah, 2022). Apart from minerals, PA was found to bind with amino acids either via direct electrostatic interactions or through indirect bridging function of multivalent cations such as zinc, iron and calcium (Chen and Xu, 2023). Moreover, the presence of PA in higher concentrations negatively affected the digestion and absorption of starch since the formation of interactions among phosphate bonds in PAs and digestive enzymes (salivary and pancreatic amylases) (Kumar et al., 2022).

Barley has been ranked as the fourth mostly produced cereal crop in the world while it is prominently utilized in brewery and animal feed sectors (Van de Velde et al., 2025). Beyond that, fermented bakery products and breakfast cereals are produced from barley malt extracts and syrups (Arif et al., 2011). As a recent growing trend, barley flour is used to partially or completely substitute wheat flour in unleavened bakery products (Lukinac and Jukic, 2022). PA content of some widely consumed cereals such as rice, oat, barley and rye was in the range 4.5-7.5 mg g⁻¹, which was lower compared to that of in pseudocereals such as millet, sorghum and corn with a PA range of 7.5-10 mg g⁻¹ (Wu et al., 2009). When concerned about whole barley grains, more than 80% of PA is available in the aleurone layer, a cellular layer located between endosperm and hull, and PAs mostly exist in association with protein globoids (Dai et al., 2007; Mayer et al., 2023).

Both wet and dry processing techniques can be applied to reduce naturally occurring PA levels in cereals (Feizollahi et al., 2021). Application of some dry techniques such as mechanical de-branning with pearling via friction and abrasion caused a reduction of 50%

of inositol phosphorus, while conventional storage of barley kernels at 41 °C and 75% RH for 3 months resulted in a slight decrease of PA content (4-9%) (Mayer et al., 2023; Feizollahi et al., 2021). When concerned about wet processing techniques, malting, which is a controlled germination process combined with kilning caused for decreasing phytic acid content by 33.50%, whereas treatment of barley bran suspension in water with high intensity ultrasound waves (24 kHz), decreased phytic acid content by 39% (Arif et al., 2011; Grgic et al., 2023).

Quantification of PA in barley using some classical methods such as Huag and Lantzsch method, and Vaintraub and Lapteva method cause for slight inaccuracies since the non-selectivity of Fe(III) ion for PA, its dephosphorylated analogues and other inorganic polyphosphates, and also due to the stoichiometric inconsistency between iron(III) and PA (Raboy et al., 2017; Kahrman et al., 2020; Marolt and Kolar, 2021; Wu et al., 2009). Compared to those, AOAC method is based on separation of PAs using anion exchange method and quantification by colour development by complexation of phosphorus with molybdate reagent (Kahrman et al., 2020). However, an overestimation of PA content can happen since the inability to differentiate between phytic acid (IP6) and its dephosphorylated forms like IP4 and IP5 (Wu et al., 2009). In addition, some advanced techniques including high performance ion exchange chromatography coupled with UV detection were applied to determine the contents of PA and other inositol isomers (Schemmer, Spillner and Sudekum, 2020). However, application of those techniques can be less practical during analysis of a larger set of samples on a routine basis due to their low throughput and time consuming nature (McKie and McCleary, 2016).

Although previous studies have examined influence of single processing techniques on phytic acid content of barley, there is limited evidence on exploring combined effect of several techniques on the phytic acid content. Most studies have investigated the effect on PA

content when processed under controlled laboratory conditions. However, previous studies have not made a comparative approach to determine phytic acid content of barley that was processed within industrial and laboratory conditions. In addition, there are no previous studies conducted to explore the roasting impact of barley at different levels on phytic acid content. Moreover, limited studies have employed highly specific enzyme assay methods such as Megazyme method (enzymatic dephosphorylation method), as a common analytical technique for quantification of phytic acid content of barley that was processed under both experimental and industrial conditions.

2. Materials and Methods

2.1. Materials

Four types of industrially processed barley samples: Raw barley or whole barley flour (RB) malted barley (MB), malted barley subjected to mild roasting (MB-l) and malted barley subjected to intense roasting (MB-i) were provided by a cereal products processing company in United Kingdom.

Concentrated sulphuric acid (Sigma cat. no. 258105), ascorbic acid (Sigma cat. no. 95210), trichloroacetic acid (Sigma cat. no. T6399), ammonium molybdate (Sigma cat. no. 277908), Sodium hydroxide (Sigma cat. no. 795429), hydrochloric acid (Sigma cat. no. 258148) were used as analytical grade chemicals. Phytic acid assay kit was purchased from Megazyme Ltd.

2.2. Sample preparation

In addition to the four industrially processed flour types, two portions of raw barley flour were ground and roasted to two different levels by using an electric oven (SQ-4870) in the laboratory. One portion (R-l) was roasted at a temperature range between 120 °C to 150 °C for 30-40 minutes, while the other portion (R-i) was roasted at a temperature range between 170-210 °C for 40-50 minutes. Colour of flour samples were measured time to time by using a CIE Lab* space colourimeter (SISCO-CM-CR8) until colour profiles of R-l and R-i samples approximately reached to colour profiles of MB-

The present study aimed to determine and compare phytic acid content of industrially processed barley flour types; whole, malted, and roasted combined to malting, alongside barley samples roasted to varying extents under controlled laboratory conditions. The current study has made a novel approach by employing a common analytical technique to comparatively explore impact of distinct processing pathways on the phytic acid content of barley flour. Through this research, it is expected to enrich food scientists and manufacturers with actionable insights to optimize barley processing for improved nutritional quality.

l and MB-i respectively (table 2). However, temperatures were maintained within above stated ranges and roasting was carried out for specified time lengths until achieving approximately similar colours. The colourimeter was calibrated against standard white reference prior to the colour measurement, and such measured colours were recorded with respect to L* (lightness), a* (red-green axis) and b* (yellow-blue axis).

2.3. Sample extraction

Each sample was extracted in duplicate. Initially 1 g of flour sample was measured into a 75 ml glass beaker. Then 20 mL of 0.66 M HCl was added. After that the beaker was covered with foil and vigorously stirred on a magnetic stirrer (Dlab MS7-H550-Pro) overnight at room temperature.

Quantification of phytic acid content and free phosphorus content

Phytic acid content of barley samples was quantified via phytic acid assay kit by following the method and calculations as described by McKie and McCleary (2016).

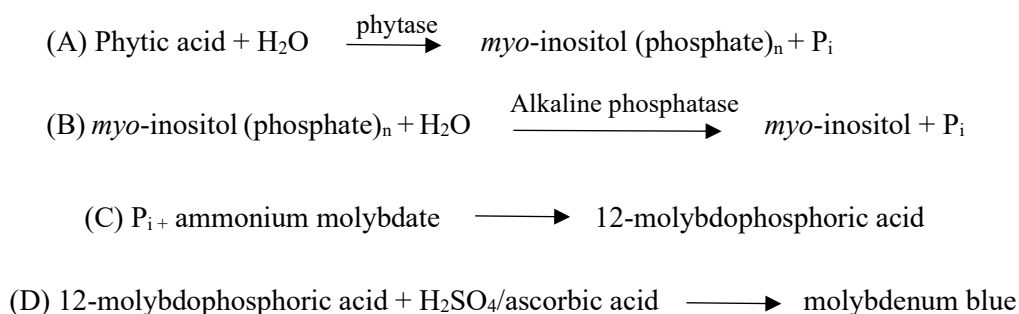
Initial free phosphorus content of each sample was determined as a pre-requisite to calculate the phytic acid content. This was included in the method described by McKie and McCleary (2016).

The principle and applicability of phytic acid assay kit

Colorimetric technique was applied to determine total phosphorus and PA content. This method is underpinned by the principle of measuring phosphate released from PA and lower inositol phosphates (InsP₁₋₅) due to the action of phytase and alkaline phosphatase enzymes in the assay kit (McKie and McCleary, 2016). Such enzymatically released phosphates were measured by a modified colorimetric molybdenum blue assay (McKie and McCleary, 2016). The same assumption of AOAC Official

Method 986.11, which presumed that the total amount of phosphorus quantified, is solely released from phytic acid instead of *myo*-inositol phosphates and other phosphate esters, was used during calculations (McKie and McCleary, 2016).

The set of reactions that involve during phytic acid determination procedure can be explained in accordance with the reaction scheme that was explained by McKie and McCleary (2016).



The assay kit measures total phosphorus content available in the samples because phytase enzyme has a broader range of specificity for lower *myo*-inositol phosphate compounds (IP₂-IP₅) in addition to phytic acid, while the action of alkaline phosphatase results in releasing last phosphate group from *myo*-inositol phosphate (McKie and McCleary, 2016). Therefore, theoretically it was presumed that the total phosphorus content of samples should fall within a narrow range since the processing involves only in altering the form of phosphorus (McKie and McCleary, 2016). Hence, for calculating PA content, the difference between free phosphorus and total phosphorus contents becomes a crucial value. Therefore, free phosphorus content is an important measurement which reflects the degradation of phytic acid when exposed to different processing conditions.

2.4. Data analysis

For each sample, 4 replicates were performed. Normality and homogeneity of variance were tested prior to conducting

statistical analysis. Mean values and standard deviations were calculated using SPSS software (version 29). Data were analysed to determine significant differences between sample means using SPSS software (version 29) by following one way ANOVA and Turkey HSD tests.

3. Results and Discussion

3.1. Phytic acid content of raw barley

Phytic acid content for two batches of raw or whole barley (RB), malted barley (MB), malted barley subjected to mild roasting (MB-l), malted barley subjected to intense roasting (MB-i), ground barley subjected to mild roasting (R-l) and ground barley subjected to intense roasting (R-i), are depicted in the table 1. Additionally, the table presents free phosphorus contents for each sample.

PA content of the two batches of raw barley samples was not significantly different ($P < 0.05$), and it fell within the range from 3.85 mg g⁻¹ to 9.85 mg g⁻¹ (0.385 – 0.985 g/100 g) and was also comparable with the mean value of 7.01 mg g⁻¹ (0.701 g/100 g) for phytic acid content according to the findings of Dai et al.

(2007). However, a non-mutant, wild type barley grains contained a greater level of PA content (1.02% on dry basis) compared to PA

content of raw barley that was determined in the present study (Ockenden et al., 2004).

Table 1. Phytic acid content and free phosphorus content of raw and processed barley samples under industrial and laboratory conditions

Barley sample	Phytic acid content (g/100g) d.b.	Free phosphorus content (g/100 g) d.b.
<i>Barley samples processed under industrial conditions</i>		
RB (Batch I)	0.859 ± 0.027 ^a	0.034 ± 0.001 ^A
MB	0.664 ± 0.021 ^b	0.054 ± 0.008 ^B
MB-I	0.712 ± 0.025 ^b	0.079 ± 0.005 ^C
MB-i	0.685 ± 0.029 ^b	0.110 ± 0.007 ^D
<i>Barley samples processed under laboratory conditions</i>		
RB (Batch II)	0.863 ± 0.027 ^a	0.045 ± 0.004 ^P
R-I	0.745 ± 0.074 ^b	0.052 ± 0.004 ^{PQ}
R-i	0.582 ± 0.003 ^c	0.060 ± 0.002 ^Q

Values are means ± SD expressed in dry basis; values with same superscript letter for each column are not significantly different (P<0.05).

Based on those previous literature findings, authors of the current study suggest that the naturally present phytic acid content in raw, unprocessed barley in current study might have been influenced by both intrinsic factors and extrinsic factors (Bloot et al., 2023). Genetic effect was determined to be the prominent intrinsic factor according to the findings of Kumar et al. (2021), who reported that Indian barley varieties cultivated at similar environmental conditions were detected with varying PA levels that fell within a significantly broader range (0.6-2.0 g/100 g).

Apart from genetic factors, phytic acid biosynthesis pathway is influenced by external environmental factors such as climate, geographical location, soil, fertilizer usage and irrigation conditions (Bloot et al., 2023). According to a presentation shared by the UK based cereal processing company (2025), 85-

90% of barley is obtained from East Anglia region. This region is well known for its light sandy soil that is naturally having a high level of phosphate, dry weather with less rainfall and more hours of sunlight per year that provide the ideal conditions for barley cultivation (<https://crispmalt.com/>). As revealed by previous studies, phytic acid content of spring barley correlated negatively with temperature, whereas it correlated positively with precipitation and application of different doses of phosphorus fertilizer (Korge et al., 2025; Reineke and Steffens, 2022).

3.2. Phytic acid content of malted barley

Malted barley sample showed a significantly (P<0.05) lower PA content compared to raw barley flour sample (figure 1). This finding reflects that PA content was subjected to a significant decrease (P<0.05) upon germination

step. This treatment accounted for reducing PA content by 22.70%, which was the second highest reduction compared to other processing conditions employed in the study (figure 3). Findings of Velde et al. (2025) revealed an approximately similar percentage reduction of PA (27.77%), which was corresponding to the fall of PA contents from 0.72 ± 0.07 g/100 g (d.b.) to 0.52 ± 0.00 g/100 g (d.b.) when subjected to germination at 20 °C for 95.5 hours. However, another study showed that PA content decreased linearly to a minimum value which ranged between $0.12 - 0.36$ mg g⁻¹ ($0.012 - 0.036$ g/100 g d.b.) from an initial PA content that fell between $4.22 - 4.74$ mg g⁻¹ ($0.422 - 0.474$ g/100 g d.b.) following germination for a period of 12 days (Bouajila et al., 2020).

The activity of phytase enzyme which is also termed as *myo*- inositol hexaphosphate hydrolase, plays a crucial role in determining the extent of PA reduction since the enzymatic action hydrolyses PA into inorganic phosphate (Bouajila et al., 2020). Major class of phytases in barley is known as purple acid phosphatase (PAP) (Madsen and Brinch-Pedersen, 2019). This enzyme results in the formation of *myo*-inositol compounds and inorganic free phosphates due to the removal of at least a single phosphate group from PA (Li et al., 2024). Enzymatic action is evident in the current study due to the presence of a significantly higher content ($P < 0.05$) of free phosphorus in MB sample compared to RB sample (table 1).

Barley cultivar influenced for varying phytase activity since there are two main genes namely PAPHy_a, which is expressed during grain filling stage, and PAPHy_b that is expressed during germination stage (Bouajila et al., 2019; Madsen and Brinch-Pedersen, 2019). Apart from intrinsic factors such as genetic makeup, extent of PA reduction was influenced by some extrinsic factors like germination conditions employed (Haraldsson et al., 2004). In the current study, the MB sample was commercially processed in diastatic form. As the initial step of malting process, barley grains were steeped in water for two days until the moisture content get increased up to 45%

causing to initiate germination. Following germination for 4-6 days, barley was subjected to a kilning process for 1-2 days to obtain malted barley (UK malted grain processor, 2025).

Absorption of water into barley grains results in the activation of phytase enzyme (Bouajila et al., 2019). Degradation of PA was positively correlated with germination time, while phytase activity was greater at 25 °C compared to lower temperatures (Li et al., 2024; Bouajila et al., 2019). In addition, findings of another study revealed that maximum phytase activity was observed after four days of germination at 20-25 °C, while the optimum phytase activity was observed at 50-55 °C and within the pH range of 5-6 (Sung et al., 2005).

3.3. Phytic acid content of malted and roasted barley

Two types of non-diastatic malted flours that were lightly roasted (MB-l) and intensely roasted (MB-i) at industrial processing conditions, were analysed in the current study. The extent of roasting varied based on temperature that ranged from 120 to 170 °C and time duration that ranged within 90 to 240 minutes (UK malted grain processor, 2025). According to findings of the present study, phytic acid content of MB, MB-l and MB-i samples did not show a significant difference ($P > 0.05$), and the PA content varied within a narrow range from 0.640 to 0.740 g 100 g⁻¹ (table 1). Those outcomes highlighted that malting followed by roasting did not cause further decrease in PA content, while malting effect was prominent over roasting for decreasing PA level. Roasting step resulted in producing flours that are enzymatically inactive since extreme temperatures beyond 55 °C terminate endogenous phytase enzyme activity (Frontela et al., 2008). However, application of processing techniques including malting alone and malting combined with roasting at two different intensities resulted in decreasing PA contents of MB, MB-l and MB-i samples significantly ($P < 0.05$) compared to RB sample (figure 1).

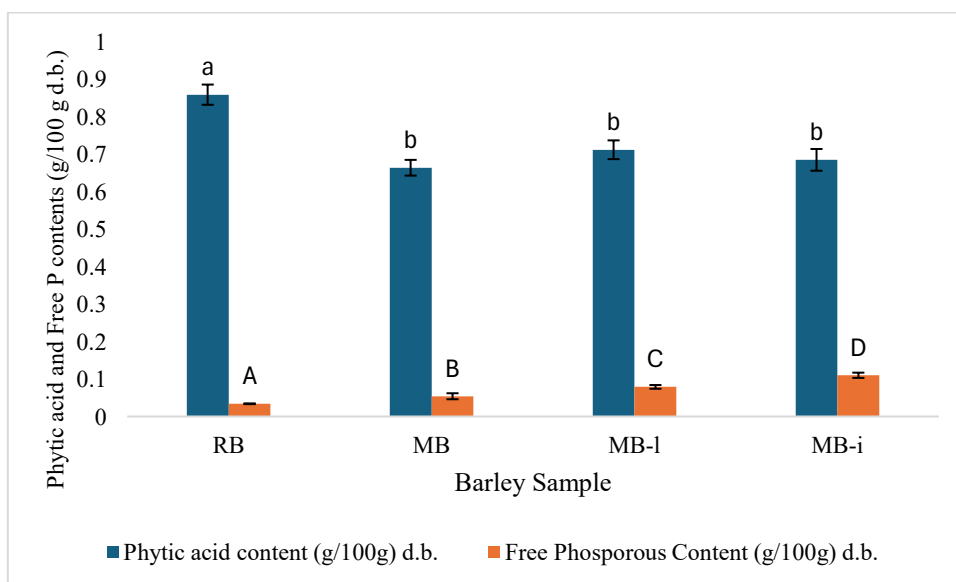


Figure 1. Phytic acid content (g/100g) and free phosphorus content (g/100 g) expressed on dry basis.

Legend: different simple letters in bar chart indicate significant differences ($P < 0.05$) between the samples with respect to phytic acid content. Different capital letters in line chart indicate significant differences between samples with respect to free phosphorus content. Samples: raw barley (RB), malted barley (MB), malted barley with light roasting (MB-l), malted barley with intense roasting (MB-i).

Content of phosphorus that existed in unbound or free form was minute compared to corresponding PA content (figure 1) of each sample. However, it showed a significant difference ($P < 0.05$) among raw (batch I) and industrially processed samples of barley and varied within a broader range from 0.033 g/100 g to 0.117 g/100 g d.b. As shown in the figure 1, processing conditions including malting alone and roasting combined with malting, caused for releasing significant levels of phosphorus ($P < 0.05$). More specifically, findings suggested that, along with intensifying roasting conditions free phosphorus content get increased. Therefore, free phosphorus content of the three samples can be arranged in ascending order as: MB, MB-l and MB-i (figure 1). It is noteworthy that both mild and intense roasting did not result in decreasing the PA content, although it increased free phosphorus content. Based on this, it can be hypothesized that malting prior to roasting caused for generating a pool of lower inositol intermediate compounds, which get dissociated further due to heat applied during roasting, while releasing free phosphorus.

Furthermore, it can be presumed that difference between thermal stabilities of phytic acid and its lower inositol phosphate compounds, is likely the main reason behind this. However, additional studies are required to investigate those mechanisms.

3.4. Phytic acid content of barley roasted in laboratory conditions

MB-l and R-l barley samples showed no significant difference ($P > 0.05$) with respect to a^* , b^* and chroma values as depicted in table 2. In addition, a^* values of MB-i and R-i samples were not significantly different ($P > 0.05$), while the percentage decrease of L values between RB and MB-l samples (12.7%) were approximately closer to that of between RB and R-l samples (14.6%). Similarly, the percentage reduction of L values among RB and MB-i samples (40.5%) were approximately closer to that of among RB and R-i samples (42.6%). Therefore, it is evident that roasting of ground barley in the laboratory had been conducted in a controlled manner in order to achieve a similar degree of roasting compared to industrially roasted samples.

Table 2. Colour parameters (L, a*, b* chroma and hue values) of raw and roasted barley flour samples

Parameter	RB	MB-l	MB-i	R-l	R-i
L	84.33 ± 0.50 ^a	73.60 ± 0.46 ^b	50.20 ± 0.10 ^c	72.03 ± 0.40 ^d	48.37 ± 0.75 ^e
a*	2.77 ± 0.32 ^a	6.34 ± 0.21 ^b	10.50 ± 0.36 ^c	4.93 ± 0.38 ^b	10.23 ± 1.06 ^c
b*	7.80 ± 0.61 ^a	17.90 ± 0.20 ^{bc}	20.83 ± 0.70 ^c	18.26 ± 0.32 ^{bc}	13.40 ± 3.99 ^b
C	8.30 ± 0.69 ^a	18.97 ± 0.25 ^b	23.37 ± 0.75 ^c	18.90 ± 0.45 ^b	17.00 ± 3.05 ^b
h	70.27 ± 0.76 ^a	70.43 ± 0.38 ^a	63.27 ± 0.15 ^b	74.90 ± 0.96 ^c	57.14 ± 2.16 ^d

Values are means ± SD. Values with same subscript letter for each row are not significantly different ($P < 0.05$). Colour expressed for samples: Raw barley (RB), malted barley with light roasting (MB-l), malted barley with intense roasting (MB-i), raw barley with light roasting (R-l), raw barley with intense roasting (R-i)

As illustrated in figure 2, PA content was significantly decreased ($P < 0.05$) when ground barley samples were subjected to both light and intense roasting. Moreover, intense roasting accounted for the highest percentage reduction (32.56%) of PA content among all treatment types as depicted by the figure 3. In contrast, an increasing trend was observed with respect to the free phosphorus content when roasting conditions were intensified (figure 2). Those outcomes aligned with the findings of Frontela et al. (2008), which revealed a significant reduction of inositol hexaphosphate (IP6) content from 639.0 ± 2.1 mg/100 g to 257.7 ± 9.0 mg/100 g (0.639 g/100 g to 0.257 g/100 g) when barley flour was roasted at 120 °C for 30 minutes. However, the corresponding percentage reduction (59.7%) achieved in the study conducted by Frontela et al. (2008), is significantly greater compared to the percentage reduction of PA achieved by roasting in the current study. It can be hypothesized that this significant difference is attributable to the type of analytical techniques employed, barley variety and some pre-processing parameters employed including milling size.

In addition, Li et al. (2023) highlighted that main possible reason behind the increase of iron and calcium contents in oven roasted cereal flours, was the elimination of antinutritional factors including phytic acid due to applied heat. Therefore, the possibility to degrade PA in samples that are in solid state due to heat can be justified based on those findings. Moreover, determination of PA content of roasted cereal flour can be considered as an important analytical test when designing infant food formulations. It is useful to maintain the molar ratio between PA and respective mineral within acceptable safe limits, while determining the content of minerals that should be added externally to cereal-based infant formulations. Apart from that, PA content of raw barley decreased when different processing conditions were applied in the current study and therefore, it can be presumed that, it results in decreasing the molar ratio (phytic acid to mineral molar ratio) below critical levels with respect to each mineral. So that, there is no effect on hindering mineral absorption and bioavailability (Abdulwaliyu et al., 2019).

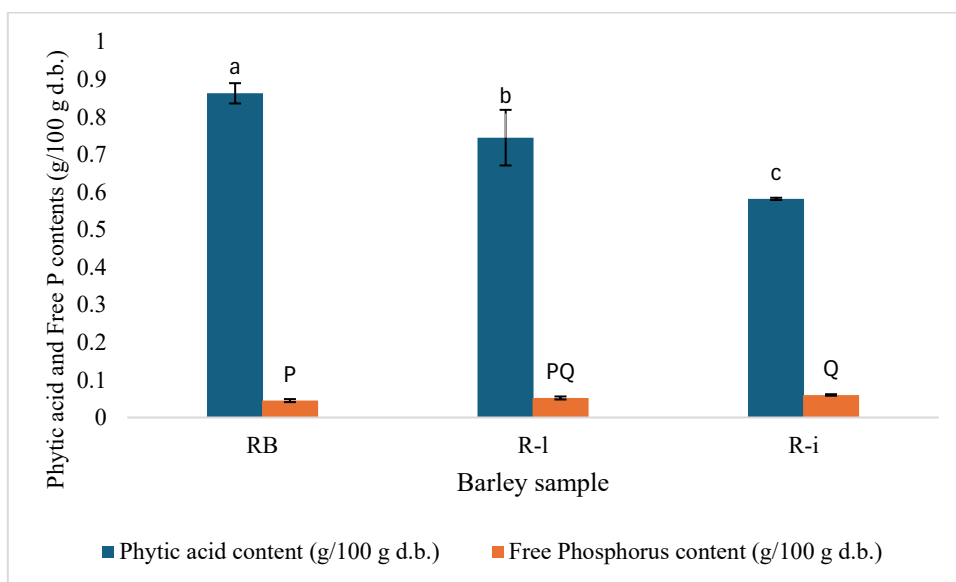


Figure 2. Phytic acid content (g/100g) and free phosphorus content expressed in dry basis.

Legend: Different simple letters in bar graph indicate significant differences ($P < 0.05$) between the samples with respect to the phytic acid content. Different capital letters in the line graph indicate significant differences ($P < 0.05$) with respect to the free phosphorus content. Samples: raw barley (RB), raw barley with light roasting (R-l), raw barley with intense roasting (R-i).

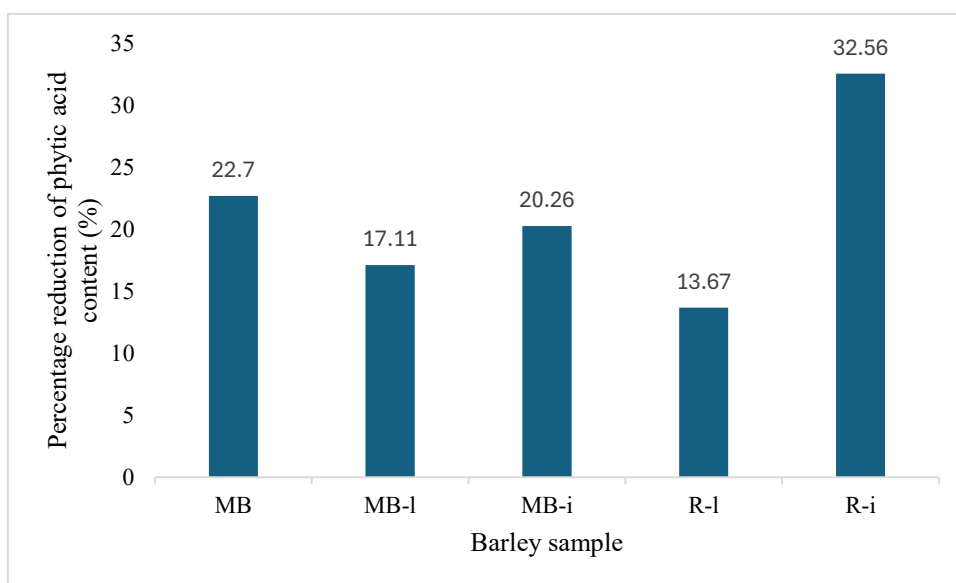


Figure 3. Bar graph demonstrates the percentage reduction of phytic acid content of barley when processed at different conditions.

Legend: MB (malted), MB-l (malted and lightly roasted), MB-i (malted and intensely roasted), R-l (lightly roasted barley), R-i (intensely roasted).

Significant decrease in PA concentration along with a simultaneous increase in orthophosphate (free phosphorus) concentration upon heating of a solid phytate sample at 60 °C for one day as revealed by Doolette and Smernik (2017), provided evidence for facile

decomposition of pure phytate due to heat. Formation of intermediate molecules such as inositol phosphates (IP_n), orthophosphate and pyrophosphate, when a phytate sample was heated at 85 °C for 36 hours, provided further evidence for thermal degradation of PA

(Watson, Smernik and Doolette, 2019). In the current study, free phosphorus content of barley samples depicted a rising trend when roasting conditions were intensified (figure 2). Therefore, decomposition of phytate in a solid sample due to heat was indicated by the increase of free phosphorus content (Doolette and Smernik, 2017).

Myo-inositol pentakisphosphates that were produced due to releasing of an orthophosphate, can exist in four different isomers namely Ins (1,2,3,4,6)P₅, DL-Ins (1,2,3,4,5)P₅, Ins (1,3,4,5,6)P₅ and DL-Ins (1,2,4,5,6)P₅ (Watson, Smernik and Doolette, 2019). Therefore, the presence of IP₅ as a mixture all four possible isomers, differentiates thermal degradation of phytic acid from its biotic pathways of degradation (Watson, Smernik and Doolette, 2019). Particularly, enzymes that generate Ins (1,3,4,5,6)P₅ and Ins (1,2,3,4,6)P₅ are known to be very rare, and presence of a combination of biota that would produce all IP₅ isomers in measurable quantities, is quite unlikely (Watson, Smernik and Doolette, 2019).

Phytate degradation mechanism at solid state was explained as a process that occurs as a result of facile transfer of a phospho group from phosphate esters to other phosphate groups present, which act as recipients (Doolette and Smernik, 2017). Orthophosphates and phosphate groups of an inositol phosphate act as recipients to generate diphosphates and organic diphosphates respectively (Doolette and Smernik, 2017). Therefore, the same mechanism can be presumed to explain phytate degradation during roasting of ground barley. However, further studies are required to elucidate the mechanism and rate of PA degradation when it is present within a natural food matrix such as ground barley.

4. Limitations of the study

This study has some limitations. Use of Megazyme assay, which specifically quantifies phytic acid (IP₆), did not measure other intermediate products such as PI₅, IP₄ and IP₃ along with other heat generated products. In addition, use of a single analytical technique

prevented cross-validation and may introduced method specific biasness. Only a single barley type was used in the present study, which narrowed down the potential to explore whether processing impacts in reducing phytic acid contents in the same pattern irrespective of the cultivar. In addition, phytase activity was not determined in the current study, which narrowed the ability to understand effects of endogenous phytase alone for reducing the phytic acid content.

5. Future recommendations

Future research should expand the scope of this study by using multiple barley cultivars to explore whether processing effects are consistent across genotypes or cultivar specific. In addition, quantifying phytase activity before and after processing would provide deeper insights into the mechanisms underpinning phytic acid degradation. Further work should also focus on employing a complementary analytical technique such as HPLC, alongside Megazyme assay to capture both phytic acid and lower inositol compounds that generated during processing. Apart from that, it is recommended to measure mineral bioavailability of samples before and after processing, to highlight the practical significance of phytic acid reduction.

6. Conclusions

Phytic acid contents of MB, MB-1 and MB-i samples were significantly ($P < 0.05$) lower compared to RB sample. However, the phytic acid contents of MB, MB-1 and MB-i samples were not significantly ($P > 0.05$) different from each other indicating that malting was the prominent causal factor compared to roasting that resulted in reducing the phytic acid content. Roasting of barley in ground form caused for a significant ($P < 0.05$) reduction of phytic acid content during both R-1 and R-i samples. Among the two roasting treatments, intense roasting showed the greatest reduction of phytic acid content. Overall, these findings provide useful insights into selecting and optimizing processing strategies of barley, while improving nutritional value of barley-based food products.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.