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Impact of germination on phenolic composition, antioxidant properties, antinutritional factors, mineral content and Maillard reaction products of malted quinoa flour

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ABSTRACT

The study aimed at improving and comparing the nutritional profile of black (BQ) and white quinoa (WQ) through malting at different germination periods (24, 48, 72 and 96 h), followed by drying at 50 °C, decluming, grinding and sieving to obtain malt flour. The changes in protein, reducing sugar, Maillard reaction products (MRPs), minerals, free and bound polyphenols and antioxidant activity were noted. Malting caused significant increase in polyphenolic content, antioxidant capacity and fluorescence of advanced MRP (FAST) index. The highest increment was noted in malts germinated for 48 and 72 h. BQ malts were marked by higher bound hydroxycinnamic acids, flavan-3-ols, magnesium, potassium and antioxidant activity, while, WQ malts had lower saponin, phytic acid but higher protein, iron, calcium, FAST index. WQ exhibited highest increment (27.23%) in antioxidant activity even though it had lower polyphenols than BQ after malting. Major loss in polyphenols and proteins occurred in malt germinated for 96 h.

1. Introduction

Bioactive compounds of plant origin have gained recent popularity, significantly owing to their numerous nutraceutical and healthful properties and as such consumer demand for such foods have increased. Among such foods, are the seeds of the crop *Chenopodium quinoa* Willd. Quinoa is a pseudo-cereal or a false cereal of the *Amaranthaceae* family, which means that unlike cereals such as wheat and rice, quinoa is a dicot and produces starch rich seeds that can be consumed and processed like conventional cereal grains (Bhargava & Srivastava, 2013). This Andean crop has a global production of 1,58,920 tonnes, with Peru chiefly producing 86,011 tonnes of quinoa cultivated across 64,660 ha, followed by Bolivia 70,763 tonnes (1,11,605 ha) and Ecuador 2146 tonnes (2048 ha) (FAOSTAT, 2018). Depending upon pigmentation, quinoa grains can be found in three different colors, namely, white or creamish gold, red and black (Tang, Li, Zhang, Chen, Liu, & Tsao, 2015). The grains are an excellent source of good quality gluten-free proteins that are rich in essential amino acids, minerals such as calcium, iron, phosphorous and zinc, vitamins namely E, C and B-complex and large amounts of flavonoids and phenolics. Owning to its highly nutritious composition, the grains exhibits good antioxidant properties, anti-obesity activity, hypocholesterolemic effect and anti-inflammatory properties. Thus, consumption of quinoa can reduce the risk of cancer, inflammatory and cardiovascular diseases (Repo-Carrasco-Valencia, Hellström, Pihlava, & Mattila 2010, Tang et al., 2015).

More so, quinoas' popularity can not only be attributed to its health promoting nutrient composition, but also to the crops' inherent unique ability to tolerate stressful abiotic agroclimatic conditions, in which

Abbreviations: WQ, White Quinoa Flour; BQ, Black Quinoa Flour; GT, Germination Time; WQM, White Quinoa Malt Flour; BQM, Blue Quinoa Malt Flour; Mg, Magnesium; Ca, Calcium; Fe, Iron; Cu, copper; K, Potassium; Mn, Manganese; Zn, Zinc; HPLC, High-performance Liquid Chromatography; SC, Saponin Content; PaC, Phytic acid Content; TC, Tannin Content; TFC, Total Flavonoid Content; TPC, Total Phenolic Content; AA, Total Antioxidant Activity; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; RuE, Rutin Equivalent; PC, Protein content; RS, Reducing sugar; CaE, Catechin Equivalent; QuE, Quercetin Equivalent; GaE, Gallic acid Equivalent; TE, Trolox Equivalent; HBA, Hydroxybenzoic acid; HCA, Hydroxycinnamic acid; MRP, Maillard reaction products; FIC, Free Fluorescent Intermediate Compounds; TRY, Fluorescence of soluble tryptophan; FAST, Fluorescence of advanced MRP; BI, Browning index; AL, Available lysine; OPA, o-phthaldialdehyde; LOPA, Loss in OPA activity; rpm, Revolutions per minute.

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other conventional and majorly consumed crops cannot thrive. The plant is a facultative halophyte that thrives well in high soil salinity, frost, frequent droughts, wind and hail at 3500 to 3900 m elevation above the sea level (Bhargava & Srivastava, 2013). However, the utilization of quinoa grains or its flour is hampered by the presence of bitter saponins and other antinutritional factors like phytic acid, tannins, oxalates and trypsin inhibitors. Therefore, appropriate techniques for processing quinoa grains are required for improving the overall sensorial and nutritional quality particularly in terms of polyphenolic compounds (Nickel, Spanier, Botelho, Gularte, & Helbig, 2016).

Malting is a simple traditional technique that can be employed to improve the nutritional quality (Aguilar, Miano, Obregón, Soriano-Colchado, & Barraza-Jáuregui, 2019) and reduce bitterness without abrasive removal of the grain pericarp, which otherwise, results in loss of dietary fibre, polyphenolic compounds and other bioactive agents concentrated in these outer layers of the grain (Hemalatha, Bomzan, Rao, & Sreerama, 2016). Malting consists of steeping the grain in water, which in itself is a unit operation capable of solubilising hydrophilic saponins, thus reducing their concentration (Nickel et al., 2016). Further, steeping causes an activation of intercellular enzymes that hydrolases stored nutrients, initiates grain germination, thus producing numerous physiological and biomolecular changes in the grains (Aguilar et al., 2019). These changes include degradation of complex carbohydrates to reducing sugar and low molecular weight compounds, and have been known to lower antinutritional components in legumes and cereals, improve protein and starch digestibility, increase polyphenolic compounds, and antioxidants such as ascorbic acid; the extent of which depends upon germination period and conditions (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010). Finally, to obtain malt the grains are dried, which results in further enhancement of flavour profile through the formation of Maillard reaction products (MRPs). Optionally, grains could also be kilned or roasted further to enhance its flavour profile. Numerous studies have proposed germination and malting as effective techniques to enhance the antioxidant capacity, polyphenolic composition and ascorbic acid (Alvarez-Jubete, et al., 2010, Abderrahim et al., 2012, Pilco-Quesada, Tian, Yang, Repo-Carrasco-Valencia, & Suomela, 2020). Malts have been found to serve as a natural enzyme delivery system with improved bioavailability of amino acids, gut friendly fermentable sugars, dietary fibre, minerals and vitamins (Ispiryan, Kuktaite, Zannini, & Arendt, 2020). Thus, malts find application in baked foods and non-alcoholic beverages as well (Bewley, Bradford, Hilhorst & Nonogaki, 2013). Impact of germination and malting has been extensively studied for various cereals. However, very few studies have focused on quinoa and there is lack of information on impact of germination period on the changes in polyphenolic composition, development of MRPs and antioxidant activity of quinoa malt. Therefore, the study was taken up to establish the impact of germination time (GT) on the proximate and mineral composition, polyphenolic profile, antioxidant capacity and antinutrient composition of quinoa malt. Further, the present study also evaluated, characterized and compared, free and bound polyphenolic composition of cream and black coloured quinoa grain malts, and assessed their potential health-promoting benefits through antioxidant assay to establish whether pigmentation yields nutritionally superior malt.

2. Materials and methods

2.1. Material

Quinoa of white and black coloured quinoa grown in India under the brand name Miltop were purchased from local market of Amritsar, India. To prepare malted flour, grains were first disinfected by dipping in 2.5% sodium hypochlorite solution for 5 min. Further, the grains were steeped in deionised water, in the mass ratio of 2:3, for 4 h at 25 °C. The moisture content of hydrated grains increased to 45–55%. After which the grains were spread in a thin layer in a tray, which was covered with a moist muslin cloth. The trays were placed in a growth chamber and the grains were allowed to germinate for 24, 48, 72 and 96 h at 24 °C, under dark conditions. The grains were sprayed with deionized water at regular intervals of 8 h. Relative humidity was maintained to 95–100%. Later the grains were dried for 24 h in an oven at 50 ± 5 °C to obtain malted grains. Both malted and germinated grains were milled (super mill) and passed through a 250 μm opening mesh, to obtain flour. The flour samples were stored at −20 °C till further analysis.

2.2. Chemical composition

The moisture of raw and malted quinoa flours was estimated with oven drying at 130 °C. The protein content (PC) was established by multiplying the nitrogen estimated by Kjeldahl method with a conversion factor of 6.25. Ash content was estimated by charring in muffle furnace (AOAC, 2002). The reducing sugar content was determined on the basis of cupric reduction (Başkan, Tütem, Akyüz, Özen, & Apak, 2016). Flour was suspended in distilled water, shaken, centrifuged and supernatant was collected. To 25 μL of this extract, 75 μL of water and 1 ml DNS (3,5-dinitrosalicylic acid) solution was added and mixture was shaken and incubated for 5 min in boiling water bath. After adding 40% sodium potassium tartrate solution (1 ml), the resulting mixture was cooled in ice bath and absorbance at 540 nm was taken. A D(+)-glucose standard calibration curve was used to report results in gGu/100g on flour dry basis.

2.3. Mineral composition

The raw and malted quinoa flours were estimated for mineral composition (K, Mg, Zn, Fe, Mn, Ca and Cu) using Atomic Absorption Spectrophotometer (Agilent Technologies) (Bhinder, Kaur, Singh, Yadav, & Singh, 2020). Briefly, quinoa flour (1 g), taken in a crucible, was charred at 600 °C in a muffle furnace to obtain mineral rich ash. About 2.5 ml of nitric acid (1 N) was added to the crucible to dissolve the ash. The solution was filtered, diluted using Milli-Q water in a 100 ml volumetric flask, and was analysed for minerals. Instrument calibration was carried out with a 1000 ppm standard solution prepared with the Agilent Single Element Standards.

2.4. Antinutritional components

2.4.1. Saponin content

To extract saponins, (1.11 g) flour was suspended and macerated (72 h with constant stirring at 200 rpm) in 10 ml of 50% ethanol. After filtration, the extract volume was made up to 10 ml in a volumetric flask with 50% ethanol. The extracts and reference standard were diluted (1:20 dilution) and Lieberman-Burchard reagent (7 ml) was added to 2 ml of diluted extract. The solution was vortexed and kept at room temperature for 30 min before taking absorbance at 528 nm on a Cary-60 UV-VS spectrophotometer (Agilent Technology). Quantification was carried with a standard desogenin curve and expressed as mg/g of flour on dry basis (Nickel et al., 2016).

2.4.2. Phytic acid content

To estimate phytic acid, flour (0.5 g) extract was prepared in 0.2 N HCl (25 ml) by gently shaking (3 h), followed by filtration. To this extract (0.5 ml), 25 ml of acidic solution of iron (III) prepared by dissolving ferric ammonium sulphate dodecahydrate (0.2 g) in 2 N HCl (100 ml) was added and the stoppered test tube was placed in a boiling water bath for 30 min. After centrifugation supernatant (1 ml) was taken in another test tube and bipyridine solution (1.5 ml) was added and readings were taken at 519 nm on UV-VS spectrophotometer. Phytic acid was quantified in terms of sodium phytate standard in mg/g of flour on dry basis (Haug & Lantzsch, 1983).

2.4.3. Tannin content

Tannin content was estimated by preparing flour (0.5 g) extract in 40 ml aqueous ethanol solution (10%) by boiling and shaking in a boiling water bath. The volume was made to 50 ml by adding methanol. To this extract (5 ml), first Folin-denis reagent (5 ml), then saturated sodium carbonate solution (10 ml) was added and volume was made up to 100 ml using deionized water in a volumetric flask. After 30 min of incubation, absorbance was taken at 760 nm on UV-VS spectrophotometer and result was calibrated and reported using tannic acid standard curve in mg/g of flour on dry basis (Makkar, Blümmel, Borowy, & Becker, 1993).

2.5. Antioxidant properties

To establish polyphenolics in free or extractable and bound or unextractable form, the method depicted by Bhinder et al. (2019) with a few modifications was followed. Quinoa flour (1 g) was ultrasonicated with 10 ml of 80% methanol (1 h), centrifuged and supernatant was collected. The residue was again reextracted with 80% methanol (10 ml), ultrasonicated, further centrifuged and supernatants were pooled. The pH of the pooled supernatants was readjusted to 4.0–4.5 with 6 N HCl and the extract was labelled as free phenolic extract. To extract bound phenolics, the residue was hydrolysed with 0.4 M NaOH (2 h) and pH was adjusted to 4.0–4.5. The supernatant obtained after centrifugation was labelled as bound polyphenolic extract.

2.5.1. Total phenolic and flavonoid content

The total free and bound phenolic (TPC) and flavonoid (TFC) content in quinoa flour was determined by following the procedure reported by Bhinder et al. (2019). The TPC was reported in gallic acid equivalents (mgGaE/g) on flour dry weight basis, while TFC was reported in rutin equivalents (mgRuE/g) on flour dry weight basis.

2.5.2. Antioxidant activity

The antioxidant activity was estimated through scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Bhinder et al., 2019). The decrease in absorbance of DPPH solution in the presence of 100 μL of extract was measured at 515 nm (UV-VS spectrophotometer). Antioxidant activity was reported in Trolox (6-hydroxy-2,5,7,28-tetramethylchroman-2-carboxylic acid) equivalent (μmolTE/g) on flour dry weight basis.

2.6. High performance liquid chromatography (HPLC)

The free and bound polyphenolic compounds were identified and quantified using the 1260 infinity Agilent technology HPLC comprising a DAD-PDA type Diode Array Detector with a binary pump, Agilent technology C18 column and rapid separation autosampler. Mobile phase A (1% acetic acid) and B (HPLC grade water:acetic acid:acetonitrile in the ratio of 67: 1: 32), at flow rate of 0.5 ml/min were passed through the column with a gradient program established as follows: 0 min, 90% A; 0–10 min, 80% A; 10–16 min, 80–60% A; 16–20 min, 60–50%, A; 25–26 min, 50–30% A; 26–30 min, 30% A; 30–40 min, 30–90% A; 40–45 min, 90% A. The injection volume was 10 μL, oven temperature was 30 °C and the chromatograms of all polyphenolic compounds were recorded at 275 nm (Deng et al., 2012) and the polyphenols were identified by comparing the retention time with working standards. The quantification of identified peaks was carried out through area normalization and results were calculated in mg/100 g of flour dry basis.

2.7. Maillard reaction products (MRPs)

For analysing the formation of MRPs, quinoa flour (50 mg) extract was prepared by incubating (30 min) the flour suspension prepared in 6 ml sodium dodecyl sulfate solution (6%) with vigorous agitation for 30 s after every 10 min. After filtration through nylon filter (0.45 μm) the extracts were employed for measurement of available lysine content FAST index, FIC (free fluorescent intermediate compounds) and browning index (BI) by following method described in previous report (Bhinder et al., 2019).

2.7.1. Available lysine

OPA assay (o-phthaldialdehyde) was used to estimated available lysine content. Fluorescence of the sample extract was estimated at excitation and emission wavelength (λ_{ex} and λ_{em}, respectively) of 340 and 455 nm using a LS5.5 Perkin-Elmer fluorescence spectrophotometer. The quantification of AL was carried out with an external standard of lysine and results were reported as mg/g of flour dry matter (Ramírez-Jiménez, García-Villanova, & Guerra-Hernandez, 2004).

2.7.2. Free fluorescent intermediate compounds

The intermediate MRPs in terms of FIC were established in arbitrary fluorescence units (FU), at 438 (λ_{em}) and 353 nm (λ_{ex}) using fluorescence spectrophotometer.

2.7.3. FAST index

FAST index (%) was evaluated as the ratio of FIC to soluble tryptophan. Soluble tryptophan fluorescence was taken at 340 nm (λ_{em}) and 290 nm (λ_{ex}) in the fluorescence spectrophotometer (Bhinder et al., 2019).

2.7.4. Browning index (BI)

The estimation of melanoidin formation in terms of BI was carried out by taking the absorbance of extract, in the Agilent Technology's Cary 60 UV-VS spectrophotometer at 420 nm. BI was expressed in arbitrary absorbance units (AU) (Małgorzata, Konrad, & Zieliński, 2016).

2.8. Statistical analysis

All experiments were carried out in triplicates and the data was expressed as mean value of the triplicate ± standard deviation. Statistical tools such as two-way ANOVA, Pearson correlation and principal component analysis was carried out on the 14.12.0 version of Minitab Software (U.S.A.) to evaluate the impact of GT on flour obtained from raw and malted white and black quinoa.

3. Result and discussion

3.1. Protein content

The proximate composition of flour from raw and malted white and black quinoa (WQ and WQM, BQ and BQM, respectively) grains is given in Table 1. The PC of WQ and BQ was 13.82% and 11.51%, respectively. F values revealed that GT and grain colour significantly affected the PC of quinoa malt flours ($P < 0.005$, Table 4a). The highest percent increment (5.56%) in PC after malting was observed in BQM germinated for 48 h. However, WQM exhibited the highest increment (5.50%) in PC after germination for 72 h Aguilar et al. (2019) studied the impact of malting on three quinoa varieties and found that only one variety (Negra Collana) exhibited a percent increase of 8.04% in PC (GT = 48 h) while a decrease in PC was observed for the other two varieties. Whereas, Pilco-Quesada et al. (2020) observed a very high increase from 9.6% to 26% in PC after GT of 72 h for WQM. The increment could be attributed to mobilization and generation of nutrient reserves in the grains. Previous study has stated that during germination of dicots like quinoa, an increase in the content of free amino acids was observed during the first 24 to 72 h (Bewley et al., 2013). However, PC of quinoa malt variants was found to decrease as GT increased. This could likely be due to protein catabolism during radical formation and the subsequent deculming during grain drying (Bewley et al., 2013). The percent decline (7.21%) in PC was the highest in BQM, germinated for 96 h indicating higher rate of metabolism in BQ.

Table 1
Effect of germination time on proximate, mineral composition and antinutritional components of raw and malted white and black quinoa flour (dry weight basis).

	White Quinoa					Black Quinoa				
Germination time (h)	0	24	48	72	96	0	24	48	72	96
PC (%)	13.82 ± 0.66^{d}	13.98 ± 0.24^{d}	14.12 ± 0.75^{e}	14.58 ± 0.35^{e}	13.08 ± 0.16^{d}	11.51 ± 0.22^{b}	11.83 ± 0.58^{b}	12.15 ± 0.32^{c}	11.37 ± 0.42^{b}	10.68 ± 0.31^{a}
AC (%)	3.44 ± 0.26^{b}	3.26 ± 0.09^{b}	3.21 ± 0.12^{a}	3.32 ± 0.35^{b}	3.3 ± 0.24^{b}	3.97 ± 0.34^{d}	3.63 ± 0.21^{c}	3.91 ± 0.14^{d}	3.77 ± 0.35^{d}	3.82 ± 0.23^{d}
Moisture (%)	9.03 ± 0.31^{e}	6.4 ± 0.15^{b}	6.72 ± 0.29^{b}	6.23 ± 0.23^{b}	5.61 ± 0.33^{a}	8.75 ± 0.21^{c}	6.58 ± 0.42^{b}	6.11 ± 0.26^{b}	6.37 ± 0.36^{b}	5.41 ± 0.31^{a}
RS (g Glu/100 g)	3.22 ± 0.16^{a}	5.34 ± 0.2^{b}	8.72 ± 0.4^{b}	11.61 ± 0.7^{c}	16.55 ± 0.67^{d}	2.68 ± 0.26^{a}	3.31 ± 0.28^{b}	7.45 ± 0.22^{b}	14.52 ± 0.51^{c}	17.86 ± 0.8^{d}
Ca (mg/100 g)	89.75 ± 3.23^{d}	83.65 ± 5.3^{c}	95.65 ± 5.89^{d}	85.54 ± 4.73^{d}	82.65 ± 3.31^{c}	56.53 ± 3.48^{a}	62.08 ± 4.52^{b}	64.65 ± 3.71^{b}	53.34 ± 1.25^{a}	50.76 ± 2.51^{a}
K (mg/100 g)	452.72 ± 26.62^{b}	421.73 ± 12.86^{a}	425.72 ± 23.06^{a}	402.94 ± 6.56^{a}	416.64 ± 17.7^{a}	562.15 ± 21.77^{e}	514.72 ± 30.63^{c}	545.31 ± 10.18^{d}	553.15 ± 27.51^{d}	527.86 ± 12.01^{d}
Mg (mg/100 g)	127.54 ± 24.25^{b}	111.56 ± 21.36^{a}	114.81 ± 13.41^{a}	121.05 ± 8.41^{b}	124.34 ± 8.72^{b}	223.9 ± 10.68^{e}	220.72 ± 25.03^{e}	215.64 ± 5.37^{e}	223.73 ± 14.05^{e}	212.55 ± 5.35^{e}
Zn (μmol/g)	2.42 ± 0.06^{b}	2.42 ± 0.02^{b}	2.12 ± 0.11^{b}	1.85 ± 0.08^{a}	1.78 ± 0.03^{a}	3.64 ± 0.22^{e}	3.51 ± 0.18^{e}	3.49 ± 0.05^{d}	3.42 ± 0.03^{d}	3.15 ± 0.03^{d}
Fe (mg/100 g)	12.84 ± 0.2^{d}	12.98 ± 0.05^{d}	13.73 ± 0.31^{e}	13.54 ± 0.23^{e}	13.67 ± 0.07^{e}	11.04 ± 0.13^{a}	10.99 ± 0.08^{a}	10.98 ± 0.07^{a}	11.1 ± 0.12^{a}	10.96 ± 0.14^{a}
Cu (mg/100 g)	0.87 ± 0.09^{a}	0.89 ± 0.02^{a}	0.87 ± 0.03^{a}	0.87 ± 0.01^{a}	0.84 ± 0.02^{a}	1.54 ± 0.09^{d}	1.53 ± 0.03^{d}	1.52 ± 0.01^{d}	1.4 ± 0.01^{cd}	1.41 ± 0.05^{d}
Mn (mg/100 g)	3.35 ± 0.07^{e}	3.39 ± 0.11^{e}	3.33 ± 0.03^{e}	3.2 ± 0.08^{ce}	3.19 ± 0.01^{c}	2.72 ± 0.05^{b}	2.68 ± 0.01^{b}	2.75 ± 0.04^{b}	2.71 ± 0.03^{b}	2.56 ± 0.08^{a}
SC (g/100 g)	1.63 ± 0.07^{c}	1.13 ± 0.04^{a}	1.18 ± 0.02^{a}	1.36 ± 0.11^{b}	0.93 ± 0.09^{a}	2.42 ± 0.16^{e}	1.69 ± 0.08^{c}	1.76 ± 0.09^{c}	1.58 ± 0.11^{b}	1.01 ± 0.07^{a}
TC (mg/100 g)	3.41 ± 0.16^{b}	3.21 ± 0.12^{b}	3.47 ± 0.17^{b}	3.13 ± 0.17^{b}	2.35 ± 0.1^{a}	4.81 ± 0.12^{d}	4.53 ± 0.2^{d}	4.62 ± 0.11^{d}	4.87 ± 0.17^{d}	3.44 ± 0.14^{b}
PaC (mg/100 g)	375.27 ± 1.7^{d}	308.2 ± 12.7^{c}	253.8 ± 14.73^{c}	158.8 ± 17.28^{a}	153.53 ± 11.02^{a}	445.73 ± 3.06^{e}	331.93 ± 10.76^{c}	250.4 ± 16.8^{c}	272.4 ± 8.98^{c}	161.93 ± 6.77^{b}

Data values with the same alphabetic superscript in a row do not vary significantly ($P < 0.05$). PC = Protein Content; AC = Ash Content; RS = Reducing Sugar Content; Glu = Glucose; SC = Saponin Content; PaC = Phytic acid Content; TC = Tannin Content; Mg = Magnesium; Ca = Calcium = Fe = Iron; Cu = copper; K = Potassium; Mn = Manganese; Zn = Zinc.

3.2. Ash and moisture content

After malting, the overall moisture and ash of both WQ and BQ showed a decline. Ash varied significantly with grain colour, while moisture varied significantly with GT ($P < 0.005$, Table 4a). The decline of ash content (AC) after malting can be accounted to the lixiviation of certain minerals in water during grain steeping and germination (Bewley et al., 2013), which could account for 8.56% (BQ) and 5.23% (WQ) percent loss in AC after malting (GT = 24 h). Aguilar et al. (2019) reported a comparable loss in AC by 5 to 37% during malting (GT = 48 h) of three different quinoa varieties. Whereas, Pilco-Quesada et al. (2020) reported a steeper decline in AC of quinoa malt after 24 h GT from 5.5% to 2.6%; however, an increase in AC to 4.5% was noted as GT increased to 72 h.

3.3. Reducing sugar

Reducing sugar (RS) of WQ (3.22 gGlu/100 g) and BQ (2.68 gGlu/100 g) showed highly significantly variation with GT ($P < 0.005$, Table 4a). Also, a highly positive significant correlation between RS and GT was observed ($r = 0.971$; $p \leq 0.05$) (Table 5). The highest increment in RS from 2.68 to 17.86 gGlu/100 g was observed for BQM (GT = 96 h) (Table 1). An increment in reducing sugar as a result of carbohydrate catabolism during germination has been reported earlier in *Chenopodium* genus as well (Abderrahim et al., 2012; Jimenez, Lobo, & Sammán, 2019; Aguilar et al, 2019).

3.4. Mineral composition

The mineral composition of raw and malted quinoa flours is shown in Table 1. Minerals are essential in human health for maintaining and promoting an overall mental and physical wellness in terms of taking integral part in the development and maintenance of body tissues, blood muscles, nerve cells, bones, and teeth (Bhinder et al., 2020). Among macrominerals (K, Mg and Ca), BQ variants had higher Mg (212.55 – 223.9 mg/100 g) and K (527.86 – 562.15 mg/100 g) than WQ variants, which had higher Ca (82.65 – 89.74 mg/100 g) (Table 1). Among microminerals, most noteworthy was Fe, which was higher in WQ (12.84–13.73 mg/100 g) and lower in BQ (10.96 – 11.04 mg/100 g) variants. AC showed a highly positive significant correlation with macrominerals ($r = 959$, $p \leq 0.005$) as shown in Table 5. After malting, loss in Zn, K and Mg was the most evident with increase in GT, while, Fe content particularly for WQM increased as GT increased. These changes could be attributed to leaching of certain minerals during steeping. Also, minerals act as cofactors aiding enzyme catalysis of carbohydrates and proteins, thus getting mobilized to the radicles, which are lost during deculming (Bewley et al., 2013).

3.5. Antinutritional factors

The main antinutritional components in quinoa are phytic acid and saponins, while other antinutrients like tannins, trypsin inhibitor and oxalates are present in low quantities (Bhargava & Srivastava, 2013). The impact of malting on saponin (SC), phytic acid (PaC) and tannin content (TC) of the two quinoa variants has been reported in Table 1.

In quinoa, saponins are mostly concentrated in the epicarp and classified as triterpene saponins which comprises up of a hydrophobic aglycone linked to a hydrophilic saccharide chain. Even though saponins have anti-inflammatory and antifungal activity and enhance drug and food assimilation through the intestinal mucosa, these compounds impart a characteristic bitter taste and reduce mineral bioavailability by forming insoluble complexes with minerals like zinc and iron and are therefore categorised as antinutrients (Suárez-Estrella, Torri, Pagani, & Marti, 2018). The SC of WQ and BQ was 1.63 and 2.42 mg/g, respectively. The perception of bitterness by human has been associated with SC higher than 1.1 mg/g in quinoa (Koziol, 1991). Depending upon the

quinoa genotype, plant growth, soil and environmental conditions (Suárez-Estrella et al., 2018), SC in quinoa varies widely from 0.1 to 50 mg/g (Stuardo & San Martín, 2008). GT and grain colour highly and significantly affected the SC ($P < 0.005$). The sharpest decline in SC by 42.94% and 58.26% in WQM and BQM germinated for 96 h, respectively, was observed. A positive significant correlation was found between GT and SC ($r = -0.684$, $p \leq 0.05$) (Table 5). SC showed a sharp decline in malts germinated for 24 h, which could be attributed to water solubility of saponins causing leaching during grain steeping. Hydration of grains causes water to penetrate into the grain mass, thus releasing greater amounts of saponins through simple diffusion However, a slight increase in TSC was observed for WQM germinated for 48 and 72 h. Studies have reported loss of saponins by grain washing, soaking and cooking (Nickel et al., 2016).

Phytic acid is a saturated cyclic acid with six reactive phosphate groups that acts as a chelating agent, thus has the ability to bind positively charged functional groups or minerals, thereby reducing their bioavailability during food metabolism. Unlike cereal grains where phytic acid is concentrated in the germ, in quinoa it is present in the outer grain layers as well as throughout the endosperm (Demir & Bilgicli, 2020). The PaC of WQ and BQ was 375.27 mg/100 g and 445.73 mg/100 g, respectively. PaC varied significantly with quinoa colour and GT ($P < 0.005$). A highly significant positive correlation was observed between PaC and GT ($r = -0.933$, $p \leq 0.005$). The highest decline of 63.67% was observed for BQM germinated for 96 h, while WQM (GT = 96 h) exhibited a decline of 59.09%. The loss in PaC could be associated to the increase in native phytase activity of the germinating grains, that hydrolyses phytic acid. After germination of 48 h, Demir and Bilgicli (2020) reported a loss of 77% in quinoa flour from 970.97 to 221.05 mg/100 g, while, Elgi, Davidsson, Juillerat, Barclay, & Hurrell (2002) reported a loss of 12% in PaC after quinoa germination of 48 h from 0.97 to 0.85 g/100 g. In another study by Padmashree, Handu, Khan, Semwal and Sharma (2019) reported a 16.37% and 29.52% loss in PaC for white and red quinoa, respectively, after 48 h of germination.

Tannins are polyphenols that form complexes with macromolecules like protein depending upon food pH thereby reducing assimilation of these macromolecules. Apart from this, tannins impart astringency to the food, impair absorption of vitamin B_{12}, iron and glucose (Bhargava & Srivastava, 2013). Quinoa had low tannin content (TC) of 3.41 and 4.81 mg TaE/100 g, WQ and BQ, respectively. A steady decline with increase in GT was not observed for TC. Even though TC increased slightly by 1.76% after malting for WQ (GT = 48 h), the highest decline of 31.08% was observed for WQM (GT = 96 h). The former could be attributed to the change in tannin content due to synthesis of macromolecular compounds from phenolic compounds such as catechins (Kim et al., 2016). Similar, increase was observed for BQM germinated for 72 h. Overall, malting resulted in a significant reduction in antinutritional factors of quinoa flours.

3.6. Maillard reaction products

Maillard reaction products (MRPs) were examined as available lysine (AL), intermediate free fluorescence compound (FIC), fluorescence of tryptophan (TU), FAST index and browning index (BI) (Table 2). Maillard reaction comprises of heat-induced complex reactions that occurs in three stages between free amino group of a protein and reducing sugar. The initial stage consists of condensation reaction between free amino and carbonyl group to form an unstable imine that results in the loss of available lysine content. (Bhinder et al., 2019; Abderrahim et al., 2012). Therefore, the reduction in AL indicates the incitation of Maillard reaction. GT and grain colour resulted in a highly significant variation in AL ($P < 0.005$) (Table 4). The AL content of WQ and BQ was 4.81 mg/g and 3.6 mg/g, respectively. After malting, an increase of 8.32% and 14.17% in AL was observed for WQM and BQM germinated for 48 h,

Table 2
Effect of germination time on Maillard reaction products and antioxidant properties of raw and malted white and black quinoa flour (dry weight basis).

	White Quinoa					Black Quinoa				
Germination time (h)	0	24	48	72	96	0	24	48	72	96
FTFC (mg RE/g)	3.12 ± 0.1^{a}	3.6 ± 0.15^{ab}	4.3 ± 0.18^{c}	4.87 ± 0.04^{d}	4.33 ± 0.09^{c}	5.47 ± 0.1^{e}	5.41 ± 0.14^{e}	6.02 ± 0.14^{ef}	5.49 ± 0.14^{e}	4.79 ± 0.11^{cd}
BTFC (mg RE/g)	2.67 ± 0.03^{e}	2.75 ± 0.02^{e}	2.25 ± 0.06^{d}	1.8 ± 0.03^{c}	1.93 ± 0.07^{c}	1.47 ± 0.06^{b}	1.29 ± 0.08^{a}	2.51 ± 0.05^{e}	1.92 ± 0.03^{c}	1.44 ± 0.05^{b}
TFC (mg RE/g)	5.79 ± 0.09^{a}	6.34 ± 0.13^{b}	6.55 ± 0.17^{b}	6.67 ± 0.04^{c}	6.26 ± 0.14^{b}	6.94 ± 0.15^{c}	6.71 ± 0.21^{c}	8.53 ± 0.18^{f}	7.41 ± 0.12^{d}	6.29 ± 0.08^{b}
FTPC (mg GAE/g)	2.39 ± 0.05^{a}	2.32 ± 0.04^{a}	2.44 ± 0.03^{a}	3.05 ± 0.02^{c}	3.09 ± 0.03^{c}	2.29 ± 0.05^{a}	2.37 ± 0.07^{a}	2.88 ± 0.07^{c}	3.54 ± 0.04^{e}	2.9 ± 0.03^{c}
BTPC (mg GAE/g)	4.71 ± 0.07^{a}	4.62 ± 0.14^{a}	5.2 ± 0.20^{b}	5.38 ± 0.07^{b}	5.16 ± 0.11^{b}	5.54 ± 0.15^{c}	5.81 ± 0.13^{c}	6.49 ± 0.07^{d}	6.08 ± 0.08^{d}	6.25 ± 0.07^{d}
TPC (mg GAE/g)	7.09 ± 0.03^{a}	7.3 ± 0.13^{a}	8.36 ± 0.15^{b}	8.56 ± 0.06^{c}	8.04 ± 0.15^{b}	7.82 ± 0.15^{b}	8.18 ± 0.2^{b}	9.38 ± 0.13^{d}	9.61 ± 0.09^{d}	9.15 ± 0.09^{d}
FAA (μmol/g)	3.61 ± 0.46^{a}	4.05 ± 0.14^{b}	4.34 ± 0.06^{b}	4.75 ± 0.25^{b}	4.46 ± 0.06^{b}	5.12 ± 0.06^{c}	5.66 ± 0.14^{c}	6.26 ± 0.08^{d}	6.9 ± 0.26^{d}	6.86 ± 0.07^{d}
BAA (μmol/g)	4.29 ± 0.11^{a}	4.44 ± 0.06^{a}	4.91 ± 0.11^{c}	5.29 ± 0.05^{d}	5.14 ± 0.1^{d}	5.05 ± 0.14^{c}	5.17 ± 0.09^{d}	5.38 ± 0.06^{d}	5.25 ± 0.06^{d}	5.2 ± 0.02^{d}
TAA (μmol/g)	7.9 ± 0.49^{a}	8.49 ± 0.09^{b}	9.25 ± 0.12^{b}	10.05 ± 0.30^{c}	$9.6 \pm 0.04b^{c}$	10.17 ± 0.2^{c}	10.82 ± 0.06^{d}	11.65 ± 0.09^{d}	12.15 ± 0.32^{e}	12.06 ± 0.06^{e}
AL (mg/g)	4.81 ± 0.23^{c}	4.77 ± 0.24^{c}	5.21 ± 0.12^{d}	5.10 ± 0.31^{d}	4.12 ± 0.18^{b}	3.6 ± 0.24^{ab}	3.68 ± 0.12^{b}	4.11 ± 0.3^{b}	3.59 ± 0.25^{a}	3.17 ± 0.15^{a}
FIC (FU)	79.3 ± 2.03^{c}	83.28 ± 3.28^{c}	85.59 ± 1.34^{d}	87.28 ± 3.98^{d}	93.28 ± 2.22^{e}	65.14 ± 2.42^{a}	70.14 ± 1.95^{b}	69.94 ± 4.26^{a}	73.75 ± 3.78^{b}	70.94 ± 2.18^{b}
TU (FU)	20.4 ± 2.67^{e}	20.41 ± 1.96^{e}	16.41 ± 1.15^{c}	17.95 ± 2.54^{d}	16.04 ± 1.58^{c}	13.23 ± 2.46^{b}	13.96 ± 0.73^{b}	15.07 ± 2.2^{c}	13.48 ± 1.53^{b}	12.36 ± 1.28^{a}
FAST	392.1 ± 39.31^{a}	409.65 ± 27.57^{b}	522.8 ± 27.42^{c}	490.98 ± 50.16^{b}	584.42 ± 41.9^{d}	500.61 ± 67.67^{c}	503.07 ± 17.24^{c}	468.01 ± 41.78^{b}	549.61 ± 35.62^{d}	576.54 ± 40.82^{d}
BI	0.291 ± 0.04^{c}	0.2945 ± 0.03^{c}	0.2925 ± 0.05^{c}	0.2962 ± 0.03^{c}	0.3177 ± 0.08^{d}	0.2219 ± 0.03^{a}	0.222 ± 0.05^{a}	0.2229 ± 0.03^{a}	0.2251 ± 0.02^{a}	0.2378 ± 0.02^{b}

Data values with the same alphabetic superscript in a row do not vary significantly ($P < 0.05$). FTFC, BTFC and TFC = Free, Bound and Total Flavonoid Content respectively;; FTPC, BTPC and TPC, Free, Bound and Total Phenolic Content, respectively; FAA, BAA and AA, Free, Bound and Total Antioxidant Activity, respectively; FIC = Free Fluorescent Intermediate Compounds; TRY = Fluorescence of Soluble Tryptophan; FAST = Fluorescence of Advanced MRPs; BI = Browning index; AL = Available lysine; RE = rutin equivalents, GAE = Gallic acid equivalents.

Table 3
Effect of germination time on level of free and bound phenolic compounds (mg/100 g) of raw and malted white and black quinoa flour (dry weight basis).

		White Quinoa					Black Quinoa				
Germination time (h)		0	24	48	72	96	0	24	48	72	96
Hydroxybenzoic acids											
Gallic acid	Free	7.59 ± 0.03^{c}	7.82 ± 0.15^{c}	8.14 ± 0.23^{d}	8.42 ± 0.11^{d}	7.42 ± 0.16^{b}	8.15 ± 0.28^{d}	8.26 ± 0.04^{d}	6.75 ± 0.32^{a}	8.4 ± 0.26^{d}	7.52 ± 0.19^{c}
	Bound	12.92 ± 0.17^{e}	12.73 ± 0.31^{e}	12.54 ± 0.2^{e}	13.25 ± 0.37^{e}	12.11 ± 0.14^{e}	8.28 ± 0.29^{b}	8.31 ± 0.14^{b}	8.73 ± 0.17^{b}	8.19 ± 0.24^{b}	7.5 ± 0.19^{a}
Protocatechuic acid	Free	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Bound	64.26 ± 2.21^{b}	62.35 ± 0.73^{a}	63.94 ± 1.34^{b}	68.98 ± 1.1^{c}	67.95 ± 2.61^{c}	72.21 ± 1.37^{d}	71.69 ± 2.45^{d}	76.87 ± 3.41^{e}	73.48 ± 1.32^{d}	70.59 ± 3.1^{e}
Vanillic acid	Free	3.22 ± 0.08^{a}	3.78 ± 0.26^{a}	6.61 ± 0.12^{d}	7.06 ± 0.3^{e}	7.18 ± 0.38^{e}	4.83 ± 0.22^{b}	5.20 ± 0.14^{c}	5.43 ± 0.45^{c}	6.52 ± 0.19^{d}	6.42 ± 0.25^{d}
	Bound	20.04 ± 2.21^{d}	20.59 ± 3.37^{e}	18.87 ± 1.44^{c}	20.52 ± 1.15^{e}	18.91 ± 0.84^{c}	16.93 ± 1.62^{b}	16.1 ± 0.93^{a}	17.22 ± 0.17^{b}	15.68 ± 0.51^{a}	16.82 ± 0.69^{b}
p-hydroxybenzoic acid	Free	1.97 ± 0.09^{a}	2.18 ± 0.11^{a}	2.64 ± 0.05^{b}	3.38 ± 0.13^{b}	2.57 ± 0.17^{b}	3.17 ± 0.1^{b}	4.63 ± 0.06^{c}	6.29 ± 0.2^{e}	5.4 ± 0.03^{d}	4.97 ± 0.11^{d}
	Bound	7.23 ± 0.25^{a}	7.29 ± 0.09^{a}	7.23 ± 0.72^{a}	6.54 ± 0.28^{a}	7.35 ± 0.15^{a}	12.82 ± 0.1^{d}	11.1 ± 0.21^{c}	13.85 ± 0.37^{d}	13.67 ± 0.09^{d}	12.32 ± 0.22^{d}
Hydroxycinnamic acid											
trans-Ferulic acid	Free	16.86 ± 0.26^{d}	16.92 ± 1.62^{d}	16.67 ± 0.6^{d}	19.38 ± 1.15^{e}	18.73 ± 0.93^{e}	11.6 ± 1.03^{a}	12.17 ± 0.59^{b}	13.13 ± 0.73^{b}	12.93 ± 1.57^{b}	12.23 ± 1.11^{b}
	Bound	61.08 ± 1.07^{b}	56.8 ± 1.0^{a}	59.47 ± 0.73^{a}	66.93 ± 2.44^{c}	63.92 ± 2.03^{b}	71.11 ± 3.03^{d}	69.04 ± 1.87^{c}	72.45 ± 2.83^{d}	73.16 ± 3.57^{d}	68.37 ± 2.48^{c}
Caffeic acid	Free	0.06 ± 0.0^{a}	0.85 ± 0.01^{b}	1.26 ± 0.03^{b}	2.82 ± 0.15^{d}	2.11 ± 0.1^{c}	–	0.06 ± 0.01^{a}	0.16 ± 0.01^{a}	0.35 ± 0.01^{a}	0.36 ± 0.01^{a}
	Bound	8.13 ± 0.26^{b}	7.29 ± 0.32^{a}	8.24 ± 0.21^{b}	9.15 ± 0.07^{c}	6.45 ± 0.19^{a}	11.46 ± 0.29^{d}	10.18 ± 0.36^{c}	9.36 ± 0.15^{c}	12.33 ± 0.31^{e}	9.72 ± 0.24^{c}
Sinapic acid	Free	19.9 ± 0.46^{b}	17.73 ± 0.07^{a}	19.32 ± 0.73^{a}	19.71 ± 1.72^{a}	20.46 ± 0.34^{b}	23.42 ± 1.73^{d}	23.75 ± 1.36^{d}	23.57 ± 0.82^{d}	24.64 ± 1.35^{e}	22.26 ± 1.02^{c}
	Bound	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
p-coumaric acid	Free	3.95 ± 0.22^{a}	4.36 ± 0.27^{b}	4.23 ± 0.06^{b}	5.43 ± 0.53^{c}	6.16 ± 0.27^{d}	4.55 ± 0.39^{b}	4.94 ± 0.14^{c}	6.27 ± 0.41^{d}	5.77 ± 0.12^{c}	5.56 ± 0.38^{c}
	Bound	1.79 ± 0.24^{a}	1.55 ± 0.23^{a}	2.76 ± 0.17^{b}	2.84 ± 0.22^{b}	4.56 ± 0.19^{d}	3.92 ± 0.14^{c}	4.27 ± 0.06^{d}	4.08 ± 0.17^{d}	6.05 ± 0.14^{f}	4.73 ± 0.2^{d}
Flavan-3-ols											
Catechin	Free	37.74 ± 0.41^{a}	36.99 ± 0.45^{a}	38.83 ± 1.32^{a}	41.92 ± 1.52^{b}	38.73 ± 1.32^{a}	45.39 ± 0.31^{d}	42.95 ± 0.44^{c}	47.11 ± 0.59^{d}	46.84 ± 0.49^{d}	42.62 ± 0.34^{c}
	Bound	3.58 ± 0.34^{a}	3.27 ± 0.23^{a}	4.95 ± 0.45^{b}	4.69 ± 0.42^{b}	4.27 ± 0.49^{a}	7.14 ± 0.14^{c}	7.63 ± 0.06^{d}	8.32 ± 0.17^{d}	9.74 ± 0.14^{e}	7.88 ± 0.7^{d}
Epicatechin	Free	0.26 ± 0.01^{a}	0.45 ± 0.003^{b}	0.93 ± 0.02^{c}	0.79 ± 0.002^{c}	0.48 ± 0.01^{b}	0.89 ± 0.01^{c}	0.73 ± 0.01^{c}	1.48 ± 0.01^{e}	1.83 ± 0.02^{f}	0.68 ± 0.02^{b}
	Bound	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Flavonols											
Rutin	Free	11.75 ± 0.49^{b}	12.42 ± 0.13^{b}	12.82 ± 0.71^{b}	13.43 ± 0.39^{c}	12.73 ± 0.49^{b}	10.38 ± 0.46^{a}	10.47 ± 0.38^{a}	12.35 ± 0.54^{b}	12.85 ± 0.11^{b}	12.29 ± 0.57^{b}
	Bound	0.27 ± 0.01^{b}	nd	0.37 ± 0.02^{b}	0.81 ± 0.02^{d}	0.2 ± 0.01^{ab}	nd	nd	0.08 ± 0.003^{a}	0.06 ± 0.01^{a}	0.11 ± 0.02^{a}
Quercetin	Free	9.04 ± 0.21^{d}	9.57 ± 0.18^{d}	8.29 ± 0.12^{c}	10.53 ± 0.32^{e}	9.54 ± 0.32^{d}	5.6 ± 0.25^{a}	6.96 ± 0.19^{b}	8.13 ± 0.24^{c}	9.07 ± 0.22^{d}	7.53 ± 0.15^{c}
	Bound	1.65 ± 0.01^{c}	0.92 ± 0.01^{b}	1.25 ± 0.02^{c}	2.59 ± 0.02^{e}	2.17 ± 0.01^{d}	0.75 ± 0.01^{b}	1.28 ± 0.1^{c}	1.79 ± 0.11^{c}	2.48 ± 0.16^{e}	0.43 ± 0.01^{a}
Kaempferol	Free	0.74 ± 0.01^{b}	0.58 ± 0.02^{b}	0.68 ± 0.01^{b}	0.85 ± 0.02^{c}	0.43 ± 0.02^{a}	1.31 ± 0.02^{e}	1.38 ± 0.02^{e}	0.98 ± 0.03^{c}	1.27 ± 0.02^{e}	1.18 ± 0.01^{d}
	Bound	0.7 ± 0.01^{b}	0.38 ± 0.01^{a}	0.63 ± 0.01^{b}	0.74 ± 0.01^{b}	0.57 ± 0.01^{b}	1.39 ± 0.01^{e}	1.36 ± 0.02^{e}	1.35 ± 0.02^{e}	0.83 ± 0.02^{b}	1.2 ± 0.07^{d}

Data values with the same alphabetic superscript in a row do not vary significantly ($P < 0.05$). nd = not detected.

Table 4a
F values from two-way ANOVA analysis of the parameters (germination time versus grain colour) in Table 1.

	df	PC	AC	Moisture	RS	Ca	K	Mg	Zn	Fe	Cu	Mn
Germination Time	4	7.53**	0.83	110.62**	946.52**	9.52**	3.44**	0.49	30.36**	9.36**	4.56**	8.66**
Grain Colour	1	222.69**	31.78**	2	0.18	419.41**	244.35**	313.52**	1219.62**	1569.26**	1266.4**	814.03**
Interaction	4	1.75	0.36	1.81	26.79**	2.12	1.59	0.38	4.55**	10.53**	2.32	2.78

df, degree of freedom; *$P < 0.05$; **$P < 0.005$. PC = Protein content; AC = Ash content; RS = Reducing Sugar Content; Mg = Magnesium; Ca = Calcium = Fe = Iron; Cu = copper; K = Potassium; Mn = Manganese; Zn = Zinc.

respectively. This could be as a result of increase in protein and free amino acid (refer to 3.1) during germination. Further, sharp decline in AL by 14.34% and 11.94% was observed for WQM and BQM germinated for 96 h, respectively, that could be attributed to the formation of protein–carbonyl complex, which act as precursors for subsequent reactions. As GT increased, reducing sugar content also increased

Table 4b
F values from two-way ANOVA analysis of the parameters (germination time versus grain colour) in Table 1 and 2.

	df	TFC	TPC	TAA	SC	TC	PA	FIC	TU	FAST	AL	BI
Germination Time	4	88.86**	185.47**	232.64**	99.56**	69.49**	428.37**	10.36**	2.23	10.38**	15.86**	0.25
Grain Colour	1	284.2**	415.24**	2169.94**	176.3**	615.03**	101.77**	221.09**	43.69**	6.96**	202.64**	21.84**
Interaction	4	44.82**	2.21	3.79*	15.17**	4.49**	26.57**	2.57	2.2	44.26**	1.33	0.02

df, degree of freedom; *P < 0.05; **P < 0.005; ; SC = Saponin Content; PaC = Phytic acid Content; TC = Tannin Content; TFC = Total Flavonoid Content, respectively; TPC, Total Phenolic Content, respectively; AA, Total Antioxidant Activity, respectively; FIC = Free Fluorescent Intermediate Compounds; TRY = Fluorescence of Soluble Tryptophan; FAST = Fluorescence of Advanced MRPs; AL = Available lysine; BI = Browning index.

Table 4c
F values from ANOVA analysis of the phenolic acid data (germination time versus grain colour) in Table 3.

		Gallic acid		Protocatechuic acid	Vanillic acid		*p*-hydroxybenzoic acid		*trans*-Ferulic acid		Caffeic acid	
	df	Free	Bound	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Germination Time	4	24.82**	15.41**	3.66*	144.87**	0.15	268.04**	15.5**	3.26*	7.33**	643.22**	101.47**
Grain Colour	1	0.74	2831.66**	90.94**	1.28	31.91**	3032.46**	2565.75**	194.45**	120.93**	3214.53**	886.68**
Interaction	4	22.94**	5.74**	5.35**	36.74**	1.21	87.14**	28.23**	2.13	3.99*	354.93**	20.15**

df, degree of freedom; *P < 0.05; **P < 0.005

(Table 1), which could account for greater availability of reducing sugars for the reaction. Also, as a result of protease activity, smaller peptides and free amino acids are readily formed during germination and drying. Previous studies have reported that carbonyl bound protein complex has been found to increase by four times during an incubation period of 5 days (37 °C) in bovine serum albumin containing high concentration of glucose (Liggins and Furth, 1997). Abderrahim et al., (2012) observed a marked increase in protein bound to carbonyl group of reducing sugar for canihua malt germinated for 72 h. Bhinder et al. (2019) reported a loss in AL of Tartary buckwheat after roasting at 130 °C to 170 °C. AL has also been reported to decrease during storage of infant cereal food formulation (Ramírez-Jiménez et al., 2004).

The FIC value, an indicator for intermediatory MRPs increased after malting from 79.30 to 93.28 FU and 65.14 to 73.75 FU, for WQ and BQ variants, respectively. The highest increase by 17.63% in FIC was noted for WQM germinated for 96 h. However, in BQ, the highest increment in FIC by 13.22% was observed for malt with GT of 72 h. Carciochi, Dimitrov and Galván (2016) reported a significant increase in FIC index for WQ malt (GT = 72 h). After malting, TU, another measure for intermediatory MRPs, decreased from 20.40 to 16.04 FU and 13.23 to 12.36 FU for WQM and BQM, germinated for 96 h, respectively. An increment of 13.91% in tryptophan fluorescence was observed for BQM germinated for 48 h. Higher the TU, lower is the glycation of protein (Abderrahim et al., 2012). FAST index is the ratio of FIC to TU and indicates the presence of advanced MRPs (Bhinder et al., 2019). GT and its interaction with grain colour resulted in highly significant variation in FAST index (P < 0.005), while grain colour had only a significant impact (P < 0.05). In case of WQ, FAST index particularly increased by 33.33% and 49.05% for malts germinated for 48 and 96 h, respectively. However, in BQM (GT = 96 h) this increase was lower (15.17%). Abderrahim, et al., (2012) reported an increase in FIC and FAST index for canihua malt germinated for 72 h.

BI, indicative of formation of melanoidins, did not vary significantly after malting, which could be due to lack of significant melanoidin formation after malting. A significant increment in BI, was observed only for malts germinated for 96 h. The transformation of fluorescence emitting MRPs to melanoidins usually takes place at higher temperatures. Therefore, unlike roasting, the lack of high temperature during malting may have led to the formation of only small amount of brown pigments. Overall results revealed that the rate of Maillard reaction, as a result of malting with longer germination period, was higher in WQ than in BQ. TU, AL and BI showed highly positive significant correlation with PC (r = 0.874, 0.977 and 0.838, respectively, p ≤ 0.005).

3.7. Total flavonoid and phenolic content

The total flavonoid and phenolic content (TFC and TPC) of WQ and BQ variants is given in Table 2. The TFC of the free polyphenolic extract (FTFC) was 3.12 mg RuE/g and 5.47 mg RuE/g for WQ and BQ, respectively. Chacaliaza Rodríguez, Espinoza Begazo, Ramos Escudero, and Servan (2016) reported a higher TFC of 9.14 and 8.69 mg RuE/g for Altiplano and Salcedo quinoa varieties. TFC of bound extract (BTFC) for WQ and BQ was 2.67 mg RuE/g and 1.47 mg RuE/g, respectively. Tang et al. (2015) compared the polyphenolic composition of white, red and black quinoa grown in Ontario (Canada) and reported that TFC of free extract was higher than base hydrolysed extract (0.5 to 2.0 mg CaE/g). Further, the study observed that black and red quinoa had 1–2 times higher free and base hydrolysable flavonoids than WQ. Abderrahim et al. (2015) studied coloured thirteen quinoa varieties from the Peruvian Atiplano region and reported that FTFC and BTFC ranged from 0.30 to 1.13 mg QuE/g and 0.28 – 1.47 mg QuE/g, with eight varieties possessing higher FTFC than BTFC.

Upon malting, a slight decrease of 1.06% in FTFC was observed in case of BQM germinated for 24 h. However, free TFC increased remarkably upon malting at all GT periods for other malt samples. Previous studies have reported a loss in phenolic content associated to leaching during steeping, complex formation with proteins or polymerization of polyphenolic compounds with low molecular weight (Khandelwal, Udipi, & Ghugre, 2010). Further, steeping stage has also been linked to the activation of enzymes such as phenylalanine ammonia-lyase that catalyses the key reactions in phenyl-propanoid pathway associated with formation of secondary metabolite (Kim et al., 2016). In case of BQM, the highest FTFC of 6.02 mg RuE/g was noted after 48 h of germination accounting for 10.09% increment in free TFC. In WQM, 55.95% increment in FTFC (4.87 mgRuE/g) was observed for GT of 72 h. Carciochi, Manrique and Dimitrov (2014) reported a sharp increase from 11.06 mg QuE/100 g to 17.65 mg QuE/100 g in FTFC of quinoa green malt germinated for 72 h. In another study, germination for 96 h has been reported to cause an increase in free TFC of *Chenopodium pallidicaule*, that is canihua (Abderrahim et al., 2012). Aguilar et al. (2019) reported a 62.2% increase in TFC due to malting. In case of BTFC, after malting, BQM germinated for 48 h showed a 70.74% increase which decreased as GT increased to 96 h, while an overall decrease for all WQM was observed.

In contrast to TFC, all quinoa flours possessed higher BTPC than FTPC. BTPC and FTPC for raw BQ was 2.29 mg GaE/g and 5.54 mg GaE/g, respectively, while WQ comprised of 2.39 mg GaE/g and 4.71 mg GaE/g, respectively. In the study by Abderrahim et al. (2015), FTPC and

Table 4d
F values from ANOVA analysis of the flavonoid data (germination time versus grain colour) in Table 3.

		Sinapic acid	*p*-coumaric acid		Catechin		Epicatechin	Rutin		Quercetin		Kaempferol	
	df	Free	Free	Bound	Free	Bound	Free	Free	Bound	Free	Bound	Free	Bound
Germination Time	4	1.32	27.38**	125.14**	26.4**	27.69**	5391.05**	20.82**	1180.55**	91.12**	431.74**	182.22**	96.58**
Grain Colour	1	103.66**	27.17**	801.34**	394.22**	887.35**	14263.04**	32.12**	4609.64**	534.65**	218.02**	5769.57**	4352.44**
Interaction	4	3.09*	13.85**	63.32**	7.03**	5.5**	1077.34**	3.18*	973.94**	43.35**	286.85**	160.81**	234.98**

df, degree of freedom; *P < 0.05; **P < 0.005.

BTPC of thirteen coloured quinoa varieties ranged from 1.23 to 3.41 mg GaE/g, and 1.28 to 4.52 mg GaE/g, respectively, with the nine varieties possessing higher BTPC than FTFC. Tang et al. (2015) reported that darker coloured quinoa grains had significantly higher proportion of TPC ($P < 0.05$) with black quinoa possessing the highest free TPC content of 5.18 mg GaE/g. However, Diaz-Valencia, Alca, Calori-Domingues, Zanabria-Galvez, & Da Cruz (2018) did not observe any significant correlation of TPC with colour of quinoa grain.

A significant positive corelation was observed between GT and TPC ($r = 0.639$, $p \leq 0.05$) (Table 5). The highest increment of about 54.58% in FTPC from raw flour after malting was observed for BQ (3.54 mg GaE/g) germinated for 72 h. However, the highest increment (29.47%) in FTPC was observed for WQM germinated for 96 h. Previous studies have reported a two-fold increase in FTPC from 0.72 to 1.47 mg GaE/g, after 82 h of germination of quinoa (Alvarez-Jubete et al., 2010). Abderrahim et al. (2012) studied effect of GT on FTPC and BTPC of canihua and reported the highest increase in FTPC for canihua germinated for 48 h. Further increase in GT was found to result in a decline of free TPC of canihua. Aguilar et al. (2019) also reported an increment of 49% in FTPC. The highest increment of 17.15% in BTPC was observed for BQM (6.49 mg GaE/g) germinated for 48 h, while WQM showed the highest increase of 14.22% in BTPC (5.38 mgGaE/g) after 72 h of germination. In regards to both WQM and BQM flours, the lowest BTPC was reported for 24 h germinated malts, which was 4.62 and 5.81 mgGaE/g, respectively. However, Abderrahim et al. (2012) reported an increase in bound TPC for canihua germinated for 24 h; further increase in GT resulted in a decline of bound TPC. Grain colour had a higher impact on FTFC ($P < 0.005$) than FTPC ($P < 0.05$), evident from ANOVA f values (Table 4). The increase in TPC and TFC upon malting has been linked either to enzymatic hydrolysis of bound polyphenolic compounds, which thereby results in an increase of free or extractable polyphenolic compounds or due to biosynthesis of new polyphenolic compounds.

3.8. Polyphenolic profile

The polyphenolic profile as determined through HPLC analysis revealed the presence of thirteen polyphenolic compounds in both raw and malted quinoa flours (Table 3). Among the various phenolics consumed in our diet, flavonoids account for roughly two-thirds of the total phenolics and the remaining one-third are contributed by phenolic acids. The eight phenolic acids detected were categorised as hydroxybenzoic and hydroxycinnamic acids (HBA and HCA, respectively).

All detected HBAs, namely gallic acid, protocatechuic acid, vanillic acid and *p*- hydroxybenzoic acid were primarily found in bound form. These bound phenolic acids are present covalently liked via their hydroxyl group to cell wall non polysaccharides through an ether linkage and via carboxylic group to structural proteins and carbohydrates through an ester linkage (Carciochi, Manrique, & Dimitrov, 2014). Gallic acid was the major HBA detected in free form and ranged from 7.42 to 8.42 mg/100 g, higher in BQ variants than WQ variants. Pellegrini et al (2018) also reported a higher content of gallic acid in BQ than WQ. After malting, the highest increment of 10.94% in free gallic acid content was observed for WQM germinated for 48 h, while, for the same GT, the highest decline in its content, by 17.19% was observed for BQM germinated for 48 h.

Among HBAs, protocatechuic acid was the most abundant polyphenolic acid and was detected in bound form for raw WQ (64.26 mg/100 g) and BQ (72.21 mg/100 g) flours. Liu et al. (2020) also established protocatechuic acid as the dominant phenolic acid in coloured red and black quinoa. Protocatechuic acid was absent in free polyphenolic extract. Repo-Carrasco-Valencia et al. (2010) did not detect the presence of protocatechuic acid in methanolic extracts of quinoa as well. While, Tang et al. (2015) reported the presence of *p*-hydroxybenzoic acids and vanillic acid in both free and base hydrolysed polyphenolic extracts. GT correlated significantly and positively with free HBA ($r = 0.650$, $p \leq 0.05$) (Table 5).

Table 5
Pearson correlation coefficients of various parameters of flour from raw and malted white and black quinoa.

	GT	*PC*	*AC*	*RS*	*SC*	*TC*	*PaC*	*TFC*	*TPC*	*TAA*	*FIC*	*TU*	*FAST*	*AL*	*BI*	*FreeHBA*	*BoundHBA*	*FreeHCA*	*BoundHCA*	*FreeFlavan − 3 − ols*	*FreeFlavonols*	*Macro − mineral*
PC																						
AC		−0.868**			0.653*	0.783*																
RS	0.971**			0.772**	−0.659*																	
SC	−0.684*																					
TC					0.788*																	
PaC	−0.933**			−0.856**	0.787*																	
TFC						0.668*																
TPC	0.639*							0.686*														
TAA		−0.792*	0.718*						0.915**													
FIC		0.780*	−0.910**		−0.716*	−0.866**																
TU		0.874**	−0.735*						−0.683*	−0.858**												
FAST	0.774**											−0.758*										
AL		0.977**	−0.806**							−0.741**	0.692*	0.833**										
BI		0.838**	0.915**		−0.637*	−0.914**				−0.752*	0.948**	0.750*		0.752*								
Free HBA	0.684*								0.916**	0.866**		−0.723*	0.741*									
Bound HBA			0.749*					0.876**	0.669*	0.703*					−0.650*							
Free HCA	0.650*						−0.698*				0.625*											
Bound HCA		−0.785*	0.845**			0.748*		0.644*	0.732*	0.872**	−0.686*	−0.828**		−0.744*	−0.837**	0.722*	0.806**					
Free Flavan-3-ols		−0.684*	0.837**			0.819**		0.834**	0.776*	0.851**	−0.697*	−0.740*			−0.848**	0.702*	0.890**		0.949**			
Bound Flavan-3-ols		−0.850**	0.832**			0.796*		0.678*	0.810**	0.929**	−0.744*	−0.863**		−0.767*	−0.903**	0.730*	0.706*		0.931**	0.921**		
Free Flavonols					−0.654*		−0.697*				0.720*							0.679*				
Bound Flavonols										0.748*								0.794*				
Macromineral		−0.898**	0.959**		0.642*	0.877**				−0.842**	−0.928**	−0.788*		−0.822**	−0.973**		0.674*		0.866*	0.851**	0.909**	
Microminerals		0.915**	−0.845**											0.904	0.809				−0.795*	−0.692*	−0.823**	−0.829**

* $P < 0.05$; **$P < 0.005$., AC = Ash Content; PC = Protein Content; DM = Dry Matter Content. TFC = Total Flavonoid Content, respectively; TPC, Total Phenolic Content, respectively; AA, Total Antioxidant Activity, respectively; FIC = Free Fluorescent Intermediate Compounds; TRY = Fluorescence of Soluble Tryptophan; FAST = Fluorescence of Advanced MRPs; BI = Browning index; AL = Available lysine; RS = Reducing Sugar Content; SC = Saponin Content; PaC = Phytic acid Content; TC = Tannin Content; Mg = Magnesium; Ca = Calcium = Fe = Iron; Cu = copper; K = Potassium; Mn = Manganese; Zn = Zinc; HBA = Hydroxybenzoic acids; HCA = Hydroxycinnamic acid.

Protocatechuic acid increased after malting by 6.45% and 7.34% in BQM germinated for 48 h and WQM, germinated for 96 h, respectively. A decline in its content was also noted, for both quinoa malt variants after germination of 24 h and also for BQM (2.24%) germinated for 96 h. Similar trend in overall loss of bound HBA upon malting was observed. The later loss of protocatechuic acid could be attributed to decluming, while the former could be attributed to the hydrolysis of bound phenolic acids. It has been suggested, previously, that as a result of soaking and the subsequent increase in grain metabolism, some bound polyphenolic components get liberated, thus increasing the content free polyphenolic compounds (Xu, Tian, Hu, Luo, Wang, & Tian, 2009).

After malting, the highest increment of 122.98% among HBAs, from 3.22 to 7.18 mg/100 g, was observed in free vanillic acid for WQM germinated for 96 h. However, for BQ variants highest increase of 98.42% from 3.17 to 6.29 mg/100 g was observed in free *p*-hydroxybenzoic acid for BQM germinated for 48 h. Carciochi, Manrique, and Dimitrov (2014) reported a sharp increase in vanillic acid content from 0.88 mg/100 g in raw quinoa to 8.54 mg/100 g in quinoa green malt germinated for 72 h. The study also reported a steady increase in p-hydroxybenzoic acid at all GT.

Among HCAs detected, *trans*- ferulic acid was the most abundant followed by sinapic acid, p-coumaric acid and caffeic acid. Chlorogenic acid was not detected in the quinoa variants. Tang et al (2015) has also reported the presence of *trans*-ferulic acid, *p*-coumaric acid and caffeic acid in both free and base hydrolysed polyphenolic extracts, with *trans*-ferulic acid and *p*-coumaric acid primarily present in bound form. Bound *trans*-ferulic acid was the most abundant HCA, with WQ and BQ containing 61.08 and 71.11 mg/100 g, respectively. The content of bound *trans*-ferulic acid showed the highest decline of 7.01% and 3.85%, in WQM (GT = 24 h) and BQM (GT = 96 h), respectively. However, unlike bound *trans*-ferulic acid, a steady increase in free *trans*-ferulic acid was observed at all GT. Kim et al. (2016) reported an overall total increase by 61.88% in free phenolic acids, while a total reduction by 42.85% was observed in bound phenolic acids suggesting the liberation of bound phenolic acid via hydrolysis of the cell wall structures to yield free phenolic acids. The highest increment of 14.95% and 13.18% was observed in free *trans*-ferulic acid of WQM (72 h) and BQM (48 h), respectively. Carciochi, Manrique, and Dimitrov (2014) also reported an increase in free ferulic acid content from 0.57 to 3.61 mg/100 g, for green quinoa malt, after germination for 72 h. Caffeic acid was predominantly present in bound form (6.45–12.33 mg/100 g), while sinapic acid was only detected majorly in free form (17.73–24.64 mg/100 g). After malting, the highest overall decline among the four HCAs was observed for bound caffeic acid, where WQM (GT = 96 h) and BQM (GT = 24 h) exhibiting a decline of 20.63% and 18.37%, respectively. The highest overall increase of 55.95% and 154.61% was reported for free and bound p-coumaric acid, respectively for WQM germinated for 96 h. While, in BQM the highest increment of 3.69% and 54.30% in free and bound p-coumaric acid, respectively was observed for malts germinated for 48 and 72 h, respectively and these values declined with further increase in GT. Carciochi, Galván-D'Alessandro, Vandendriessche, and Chollet, (2016) observed a progressive increment in the contents of p-coumaric acid and ferulic acid of white quinoa malt as GT increased from 0 to 72 h. Carciochi, Manrique, and Dimitrov (2014) also noticed a similar trend. Some polyphenols namely, phloretic acid, *trans*-cinnamic acid, and naringenin have been reported, previously, to increase after germination as a result of phenolic acid synthesis from amino acids like L-tyrosine or L-phenylalanine and other phenolic acids and via the shikimate pathway (Kim et al., 2016). Moreover, *p*-coumaric acid has been reported to get synthesised readily from amino acid (Lise, Charles, & Jean-Francois, 2002). Also, GT correlated positively and significantly with free HCA ($r = 0.650$, $p \leq 0.05$).

The presence of flavan-3-ols, namely, catechin and epicatechin and flavonols, namely, rutin, quercetin and kaempferol were detected. Quinoa variants were rich in catechin, followed by rutin and quercetin and limiting in kaempferol and epicatechin. Epicatechin was not detected in bound form. Quercetin was found to be present in higher amounts in WQ variants, while no significant difference was observed in the content of rutin among the two quinoa variants as well as between raw and malted flours. Pellegrini et al. (2018) reported the presence of rutin, kaempferol and quercetin, while Tang et al. (2015) reported the presence of epicatechin as well in quinoa grains. The highest increment of 62.01% in quercetin was observed for BQM germinated for 72 h. Free catechin content was found to decrease by 2.0% and 5.38% for WQM and BQM germinated for 24 h, respectively, which could be attributed to steeping losses. While, Bound catechin content showed a steady increase by 6.79 to 36.33% as GT increased from 0 to 72 h in BQ variants. The increase in both bound catechin and *p*-coumaric acid after germination in quinoa malt could be attributed to the synthesis of polymeric bound form as a result of plant growth. Also, the loss of free catechin and free *p*-coumaric acid has been associated with tannin and lignin biosynthesis (Kim et al., 2016).

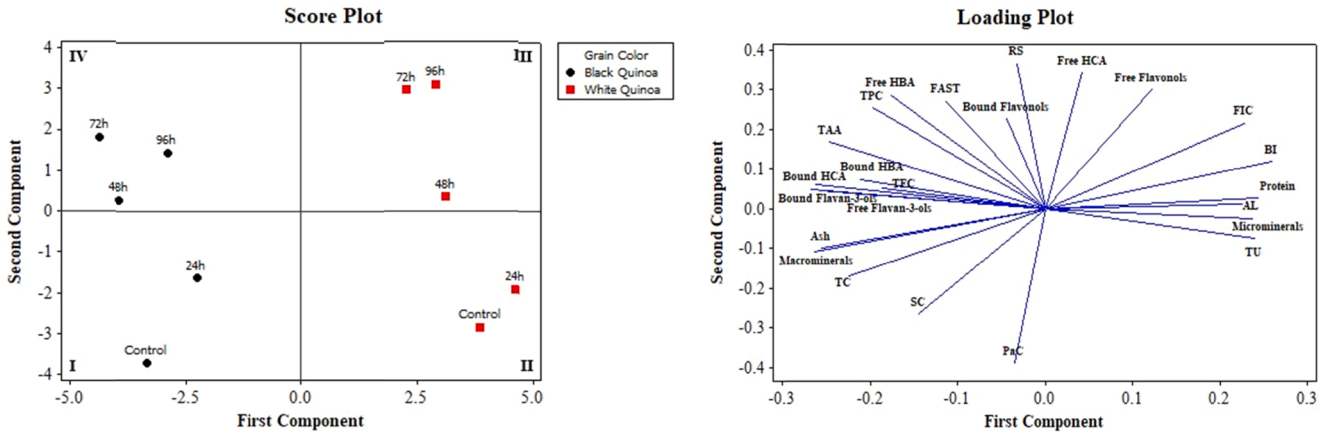
Overall, the changes in polyphenolic contents could be due to hydrolysis of polymeric polyphenols due to the enzyme glucosidase or esterases that yields free or extractable polyphenolic compounds, formation of insoluble or unextractable complex with proteins, polymerization of some polyphenols with low molecular weight, decluming, leaching during grain soaking or antioxidant synthesis (Kim et al., 2016).

3.9. Antioxidant activity

The antioxidant activity (AA) of raw and malted quinoa flours is given in Table 2. The total antioxidant activity (TAA) of WQ and BQ was 7.90 and 10.17 μmol TE/g, respectively, with the free polyphenolic extract (3.61 μmol TE/g) of WQ exhibiting lower AA than bound polyphenolic extract (4.29 μmolTE/g). However, BQ showed higher free antioxidant activity (FAA) (5.19 μmol TE/g) than bound antioxidant activity (BAA) (5.05 μmol TE/g). Malting resulted in a highly significant variation in TAA, with grain colour contributing more to this variation ($P \leq 0.005$) (Table 4b). The highest increment of 34.76% and 31.57% was observed in FAA for WQM and BQM germinated for 72 h, while BAA exhibited an increment of 23.31% and 6.53% for WQM and BQM, respectively. Aguilar et al. (2019) reported an increment in AA after malting (GT = 48 h) and also reported that coloured quinoa had higher AA than white quinoa. TAA showed highly significant positive correlation with TPC ($r = 0.915$, $p \leq 0.005$), while the correlation between TAA and TFC was only significantly positive ($r = 0.625$, $p \leq 0.005$) indicating perhaps the role of MRPs formation that exaggerates TPC and also exhibit antioxidant properties. Malting and germination has been found to enhance antioxidant activities of several cereals, legumes and pseudocereals through the biosynthesis of antioxidants of low molecular weights (Alvarez-Jubete et al., 2010) or liberating bound polyphenols through enzyme induced hydrolysis of cell wall matrix comprising up of hemicellulose, cellulose, lignin, pectin and structural proteins (Bewley et al., 2013). Further, TAA and FAST index ($r = 0.625$, $p = 0.053$) had positive correlation even though it was not significant at $p \leq 0.05$. A highly negative significant correlation with TU and BI (r = -0.858 and −0.752, respectively, $p \leq 0.005$). Abderrahim et al. (2012) reported the formation of significant advanced MRPs in canihua malt, particularly for malt with the GT of 72 h, which also had the highest antioxidant activity. The advanced end products of glycation, as assessed by FAST index, have been suggested to potentially regulate the biosynthesis of certain antioxidants, thereby improving antioxidant properties (Wu et al., 2011).

3.10. Principal component analysis

To characterize each sample on the basis of nutritional property and establish relation among properties, principal component analysis (PCA) was carried out (Fig. 1 & Table 1S). The first-three principal components (PC) (eigen value > 1), were found to account for 90.3% variability, with



(A) (B)

Fig. 1. Principal component analysis (PCA): (A) score plot and (B) loading plots; describing and comparing the relationship among various nutritional parameters of raw and malted black and white quinoa flours. Where, TFC = Total Flavonoid Content, respectively; TPC, Total Phenolic Content, respectively; AA, Total Antioxidant Activity, respectively; FIC = Free Fluorescent Intermediate Compounds; TRY = Fluorescence of Soluble Tryptophan; FAST = Fluorescence of Advanced MRPs; BI = Browning index; AL = Available lysine; RS = Reducing Sugar Content; SC = Saponin Content; PaC = Phytic acid Content; TC = Tannin Content; Mg = Magnesium; Ca = Calcium = Fe = Iron; Cu = copper; K = Potassium; Mn = Manganese; Zn = Zinc; HBA = Hydroxybenzoic acids; HCA = Hydroxycinnamic acid.

PC1, PC2 and PC3 individually accounting for 55%, 24.2% and 11.1% variability, respectively, in the data set. Variables such as, free flavan-3-ols (-0.267), total macromineral content (-0.262), BI (0.259) and bound flavan-3-ols (-0.257) primarily contributed to the variability accounted by PC1. While, variability of PC2 was largely attributed to phytic acid (-0.389), RS (0.367), free HCA (0.343), free flavonols (0.301), free HBA (0.287) FAST index (0.271) and saponin (-0.265). The two-dimensional score plot (Fig. 1A) between PC1 and PC2 clearly shows the impact of GT and grain colour on nutrient composition of raw and malted quinoa flours. In terms of grain colour, BQ (quadrant I and IV) and WQ (quadrant II and III) variants differed mainly on the basis of flavan-3-ols, macrominerals (Ca, K and Mg), bound HCA and TAA, with the former having higher concentration of these nutrients. While, WQ variants had higher protein and BI. After malting, a gradual shift can be seen in the score plot from quadrant I and II to quadrant III and IV. The shift was mainly attributed to the gradual loss of phytic acid and saponins, increase in RS, free phenolics (HCA, flavonols and HBA) and FAST index. Also, this shift in the nutrient concentration was quite rapid during the first 48 h of germination in BQ than WQ as evident from the wider distance between raw and malted counter parts. Thus, indicating higher metabolic activity in BQ during the first-two days of germination. However, changes in aforementioned nutrients greatly increased after 72 h of germination in case of WQ variants. At GT of 72 h and 96 h, WQM had higher MRP formation, lower antinutrient components than corresponding BQM. From loading plot (Fig. 1B) it is clear that TPC showed close positive relation with free HBA. Further, FAST index, an indicator of advanced products of glycation, closely corelated with TPC and TAA, pointing towards the potential role of advanced MRPs in increasing antioxidants.

4. Conclusion

In conclusion, malting was found to mobilise nutrient reserves through enzymatic activity to significantly increase polyphenols, antioxidant activity, protein and reducing sugar. Free and bound flavan-3-ols, macrominerals, bound HCA and HBA influenced grain colour, with BQ variants possessing higher amounts of these nutrients. In contrast, WQ variants had higher protein, MRPs formation and lower antinutrients. Among antinutrients, increase in GT caused the highest decrease in PaC followed by SC. However, tannin content showed a slight increase when GT increased to 48 h and 72 h. Even though BQ variants had higher TPC and TFC content, WQ variants had the highest increment (27.23%) in TAA, that could be attributed to greater MRP formation which likely enhanced TAA. Also, the metabolic changes that occur over the course of germination, were higher in BQ during the first 48 h, as indicated by the greater proportion of phenolics in BQM at 24 and 48 h of germination. While, in WQ this increase was observed at GT of 72 h. Therefore, BQM and WQM germinated for 48 and 72 h were nutritionally the most superior and therefore could be used as gluten-free adjunct in cakes, cookies and breads. Overall, nutritional quality of malts germinated for 96 h was inferior in terms of loss in antioxidants, minerals and protein as a result of declumming.

CRediT authorship contribution statement

Seerat Bhinder: Investigation, Data curation, Validation, Writing - original draft. **Supriya Kumari:** Investigation, Data curation, Formal analysis. **Balwinder Singh:** Conceptualization, Formal analysis, Resources, Writing - review & editing. **Amritpal Kaur:** Conceptualization, Investigation, Funding acquisition, Project administration, Resources, Formal analysis. **Narpinder Singh:** Conceptualization, Resources, Validation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128915.

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