Malting process optimization for protein digestibility enhancement in finger millet grain

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Abstract Finger millet (*Eleusine coracana*) is a nutritious, gluten-free, and drought resistant cereal containing high amounts of protein, carbohydrate, and minerals. However, bio-availability of these nutrients is restricted due to the presence of an excessive level of anti-nutrient components, mainly phytic acid, tannin, and oxalate. It has been shown that a well-designed malting/germination process can significantly reduce these anti-nutrients and consequently enhance the nutrient availability. In the present study, the effects of two important germination factors, duration and temperature, on the enhancement of in-vitro protein digestibility of finger millet were thoroughly investigated and optimized. Based on a central composite design, the grains were germinated for 24, 36, and 48 h at 22, 26, and 30 °C. For all factor combinations, protein, peptide, phytic acid, tannin, and oxalate contents were evaluated and digestibility was assessed. It was shown that during the malting/germinating process, both temperature and duration factors significantly influenced the investigated quantities. Germination of finger millet for 48 h at 30 °C increased protein digestibility from 74 % (for native grain) up to 91 %. Besides, it notably decreased phytic acid, tannin, and oxalate contents by 45 %, 46 %, and 29 %, respectively. Linear correlations between protein digestibility and these anti-nutrients were observed.

Keywords Finger millet · Germination process · In-vitro protein digestibility · Anti-nutrient components

Introduction

According to FAO, in 2014, the worldwide production of millet grains was 30 million tones, which made it the 6th most important agricultural cereal crop (FAOSTAT 2014). Millet is one of the most drought-resistance grains; hence, it is widely grown in arid and semi-arid areas of Asia and Africa (Saleh et al. 2013). Furthermore, millet grain is gluten-free, has a short growing season, has low production costs, and possesses a good nutritional profile, which is comparable or better than the other vastly consumed cereals (Omary et al. 2012; Saleh et al. 2013).

Based on their annual production volumes, four millet varieties are more common: Pearl millet (*Pennisetum typhoideum*), Foxtail millet (*Setaria italica*), Proso millet (*Panicum miliaceum*), and Finger millet (*Eleusine coracana*). Pearl millet is more produced and consumed worldwide and therefore, it has been the subject of more researches (Abdelrahman et al. 2007; Omary et al. 2012). Nevertheless, finger millet has been attracting increasing interest since it contains higher amounts of essential amino acids, especially lysine, threonine, and valine (Ravindran 1991). This makes finger millet superior comparing to other millets and even other cereals, particularly to be used in functional foods, like weaning products. Finger millet typically has a carbohydrate content of 72 %, protein 7.3 %, fat 1.3 %, dietary fiber 11.5 %, and mineral 2.7 % (FAOSTAT 2014).

Despite the appropriate nutrition profile of finger millet, similar to other cereals, the bio-availability of its nutrients is restricted due to the presence of high levels of anti-nutrient components and enzyme inhibitors. Anti-nutrients like

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phytate and oxalate, that bind essential minerals and proteins, along with tannins that complex proteins and inhibit enzymes, all negatively affect the nutrient bio-availability of finger millet and reduce its digestibility (Pushparaj and Urooj 2014; Ravindran 1991).

Among millet varieties, finger millet has been reported to contain higher amounts of tannin (0.04 to 3.74 % of catechin equivalents) (Antony and Chandra 1999; Rao 1994). Besides, the grain had considerable amounts of phytic acid (500–600 mg/100 g) (Antony and Chandra 1999). Studies have shown that the in-vitro protein digestibility was negatively associated with the tannin (Mbithi-Mwikya et al. 2000b; Ramachandra et al. 1977) and phytic acid (Mbithi-Mwikya et al. 2000b) contents in finger millet. Surprisingly, very few studies have been performed on the assessment of the oxalate content of finger millet. Ravindran (1991) reported that finger millet contained 29 mg/100 g of oxalate. A more recent study claimed that oxalate in raw finger millet was 11.3 mg/100 g (Amalraj and Pius 2015). In both studies, it was shown that an appropriate soaking step significantly reduced the oxalate content of the grains (Amalraj and Pius 2015). This is due to the fact that in millet, oxalate mostly exists in its water-soluble form.

It is recognized that simple traditional food processing treatments, like soaking and malting/germination may significantly reduce the anti-nutrient contents of cereal grains and improve their nutrients' bio-availabilities (Najdi Hejazi et al. 2016). The increased phytase activity during the germination step is the reason for reduction of phytic acid in sprouts, since phytase hydrolyzes phytic acid. Khetarpaul and Chauhan (1989) reported a 40 % reduction in phytate level after 24 h germination of pearl millet. Chavan et al. (1989) reported an overall trend of phytic acid reduction in sprouts. Similarly, germination decreased the tannin content by 54 % in finger millet (Rao 1994). In addition, soaking and germination have been found to be very effective in increasing the extractability of trace elements, like Ca, Fe, Cu, Zn, and Mn. This is due to the fact that phytates form complex matrices with these essential elements, which would be broken throughout the food processing steps (Rateesh et al. 2012). In recent investigations, it has been confirmed that well-designed soaking and germination stages significantly decreased the phytate and tannin contents in millet grains (Abdelrahman et al. 2007; Swami et al. 2013).

A study on 32 varieties of finger millet showed relatively low *In-Vitro* Protein Digestibility (IVPD) ranging from 55.4 to 88.1 % (Ramachandra et al. 1977). Antony and Chandra (1999) reported slightly lower values for IVPD of finger millet ranging from 50 to 65 %. Besides, the authors observed a negative correlation between IVPD and phytates and tannin contents. Studies have shown that the IVPD of millet increased after a malting/germination process (Omary et al. 2012). Germination decreased the tannin content in millets and consequently improved their protein bio-availability

(Mbithi-Mwikya et al. 2000b). Khetarpaul and Chauhan's (1990) experiments showed a notable increase (51 %) in IVPD of pearl millet grains after soaking for 12 h and germinating for 24 h at 30 °C. Similarly, 59 % of IVPD enhancement was observed by Chaturvedi and Sarojini (1996) for 72 h germinated pearl millet sprouts.

Effect of germination temperature and duration on the nutritional improvement of cereal grains has been studied by a few researchers (Saleh et al. 2013; Swami et al. 2013). Due to different soaking practices, germination duration and temperature, and other influential factors, a few contradictory results were reported in the literature. However, regarding protein content and IVPD, the majority of the studied millet species declared a direct positive correlation with germination duration (Saleh et al. 2013; Swami et al. 2013). Swami et al. (2013) stated that as the germination time increased, the protein availability of finger millet increased. Indeed, in their study, the protein content of samples increased from 14 to 17.5 %, when the germination time increased from 8 to 24 h.

Crude protein of Proso millet showed 16 % increase in seeds that were germinated up to 8 days at room temperature (Parameswaran and Sadasivam 1994). Mbithi-Mwikya et al. (2000b), observed 30 % increase in total protein content of finger millet after 96 h of germination at 30 °C. In addition, they observed significant decreases in the anti-nutrient factors, where tannins and phytates decreased to undetectable levels at the end of the treatment. On the other hand, their study reported 13.3 % loss of the seeds' dry matter throughout the germination process. Since the most significant nutrient changes occurred in the first 48 h of germination, it was concluded that germination of finger millet beyond 48 h was not necessary (Mbithi-Mwikya et al. 2000b; Najdi Hejazi and Orsat 2015). Abdelrahman et al. (2007) investigated effects of germination duration (up to 6 days) on the anti-nutrient elements of pearl millet. They showed that the phytic acid content decreased significantly ($P < 0.01$) within the first 2 days of germination. Thereafter, the drop-rate was considerably reduced.

Unlike for the duration, effects of temperature have not been well investigated for the germination of finger millet grains. In the past studies, germination was usually conducted at room temperature (Omary et al. 2012). Since temperature directly affects the enzyme activities during sprouting, it is important to precisely monitor its effect. In the present study, the two main important factors in the germination of finger millet, duration and temperature, were thoroughly investigated. As the main anti-nutrient components in finger millet, phytate, tannin, and oxalate contents were monitored. Proteins and peptides were evaluated for each germination treatment and after digestion by pepsin and pancreatin enzymes. Finally, the protein digestibility was obtained and optimized for the selected germination factors over their specified ranges.

Materials and methods

Materials

The sproutable finger millet was obtained from University of Agricultural Sciences (Dharwad, India). All the chemicals used in the study were of analytical grades. HPLC graded water (double distilled water (ddH₂O)) was supplied using Simplicity™ water purification system (Millipore, USA). Hydrochloric acid, pepsin, pancreatin, o-phthaldialdehyde (OPA), sodium tetraborate, β-mercaptoethanol, ascorbic acid, and leucine-glycine (Leu-Gly) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, sodium phosphate, vanillin, ammonium molybdate, sulphuric acid, and methanol were provided by Fisher Scientific (Fair Lawn, NJ, USA). Sodium Dodecyl Sulfate (SDS) was obtained from Bio-Rad. Ethanol was purchased from Commercial Alcohols (Industrial and Beverage Alcohol Division of Green-field Ethanol Inc., Brampton, Ontario, Canada).

Malting process

Grains were steeped in water at a ratio of (1:5 w/v) for 24 h at room temperature. Water was drained off and the soaked seeds were germinated for three different durations (24, 36, and 48 h), each at three different temperatures (22, 26, and 30 °C). The sprouted and native seeds were freeze-dried and ground to flour for further analysis.

Statistical design and analysis

The Face-centered Central Composite Design (CCD) was used for the experimental design in the current study. Two factors, malting temperature (Temp), and duration (Time), each at three levels, (22, 26, 30 °C) and (24, 36, 48 h), respectively, were used for preparing the experimental design with a total of 12 treatment combinations. This consisted of 4 factorial (GM1, GM3, GM7, and GM9), 4 axial (GM2, GM4, GM6, and GM8), and 4 central (GM5.1, GM5.2, GM5.3, and GM5.4) point combinations (Figs. 1, 2 and 3). Based on the literature, due to the loss of seeds' dry matter and decrease in process efficiency, the germination duration in the present study was restricted up to 48 h (Mbithi-Mwikya et al. 2000b; Vidyavathi et al. 1983). In addition, the temperature range was chosen close to room temperature to avoid the increased cost due to heating requirements. JMP software version 11 (SAS Institute Inc., Cary, NC, USA) was used for the experimental design and analysis. Using ANOVA analysis and regression models, the individual (linear), quadratic, and combined (bi-linear) effects of the factors were evaluated. The resulted response

surface for each investigated quantity, Z , was constructed as follows;

$$Z = \text{Intercept} + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

In this equation, X_1 = temperature and X_2 = duration were the design factors. β_1 and β_2 represented the regression coefficients of the linear, β_{11} and β_{22} were the coefficients of the quadratic, and β_{12} expresses the interactive or bilinear effects. These coefficients were assessed using ANOVA analysis, while their significance were evaluated statistically based on the F-values at the probabilities; $p < 0.0001$, $p < 0.0005$, $p < 0.001$, $p < 0.005$, $p < 0.01$, and $p < 0.05$.

Protein content determination

Protein content was quantified using the Bradford method according to the instructions of the kit manufacturer (#500–0006, Bio-Rad, Mississauga, Ontario) (Bradford 1976). The assay is based on the ability of proteins to bind Coomassie brilliant blue G 250 and shift the color from red to blue.

Briefly, 20 μL of the sample was mixed with 980 μL of dye reagent (1 part Bradford reagent in 4 parts ddH₂O) and incubated for 5 min at room temperature. The resulting color intensity was directly proportional to the concentration of protein and was determined spectrophotometrically (Ultraspec 1000, Amersham Pharmacia Biotech, NJ, USA) at the wavelength of 595 nm. The protein concentrations were compared to a standard curve of bovine serum albumin (0–2 mg/mL). Proteins with molecular weight of more than 3–5 kDa were detected using this approach.

In-vitro enzymatic protein digestion

In-vitro protein digestibility was assessed by employing the pepsin and pancreatin digestion method of Maria Vilela et al. (2006) with small modifications. The undigested protein residues before pepsin digestion, after pepsin digestion, and after pancreatin digestion were evaluated using the Bradford method (Protein content determination section). The in-vitro protein digestion percentage was calculated by subtracting the undigested protein from the initial total protein of the sample as follows;

$$\text{IVPD}(\%) = \frac{\text{Initial protein} - \text{final undigested protein}}{\text{Initial Protein}} \times 100 \quad (2)$$

Peptide content determination

The peptide content was determined by modification of the Church et al. method (1983) The method is based on binding

Fig. 1 The measured (a) protein and (b) peptides before and after pepsin and pancreatin digestion for the selected experimental design factor combinations

of the released α -amino groups with OPA reagent, causing an invisible color change, which can be detected spectrophotometrically.

Initially, 50 mL of OPA stock solution (25 mL of 100 mM sodium tetraborate solution, 2.5 mL of 20 % w/w SDS, 40 mg o-phthalaldehyde in 1 mL ethanol, 100 μ L of β -mercaptoethanol and adjust the volume to 100 mL using ddH₂O) was prepared. Subsequently, 20 μ L aliquots of each sample were collected before and after digestion with pepsin and pancreatin and added to 980 μ L of OPA solution and incubated precisely for 2 min. The resulting color intensity

was directly proportional to the concentration of peptide and was determined spectrophotometrically at the wavelength of 340 nm. The peptide concentration was compared to a standard curve of Leu-Gly (0–8 mg/mL).

Oxalate content determination

Total oxalate was quantified with an enzymatic kit (procedure 591, Trinity Biotech, Newark, NJ) according to the method of Horner et al. (2005). The kit principle is based on the oxidation of oxalate in the presence of oxalate

Fig. 2 Measured anti-nutrient contents. Left vertical axis presents phytic acid (g/100 g db) and tannin (g CE/100 g db) and the right axis quantifies the oxalate content (mg/100 g db)

oxidase (Chiriboga 1963). Hydrogen peroxide (H_2O_2) that is produced during the peroxidase-catalyzed reaction is measured spectrophotometrically at 590 nm.

Briefly, 100 mg malted finger millet flour was weighed in 15 mL falcon tube and autoclaved for 20 min. Next, 4 mL sample diluent (EDTA) was added to each tube, vortexed, and

Fig. 3 **a** IVPD (%) evaluated for all design factor combinations, **b** The response surface for the IVPD (%), and **c** the leverage plot based on the ANOVA analysis of IVPD

sonicated in a sonicator bath (Branson 5510, Kell-Strom, Wethersfield, CT) for 6 min. The respective tubes were centrifuged at 2000 rpm or 1500×g for 5 min after 24 h incubation at 55 °C. Finally, 1 mL oxalate reagent A (3-methyl-2-benzothiazolinone hydrazone, 3-dimethylamino benzoic acid), 50 µL supernatant, and 100 µL oxalate reagent B (oxalate oxidase; 3000 µ/L, peroxidase; 100 µ/L) were added into each tube and immediately mixed by gentle inversion. The intensity of the resulted indamine dye was proportional to the concentration of oxalate and was detected by spectrophotometer at 590 nm after 5 min of incubation at room temperature.

Phytic acid content determination

Phytic acid content was determined using Megazyme phytic acid (Phytate/Total Phosphorous) assay kit (#K-PHYT, Megazyme International, Ireland). Principle of the kit is based on the hydrolysis of phytic acid into myo-inositol (phosphate)_n and inorganic phosphate (Pi) in the presence of phytase and alkaline phosphatase (ALP) (Loewus and Murthy 2000). Ammonium molybdate and Pi react to form 12-molybdophosphoric acid, which is subsequently reduced under acidic conditions to molybdenum blue. The absorbance of molybdenum blue, which is proportional to the amount of Pi in the sample, is measured spectrophotometrically at 655 nm.

Briefly, sample extraction was carried out with addition of 20 mL hydrochloric acid (0.66 M) into 2.5 g of sample with vigorous stirring overnight. The extract was centrifuged at 13,000 rpm for 10 min. Thereafter, 20 µL of phytase was added to 50 µL of sample extract, vortexed and incubated in a water bath at 40 °C for 10 min. Subsequently, 20 µL of ALP was added, vortexed, and incubated at 40 °C for 15 min. Sample tube was re-centrifuged at 13,000 rpm for more than 10 min. Colorimetric determination of phosphorous was performed using color reagent (1 part ammonium molybdate (5 % w/v) into 5 parts ascorbic acid (10 % w/v)/sulphuric acid (1 M)) at 655 nm against a phosphorus calibration curve.

Tannin content determination

Tannin content was estimated using the vanillin hydrochloride method, developed by Price et al. (1978). The principle of the method is based on the reaction of vanillin reagent with any phenol that has an un-substituted resorcinol or phloroglucinol nucleus (Siwela et al. 2007). The intensity of the resulted color is proportional to the amount of tannin and is measured using catechin as a standard curve (10–100 µg/mL).

In order to prepare the extract, mixture of 1 g of sample in 50 mL 100 % methanol was shaken for 24 h. The sample was centrifuged at 1200 g for 10 min at 25 °C and supernatant was collected. About 1 mL of extract was mixed with 5 mL of vanillin hydrochloride reagent (8 % hydrochloric acid in methanol and 4 % vanillin in methanol) and incubated at

30 °C for 20 min. Absorbance was measured at 500 nm and data were expressed as g Catechin Equivalent CE/100 g db.

Results and discussions

In the present study, ANOVA analyses were employed to investigate the combined effects of germination design factors on the protein and anti-nutrient contents of finger millet grain. The goal was to optimize the *in-vitro* protein digestibility of the finger millet malt by selecting the best combination of the involved design factors. The experiments were developed using a face-centered Central Composite Design (CCD) with two factors, germination duration (Time) and temperature (Temp). For each factor, three levels were selected and the data were analyzed using the response surface methodology. All the experiments were conducted in triplicate for each design combination. The obtained averaged data as well as their corresponding Standard Deviation (SD) errors are presented in Figs. 1, 2 and 3.

First, crude protein contents were evaluated for the selected design combinations as described in “Protein content determination” section. The obtained results are presented in Fig. 1(a). From the figure, it was observed that the crude protein content was significantly influenced by both germination duration and temperature. While for the control sample (GM0, native grain), protein was estimated at 6.42 g/100 g db, this value increased for GM9 sample up to 7.32 g/100 g db. Therefore, germination of finger millet for 48 h and at 30 °C increased the crude protein content by 14 %. These results were aligned with findings of other researchers (Mbithi-Mwikya et al. 2000b; Swami et al. 2013). Protein content of finger millet is roughly around 7 %, but a large variation in this nutrient, from 5 to 13 %, has been reported in the literature (Singh and Raghuvanshi 2012). Mbithi-Mwikya et al. (2000b) observed around 30 % increase in the available protein content of finger millet after four days of germination at room temperature. Swami et al. (2013) reported a linear increase in the available protein content of finger millet grains that were germinated up to 24 h. In their study, protein availability enhanced from the initial 14 % up to the final 17.5 %, indicated 25 % increase. In the present study, a similar positive relationship between total crude protein availability and germination duration was observed.

The physical mechanisms and bio-chemical reactions that explain the observed increase in bio-availability of protein content during germination process are strongly associated with the morphology of the finger millet seed. In finger millet, endosperm represents the largest portion of the grain, which consists of an aleurone layer and three distinct starchy sections, peripheral, corneous, and floury endosperms. Peripheral endosperm contains small and tightly packed cells of protein bodies that are embedded in fiber-starch-protein

matrices. Corneous is the largest portion of the endosperm containing predominantly protein matrices. Finally, the floury endosperm is made up of compound starch granules, where protein bodies and protein matrices rarely exist (Belton and Taylor 2002). The observed increase in the crude protein level during germination was the outcome of an enhancement in the plant amylolytic activity (plant α -amylase) (Traoré et al. 2004). The activity of this enzyme resulted into the starch granules breakdown, which lead to the release of the protein from the packed cells and consequently, increased its bio-availability.

Peptide content of the samples before the digestion process was assessed (as described in “**Peptide content determination**” section) and the results are presented Fig. 1(b). While the initial peptide level of the raw finger millet (GM0) was around 0.79 g/100 g db, germination of the seeds for 48 h and at 30 °C (GM9) increased it up to 3.74 g/100 g db.

Hamad and Fields (1979) showed that the total free amino acids notably increased by about 4.5 fold during germination of finger millet. This increase may be associated to the activation of proteases enzyme during germination, which consequently enhances protein hydrolysis. Protease breaks down the protein into smaller polypeptides and amino-acid groups and facilitates the digestion procedure. Because of that, germination has been known as a natural pre-digestion step. Vidyavathi et al. (1983) showed that the protease activity in finger millet increased with germination time, maximized on the third day, and decreased afterward. They reported that protease inhibitors disappeared during the germination process. It is proposed that degradation of prolamins (the main fraction of finger millet protein) into lower peptides and free amino acids supplies α -amino groups, which may be used through transamination in the synthesis of lysine and other essential amino acids (Mbithi-Mwikya et al. 2000a). The observed elevation in peptide content is favorable as the protein quality of a food not only depends on its protein level, but also on the availability of its peptides.

In the next step, the samples were digested with pepsin enzyme as described in “**In-vitro enzymatic protein digestion**” section and their protein and peptide contents were monitored. The results are graphically presented in Fig. 1. It was noticed that the enzymatic digestion with pepsin remarkably dropped the protein content, with a proportional increase in the peptides. In fact, a major fraction of protein was digested in this step. The importance of pepsin digestion is more highlighted if it is compared with pancreatin digestion, where only a little further reduction in protein (Fig. 1(a)) and slight increase in the peptide levels (Fig. 1(b)) are observed. Therefore, in the employed *in-vitro* digestion simulation, although protein digestion was improved after using pancreatin enzyme, the enhancement was not as notable as with pepsin enzyme.

The IVPDs were calculated following Eq. (2) and the results are presented in Fig. 3(a). A relatively low digestion

value was obtained for the control (≈ 74 %), while for case GM9, IVPD improved up to 92 %. Mittal (2002) reported 62.94 % for the IVPD of native finger millet flour. The increase in IVPD with respect to germination duration has been frequently reported in the literature (Chaturvedi and Sarojini 1996; Desai et al. 2010; Khetarpaul and Chauhan 1990). Germination of cereals involves complex enzymatic reactions that decompose macromolecules (e.g. proteins) into smaller units (e.g. peptides) and makes them more digestible. Partial solubilization and some proteolysis, which usually occurs during germination, improves the IVPD (Mbithi-Mwikya et al. 2000b). Further ANOVA analysis on the obtained IVPD values will be presented shortly.

In order to quantify the major anti-nutrients and investigate any possible correlation between their levels and the IVPD, phytic acid, tannin, and oxalate contents were measured for all experimental design combinations. Unlike oxalate, phytic acid and tannin have been previously the subject of many investigations. The obtained results are graphically presented in Fig. 2, where for phytic acid (g/100 g db) and tannin (g CE equivalent/100 g db), the left vertical axis and for oxalate (mg/100 g db), the right axis are used as the reference scales. It is observed that all of these anti-nutrients were significantly reduced throughout the germination process. Comparing the values between the control sample (GM0) and the sample that was germinated for 48 h at 30 °C (GM9) illustrated that phytic acid, tannin, and oxalate levels decreased by 45 %, 46 %, and 29 %, respectively. Besides, from the figure, it was observed that, while germination duration was important and influential for all the investigated quantities, temperature was more influential in the case of oxalate, not tannin and phytic acid. This will be further verified through ANOVA analysis.

The reduction of tannin and oxalate contents during soaking and germination stages has been mostly attributed to their leaching into the sprouting medium (Mbithi-Mwikya et al. 2000b). Tannin reduction may also be due to the high activity of polyphenol oxidase and other catabolic enzymes during germination (Mbithi-Mwikya et al. 2000b). Sriprya et al. (1997) reported a reduction in tannin content during germination, which occurred by its hydrolysis into lower molecular weight phenols. Germination significantly reduces the phytate content, due to the increment in endogenous phytases action, which degrades the phytate into inorganic phosphorous and inositol (Traoré et al. 2004). The observed decrease in phytate content in the present study (45 %) was lower compared with that reported by Traoré et al. (2004) (67 %). The difference was probably due to having shorter germination duration in this investigation (48 h) comparing to their study (62 h).

The obtained IVPD data were analyzed using response surface methodology. The results are summarized in Table 1. Based on the obtained data both temperature and duration

Table 1 Summary of the ANOVA analysis of the responses for IVPD (%) and the corresponding parameter estimates of the different terms

Term	Estimate	Std Error	t Ratio	Prob > t
Intercept	88.9063492	0.3115380364	285.38	<0.0001
Temperature	2.09622640	0.2786480908	7.52	0.0003
Duration	2.78008463	0.2786480908	9.98	<0.0001
Duration × Temperature	−1.84089555	0.3412728202	−5.39	0.0017
Temperature × Temperature	−0.44866013	0.4179721362	−1.07	0.3243
Duration × Duration	−0.22667274	0.4179721362	−0.54	0.6071

were significant elements with $p < 0.0005$ and $p < 0.0001$, respectively. Their positive coefficients indicated a direct relation between the linear terms of the design factors and IVPD. The cross product term, duration × temperature, was significant as well ($p < 0.005$). The obtained negative coefficient of this bi-linear term illustrated its reverse influence on

the IVPD, when duration and temperature simultaneously were changed. Therefore, a maximum or saddle point in the IVPD response surface would be expected. It was observed that effects of quadratic terms were not significant. Based on the obtained results, IVPD was related to the selected design factors, X_1 and X_2 , as follows;

$$\text{IVPD}(\%) = 88.906 + 2.09623 X_1 + 2.78008 X_2 - 0.44866 X_1^2 - 0.22667 X_2^2 - 1.84090 X_1 X_2 \quad (3)$$

This response surface is plotted in 3D format in Fig. 3(b). The surface had a saddle point at 31.6 °C and 41.5 h, which was slightly outside of the selected domain of operation. The predicted value at this point was IVPD = 91 %. The corresponding leverage plot is presented in Fig. 3(c). Since the confidence curves (dashed-line curves) crossed the horizontal line, the correlation of design factors was significant ($p < 0.05$). According to the obtained response surface, the local IVPD maximum in the investigated range of design factors was roughly around 26 °C and 48 h. Although based on the original experimental data, germination at 30 °C and 48 h provided slightly better IVPD, the difference was negligible.

Similar procedures were performed for other investigated quantities. The response surfaces were constructed and the

linear, quadratic, and bi-linear effects of the germination factors were analyzed. The results are presented in Table 2.

In this table, the factors that were significant are highlighted in bold character. While germination duration was notably influential on all investigated parameters, germination temperature was not significant for phytic acid and tannin contents. Besides, similar to the IVPD, the bi-linear term was important in the assessments of oxalate and protein after pepsin and pancreatin digestions. Finally, only the quadratic term of germination duration was important in the case of peptide and phytic acid. All surfaces had an acceptable R^2 and RMS Error values.

To investigate the existence of any correlation between the IVPD and anti-nutrient component levels, the IVPD values

Table 2 Summary of the ANOVA analysis of the responses for all protein, peptides, and anti-nutrient contents

	Protein content			Peptides content			Anti-nutrient Content		
	Before Digestion	After Pepsin Digestion	After Pancreatin Digestion	Before Digestion	After Pepsin Digestion	After Pancreatin Digestion	Phytic Acid	Tannin	Oxalate
Intercept	7.0088^a	0.8077^a	0.7763^a	2.1000^a	7.8458^a	8.4283^a	0.4692^a	1.7463^a	35.3763^a
Temp	0.2933^c	−0.1028^d	−0.1094^d	0.4833^b	0.3267^f	0.3483^c	−0.0050	0.0350	−1.7383^a
Time	0.1900^e	−0.1913^b	−0.1669^b	0.7783^a	1.4617^a	1.2717^a	−0.0933^a	−0.2483^b	−2.3533^a
Time × Temp	0.0175	0.1602^d	0.1181^d	0.1575	0.0200	0.0650	−0.0075	0.1050	−0.7450^f
Temp × Temp	−0.0313	0.0177	0.0242	0.0500	−0.0075	−0.3200	0.0075	0.0413	0.3613
Time × Time	−0.0913	0.0670	−0.0005	0.3350^f	−0.7825^e	−0.6200^d	0.0425^b	0.1113	0.3063
R^2	0.909	0.955	0.950	0.971	0.962	0.976	0.989	0.908	0.978
R^2 adj	0.833	0.918	0.908	0.944	0.930	0.956	0.981	0.832	0.960
RMS	0.113	0.056	0.051	0.169	0.316	0.224	0.010	0.089	0.448

a: $p < 0.0001$, b: $p < 0.0005$, c: $p < 0.001$, d: $p < 0.005$, e: $p < 0.01$, f: $p < 0.05$

were plotted as scattered data with respect to phytic acid, tannin, and oxalate contents (Fig. 4). Based on the plotted linear regression curves, it was concluded that there may be a linear correlation between the level of these anti-nutrients and the desired investigated IVPD. Generally, for all selected anti-nutrient factors, IVPD increased as the level of anti-nutrient decreased.

Fig. 4 Linear regression curves representing the possible correlations between the IVPD (%) and the anti-nutrient contents, **a** oxalate **b** phytic acid, and **c** tannin

Conclusion

Protein quality determination is an important factor in the manufacture of nutritionally improved cereal products. The nutritive value of protein primarily depends on its capacity to supply needs of nitrogen and essential amino acids. Protein quality improves as the biological availability of its building block components, amino acids, increases. The availability of amino acids and peptides depends upon the extent of digestibility of proteins by the proteolytic enzymes of the alimentary tract. Malting/germination is a simple and low-cost traditional food processing approach, which improves the nutritional quality of cereals through its involved biochemical modifications. Germination decreases anti-nutrient contents and enzyme inhibitors; hence, increases the protein digestibility. In the present study, the in-vitro protein digestibility of finger millet was enhanced by 17 % after germinating the seed for 48 h at 30 °C. In addition, phytic acid, tannin, and oxalate contents notably decreased by 45 %, 46 %, and 29 %, respectively. In addition, negative correlations between the protein digestibility and these anti-nutrient components were observed.

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