Development of functional foods using psyllium husk and wheat bran fractions: Phytic acid contents

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ABSTRACT
Wheat grain is a rich source of phosphorus which is present mostly as phytic acid and is distributed mainly in the bran and germ fractions. Phytic acid has now been recognized as an important phytochemical having antioxidant properties. This study deals with the determination of total as well as phytic phosphorus contents of psyllium (PS), course (CB) and fine wheat bran (FB) enriched pan bread and Arabic flat bread. The concentration of phytic acid in CB, FB, wheat germ, whole grain wheat flour (WGF), white wheat flour (WWF), and psyllium were found to be 8.86 mg/g, 8.52 mg/g, 6.05 mg/g, 1.74 mg/g, 0.46 mg/g and 0.02 mg/g, respectively. Most of the phosphorus existed as phytic phosphorus (74.7–90.8%) in FB, CB, germ, and WGF as compared to only 42.6% in WWF. The level of phytic phosphorus in pan bread containing 10% CB, 20% FB (both containing with 5% PS) was found to be 0.63 mg/g and 1.53 mg/g respectively, as compared to only 0.34 mg/g in WWF pan bread, and 0.90 mg/g in WGF pan bread. The phytic phosphorus content in Arabic bread made with WGF and 3% psyllium was 1.32 mg/g as compared to only 0.48 mg/g in WWF Arabic flat bread. The results obtained indicate that the level of phytic phosphorus significantly increased in bread formulations containing CB, FB, and WGF, but no change with psyllium addition was observed. Adding these wheat mill fractions, and psyllium will enable bakeries not only to produce fiber-enriched pan bread and Arabic bread but would also benefit consumers to increase their dietary fiber intakes, and health-promoting phytochemicals coming from wheat bran and germ fractions.

1. Introduction
The mounting evidence of better nutritional value has increased the demand for bakery products made with whole wheat flour (Parenti et al., 2020). Bran, a by-product of wheat milling, is one of the easily available and the cheapest sources of dietary fiber (Liu et al., 2019; Jabobs et al., 2018). Qaddoumi et al. (2019) have reported that the mean fasting blood glucose and HbA1c levels (9.6 ± 3.8 mmol/L and 8.5 ± 1.8%, respectively) are affected with dietary fiber intake. According to them, the type-2 diabetes leads to many complications, such as, dyslipidemia (46%) and hypertension (40%) with nephropathy (36%) and neuropathy (35%). Kim et al (2014) have reported that intake of myo-inositol and phytic acid enhances insulin sensitivity in adipocytes, increases glucose uptake, inhibits lipolysis, and increases lipid storage capacity of the adipocytes. Unfortunately, the addition of bran to wheat flour results in certain nutritional problems related to absorption of trace minerals due to binding with phytic acid (Rebellato et al., 2017; Khodaii et al., 2019; Lemmens et al., 2019).

Psyllium is husk of a grain (Psyllium ovata) and is rich in water soluble dietary fiber (about 70%), providing many health benefits in addition to improving the functionality of dough for producing healthy baked goods (Nazario-Franco et al., 2020). Psyllium is known for its beneficial effects in lowering low density lipoprotein, attenuating blood sugar levels in diabetes and to prevent constipation (Cassidy et al., 2018; Jane et al., 2019). Psyllium supplementation as a snack for the T2D patients with chronic constipation has been shown to increase HDL cholesterol, decrease constipation,
body weight, glycemic, LDL cholesterol (Noureddin et al., 2018). Psyllium when taken together with LDL lowering statins, gave reduction in LDL cholesterol equivalent to doubling the statin medicine dose (Brum et al., 2018). Feeding of psyllium husk has been shown to enhance the production of heat shock proteins which improve the intestinal epithelial cell function, and integrity thus providing beneficial effects on intestinal health (Ogata et al., 2017). Psyllium fiber has also been shown to possess antioxidant and hepatoprotective effects against carbon tetrachloride induced hepatic toxicity (Wahid et al., 2020). Phytic acid is widely distributed in many commonly consumed foods. It is found in high concentrations in the seeds of grains, pulses, and oleaginous products (Dahdouh et al., 2019). Although earlier, phytic acid has been considered only as anti-nutritional factor, but now it is recognized as one of the important antioxidants providing protection against cancer (Karaman et al., 2018). Spaggiari et al. (2020) have reported that solid state fermentation of wheat bran activated phytase enzyme and reduced the phytic acid and enhanced the bioactive compounds. Demirkan et al. (2017) have isolated, partially purified a phytase enzyme from Bacillus megaterium EBD 9-1 strain, which is stable at higher temperature and alkaline pH to make more phosphate available from phytate.

Enhancement of phytase improves intestinal calcium absorption, thus showing the capacity of the small intestine to adopt to diets rich in phytic acid and poor in calcium. Health professionals are now looking at the wheat bran not only as a source of dietary fiber, but also for its other health promoting phytochemicals (Benitez et al., 2018). Considering the importance of phytic acid present in wheat mill fractions for their nutritional benefits in our diet, the major objective of study was, therefore, to estimate the phytic acid contents of pan bread, hamburger buns and Arabic flat bread made with the addition of psyllium husk, different wheat mill fractions (CB, FB, wheat germ) and the results are presented here.

2. Materials and methods

2.1. Raw materials

Wholegrain wheat flour (WGF), white wheat flour (WWF), coarse wheat bran (CB), fine wheat bran (FB), and wheat germ (WG) samples were obtained from the Kuwait Flour Mills & Bakeries Co., Shuwaikh. Psyllium husk was procured from India through local importer. These samples were analyzed for moisture (method 44–19), crude protein (Nx5.70) (method 46–12), crude fat (method 30–25), crude fiber (method 32–10), and ash (method 08–01) contents, according to standard AACC methods (AACC, 2000).

2.2. Baking studies

Wholegrain wheat flour, white wheat flour, wheat germ, coarse wheat bran and fine wheat bran samples collected from the Kuwait Flour Mills & Bakeries Co., Kuwait, were used in the baking studies. Fine granulated sugar, common salt, bakery shortening (Wesson brand, USA), instant dry yeast and non-fat dry milk were procured from the local market. Sodium stearoyl-2-lactylate (SSL) and diacetyl tartaric acid esters of mono- and di-glycerides (DATEM) were provided free of cost by American Ingredient Co., Kansas City, Missouri, USA. For pan bread-making, wholegrain wheat flour was used as a control while varying levels of coarse wheat bran, fine wheat bran, were added to the white wheat flour and baked as per the optimized straight-dough bread-making method 10-10B (AACC, 2000). The white wheat flour (WWF) was unleached straight-grade flour with about 72% extraction and was used as control in baked products made from WWF. The Arabic bread was made as per the procedure reported earlier by Qarooni et al. (1988). The baked bread samples were freeze-dried (make: Virtis, model: Unifree 800L, USA). The freeze-dried samples were coded and stored in air-tight containers at -20 °C till further use for the chemical analysis.

2.3. Phytic acid determination

Known weight of sample (1) was extracted with 25 ml 3% sulfuric acid by stirring for 30 min at room temperature using a magnetic stirrer. The suspension was centrifuged at 400 rpm for 10 min and filtered using Whatman No.1 filter paper. Phytic phosphorus was precipitated using ferric precipitation as described by Plaani and Kumpulainen (1991). Total phosphorus was determined by wet-digesting using a nitric acid-perchloric acid (3:1) mixture (AOAC, 1995). After making up the volume, the extract was analyzed for total phosphorus by the inductively coupled plasma technique using a Jobin Yvon spectrophotometer (Model Jy-24, Japan) as described by Al-Hooti et al. (1998). All the chemicals used in this study were of analytical grade.

2.4. Statistical analysis

The experimental data obtained were statistically analyzed for analysis of variance, using Duncan’s New Multiple Range Test (SAS Program Windows Version 6.08) and significance was accepted at P < 0.05 level. The analyses were conducted in triplicate, and the mean values ± S.D. for phytic phosphorus and total phosphorus (mg/g) on 14% moisture basis, are reported here.

3. Results and discussion

3.1. Phytic phosphorus in wheat mill fractions

All the baked products made in the laboratory (pan bread, hamburger buns and Arabic bread) were analyzed for total as well as phytic phosphorus contents by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and the results are summarized in Tables 1–6. Among the wheat mill fractions used in these baking trials, phytic acid was found to be mainly concentrated in fine wheat bran (8.86 mg/g), coarse wheat bran, (8.52 mg/g) and germ (6.05 mg/g) fractions. Phytic acid was significantly higher in WGF (1.74 mg/g) than that of WWF (0.46 mg/g), whereas psyllium had only a negligible amount of phytic acid (Table 1). The total phosphorus contents followed the same trend as that of phytic acid in these fractions. Most of the phosphorus existed as phytic phosphorus.

Table 1

| Sample Description | Total Phosphorus, mg/g | Phytic Phosphorus, mg/g | Phytic Phosphorus, as % of Total Phosphorus |
|--------------------|------------------------|-------------------------|--------------------------------------------|
| Wholegrain Wheat Flour (WGF) | 2.33 ± 0.15a | 1.74 ± 0.13a | 74.7 |
| White Wheat Flour (WWF) | 1.08 ± 0.06b | 0.46 ± 0.06b | 42.6 |
| Coarse Wheat Bran (CB) | 9.83 ± 0.34c | 8.52 ± 0.25c | 86.7 |
| Fine Wheat Bran (FB) | 9.76 ± 0.19d | 8.86 ± 0.20d | 90.8 |
| Wheat Germ | 6.98 ± 0.21e | 6.05 ± 0.40e | 86.7 |
| Psyllium husk | 0.07 ± 0.01f | 0.02 ± 0.01f | 28.6 |

Mean values with same superscripts do not differ significantly in a column (P > 0.05). * Mean ± S.D.; S.D. = Standard Deviation.
3.2. Phytic phosphorus in pan bread

The level of phytic phosphorus in pan bread formulations containing different levels of mill bran fractions and psyllium are presented in Table 2. As the level of bran increased from 10 to 20%, there was a corresponding and significant increase in the amount of phytic phosphorus found in pan bread. Addition of psyllium in these formulations had no significant effect on their phytic phosphorus contents. Phytic phosphorus as a percentage of total phosphorus ranged from a lower value of 41.5% in WWF control pan bread to the highest value of 73.9% in 20% coarse wheat bran pan bread. Phytase a naturally occurring enzyme in cereal flours is shown to catalyze the hydrolysis of phytates resulting in decreased content of phytate (Lemmens et al., 2019). In addition to the indigenous phytase enzyme of wheat, yeast phytase is also known to reduce the level of phytic phosphorus in baked products during the bread making process (Chhabra and Sidhu, 1988). These higher levels of phytic phosphorus still present in pan bread samples containing 20% coarse wheat bran may be due to the feedback inhibition of this hydrolytic reaction caused by the accumulated inorganic phosphorus in baked products after hydrolysis by the

Table 2
Effect of adding psyllium and wheat bran fractions on the phytic phosphorus content* of pan bread made in the laboratory (14% moisture basis).

| Sample Description | Total Phosphorus, mg/g | Phytic Phosphorus, mg/g | Phytic Phosphorus, as % of Total Phosphorus |
|--------------------|------------------------|-------------------------|--------------------------------------------|
| WWF + 0% PS        | 0.82 ± 0.04 a          | 0.34 ± 0.02 a           | 41.5                                       |
| WWF + 5% PS        | 0.84 ± 0.03 a          | 0.35 ± 0.05 a           | 41.7                                       |
| WGF + 0% PS        | 1.69 ± 0.06b           | 0.90 ± 0.09b            | 53.3                                       |
| WGF + 5% PS        | 1.60 ± 0.07b           | 0.89 ± 0.08b            | 55.6                                       |
| WWF + 10% CB       | 1.20 ± 0.09c           | 0.70 ± 0.03c            | 58.3                                       |
| WWF + 10%          | 1.14 ± 0.08b           | 0.63 ± 0.06b            | 55.3                                       |
| CB + 5% PS         | 2.20 ± 0.11 d          | 1.58 ± 0.10 d           | 71.8                                       |
| WWF + 20% CB       | 2.11 ± 0.12 d          | 1.44 ± 0.10 d           | 68.3                                       |
| FB + 5% PS         | 2.55 ± 0.12 e          | 1.72 ± 0.10 e           | 67.5                                       |
| FB + 20%           | 2.42 ± 0.14 e          | 1.53 ± 0.11 e           | 63.2                                       |
| WGF = Wholegrain wheat flour, WWF = White wheat flour, PS = Psyllium husk, CB = Coarse wheat bran, FB = Fine wheat bran. Mean values with same superscripts do not differ significantly in a column (P < 0.05). * Mean ± S.D.; S.D. = Standard Deviation.
yeast phytase. The psyllium husk added to the bread formulations made with WWF and CB or FB addition, gave consistently lower content of phytic phosphorus (as %age). It may possibly be due to the dilution effect on wheat bran concentration on the inorganic phosphorus, lowering its feedback inhibition effect on the activity of phytase enzyme.

3.3. Phytic phosphorus in hamburger buns

In case of hamburger buns made from WWF and WGF with varying levels (3–5%) of psyllium, the phytic phosphorus levels ranged from 0.32 to 0.36 mg/g and 0.55 to 0.71 mg/g, respectively (Table 3). In terms of percentages, the phytic phosphorus in these bun samples ranged from 46.4 to 62.5%. In hamburger buns made from WWF with added CB fraction with psyllium husk at 3 and 5%, the phytic phosphorus levels ranged from 0.38 to 0.42 mg/g at 10% CB and 0.71 to 0.74 mg/g at 20% CB, respectively (Table 4). As the level of CB increased from 10 to 20%, more of phytic acid (62.3–66.1%) remained unhydrolyzed in the finished product. The higher percentage of phytic acid in the baked products enriched with higher levels of CB can be explained again due to the feedback inhibition of phytase enzyme by the inorganic phosphate (Chhabra and Sidhu, 1988). As the level of psyllium husk was increased from 3 to 5%, the phytic phosphorus (as %age) was lowered, possibly due to the dilution effect of inorganic phosphate as well as the dilution of added CB with psyllium husk to the white wheat flour. Similar trends were observed in the phytic acid contents of hamburger buns when FB was added at 10 and 20% level to the white wheat flour (Table 5). As expected, the phytic acid contents were higher than those obtained with CB, as the FB comes from the region of wheat grain close to the aleurone layer known to be rich in phytic acid (Jacobs et al., 2018). The phytic acid in hamburger buns made with WWF with added FB, along with psyllium husk at 3–5%, ranged from 56 mg/g with 10% FB and 1.12–1.20 mg/g with 20% FB, respectively. As the FB level was increased from 10 to 20%, more of phytic acid (67.9–70.6%) remained unhydrolyzed in the hamburger buns, possibly again due to the feedback inhibition of phytase enzyme by the inorganic phosphate.

3.4. Phytic phosphorus in Arabic bread

Obviously, the Arabic flat bread having a lean formula and also being fermented for a shorter time (about 65 min) compared with the pan bread (90 min), it retained much higher amounts of hydrolyzed phytic acid (62.7–72.9%) than the pan bread (41.7–53.6%). With the addition of psyllium husk at 3 and 5% levels, the absolute amounts of phytic phosphorus in Arabic whole grain wheat flour bread were, obviously, significantly higher (1.46–1.61 mg/g) than the Arabic white wheat flour bread (0.45–0.51 mg/g), which is mainly due to the inclusion of bran in the WGF and due to shorter fermentation time that the dough undergoes during Arabic bread making process (Table 6). The addition of psyllium at 3 or 5% level did not change the phytic phosphorus content in any of the fiber-enriched bakery products reported in this study. The inorganic phosphorus released from the phytic acid present in the bran particles of whole grain wheat flour during the preparation of bread has been reported to lower the phytase activity due to feedback inhibition (Chhabra and Sidhu, 1988). Neither the CB nor the FB fractions were added to the Arabic bread, because the flat breads have higher surface area than the pan breads, thus, leading to a faster loss of moisture which gives harder texture, poor eating quality and acceptability among the consumers (Sidhu et al., 1988).

During the process of bread making, when phytic acid (Inositol hexaphosphate, IP6) is hydrolyzed by the phytase into various degradation products, right up to inositol, these not only help in enhancing the availability of trace minerals (Zn, Fe, Cu), but also offer many other health benefits. Inositol is reported to be an anti-carcinogenic compound, as it works synergistically with IP6, to inhibit growth of cancer cells (Shamsuddin, 2002). Additionally, IP6 reduces cell proliferation, and differentiation of malignant cells, often leading to reversion of these cells to normal phenotypes. IP6 is easily absorbed from the small intestine, quickly taken up by malignant cells, converted to inositol and IP1-5. The varying amount of IP6 has been detected in human urine, blood, and other body fluids, depending upon the intake of fiber-rich foods. Therefore, from the epidemiological studies, the adequate intake of dietary fiber may partially explain the lower incidence of certain cancers. Irshad et al. (2017) have reviewed in details the role of phytase/phytate system in preventing certain types of cancers and providing various other health benefits. The production of these wheat bran-enriched bakery products would offer many health benefits to the consumers.

To tackle the harmful nutritional effects of consuming intact phytic acid, a few approaches have been suggested to reduce the phytic acid content in such fiber-enriched baked products. Fekri et al. (2020) have suggested the use of a combination of phytate-degrading probiotic lactic acid bacteria and yeast strains in the sour dough of Iranian bread. The Kluyveromyces marxianus strain produced the highest activity of phytase enzyme (1.64 Units/ml) giving the lowest value of phytic acid content (3.5 mg/g) in the bread, however, the Kluyveromyces aestuarii strain gave the highest porosity percentage (70.43%) and the lowest hardness (508.71 g) value for bread. In their study, in addition to functional improvements in bread, the improvements in nutritional quality in terms of phytate reduction, and increase in antioxidant capacity, phenolic compounds, exopolysaccharides and in vitro starch digestion were also observed by them.

The incorporation of wheat bran fraction in the bread formulation also brings in the subaleurone and aleurone layers which are not only rich in vitamins, minerals, health promoting phytochemicals, but also add bran-associated subaleurone endosperm proteins known to strengthen the gluten structure (Jacobs et al., 2018). The addition of bran not only weakens the dough due to dilution effect (Hemdane et al., 2016), but the presence of bran also hinders the polymerization of gluten proteins for proper dough development by hindering the contact between flour particles (Sanchez Penella et al., 2008). Phytate has also been suggested to interact with glutenin by chelation of iron and adversely affects the oxidative cross linking of glutenin molecules during dough mixing process, resulting in weaker dough leading to poor quality bread (Park et al., 2016). The wheat fine bran obtained from the commercial mill, is always contaminated with germ fraction that is rich in glutathione, a reducing compound that has been shown to exhibit weakening effect on the gluten network through disulfide interchange (Joye et al., 2009). Although, the addition of bran and germ does impart higher nutritional value to the finished product, but it also negatively affects the technological performance of the wheat flour. It is, therefore, recommended to develop new strategies to specifically focus on improving the eating quality of bran-enriched bakery products. The bakers, therefore, must overcome these negative effects of wheat bran and germ addition through a judicious use of many permitted flour additives to produce acceptable quality baked products.

4. Conclusions and recommendations

Results presented in this paper show the contribution of added wheat bran and germ fractions in enhancing the phytic acid contents in the commonly consumed baked products in Kuwait. Due to yeast phytase activity during dough fermentation and proofing, significant amount of phytic acid was hydrolyzed into inorganic...
phosphorus in these bakery products. Hydrolysis by yeast was more effective in lowering the phytic acid contents in baked products made from white wheat flour (WWF) than the whole grain wheat flour (WGF), probably due to the feedback inhibition of this enzyme by one of the hydrolytic end-products, inorganic phosphorus. The addition of psyllium at 3 or 5% level did not change the phytic phosphorus content in any of the fiber-enriched bakery products. The results of this study bring out the importance of producing phytochemicals-rich baked products made from white wheat flour supplemented with various wheat bran fractions. Evidently, consumption of such high-fiber baked products would enhance the level of dietary fiber as well as various health-promoting phytochemicals (e.g., phytic acid) in the diet of consumers of these baked products.

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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All authors contributed to the research. Dr. Sidhu conceived the research idea and guided the research team, Dr. Ahmed, Dr. Fatima, Dr. Sharifa developed and put together the manuscript, Mr. Bhatti and Mr. Al-Foudari carried out the chemical analyses work. All the authors have read and approved the final manuscript for submission to this journal.

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