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Effect of Calcium Fortification on Whole Wheat Flour Based Leavened and Unleavened Breads by Utilizing Food Industrial Wastes

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The current study was aimed to overcome the calcium deficiency in wheat with fortification by utilizing food industrial waste. Leavened breads and unleavened breads were prepared from whole wheat flour fortified with chicken bone extract powder, egg shell powder and $CaCO_3$ (0.5, 1 and 1.5 % each). Breads were evaluated for proximate composition, minerals profile and sensory attributes. The proximate composition of both leavened breads and unleavened breads, fortified with bone extract powder, egg shell powder and $CaCO_3$ in different levels were found in high concentration as compared to control. Similarly, the minerals (especially calcium) contents were also increased ($P \leq 0.05$) in fortified breads. However, mineral profile of breads fortified with bone extract powder and egg shell powder were higher than $CaCO_3$ fortified bread. Breads fortified with bone extract powder were less acceptable to the panelists in terms of colour, taste, texture and over all acceptability than egg shell powder and $CaCO_3$ fortified breads. Therefore, fortification of whole wheat flour with bone extract powder, egg shell powder and $CaCO_3$ with different concentrations had produced leavened breads and unleavened breads with a wide range of chemical and sensory qualities. However, a further study is needed on the bioavailability of calcium contents of both leavened breads and unleavened breads.

Keywords: Whole wheat flour, Bread, Fortification, Industrial waste.

INTRODUCTION

Cereals are the main source of food and directly consumed by humans. Among cereals, wheat is the third widely produced in the world after maize and rice and the second mainly consumed diet after rice [1]. About 65 % of wheat is utilized as a food, 17 % as animal feed and the remaining for various industrial purposes [1]. Wheat flour being a staple food of Indian sub-continent, gives a considerable amount of energy (> 50 %) [2]. In Pakistan, wheat is consumed as an average intake of 318 g/person/day, while in countries like Syria, Algeria, Turkey and Iran, average intake is up to 600 g/person/day [2].

Wheat is a good source of proteins, fats, vitamins B_1, B_2, B_6 and E, as well as a rich source of minerals. Among the minerals calcium, phosphorus, iron, sodium, magnesium and potassium are found in significant amount. These nutrients are mostly present in external parts of the grain (bran and germ) and 50 % of these nutrients are lost during milling process [3]. Cereals are the best vehicle for the fortification mostly in developing countries as around 95 % of the population consumes cereals as a dietary staple food. Furthermore, wheat is widely grown and utilized worldwide with relatively reasonable price to all the economic classes [1].

Fortification is an intentional addition of micronutrients in foods, particularly cereals for enhancing its nutritional quality. It plays an important role to provide sufficient nutrients to the body and eradication of common diseases, like anemia, rickets *etc*. [4]. Among the cereals, wheat is the first recommended cereal by the World Health Organization (WHO) for the fortification and being widely fortified [5]. Wheat flour fortification was initiated by United States and Canada in 1940s, followed by Chile in 1950s and some Latin American countries in 1960s. Later on, fortification has been started by some Asian Countries *i.e.* Azerbaijan, Kazakhstan, Kyrgyz Republic, Mongolia, Tajikistan and Uzbekistan [6]. Vietnam, Thailand, The People's Republic of China, Pakistan and Indonesia also signed the agreement on wheat flour fortification. Now, it has successfully been implemented in Pakistan to reduce micronutrient deficiency [7].

Minerals are inorganic materials, play a vital role in structure and function of human body. Minerals are of two types, major and minor and are required 100 mg and less than 20 mg per day, respectively [8]. Calcium is one of the major mineral and present in many foods like milk products, grains, vegetables and fruits. Acceptable intake of calcium varies with age, body weight, pregnancy and lactation. In human body 99 % of calcium is stored in teeth and skeleton and keep them strong, while the residual 1 % is present in blood and soft tissues. Improper growth of bones and osteoporotic fracture with age especially in women are due to calcium deficiency [8]. Now-a-days, osteoporosis is one of the important degenerative diseases worldwide, particularly in the Asia. Successive hip fracture in females is due to the consumption of low calcium diet and is a major public health concern in the Asia [8,9]. For Asian women, daily intake of calcium is far below than the recommended requirement (1000 mg/day). Calcium and vitamin D supplementation is widely recognized as an important strategy for the prevention of low bone density and osteoporosis.

Traditional dairy products are the major sources of calcium. However, consumers demand is increasing for nontraditional calcium sources. Furthermore, lactose intolerance people or who do not prefer dairy products need an alternate source of calcium for proper intake. To overcome such problems, calcium fortification is one of the methods to maintain the proper nutritional status which is a challenge for the food industries.

Various studies have been conducted to fortify food product with calcium from different sources. Ekbote *et al.* [9] studied the bioavailability of calcium from cereal-legume snack (*laddoo*) fortified with calcium carbonate. Romanchik-Cerpovicz and Rebecca [10] fortified all purpose wheat-flour tortillas with calcium lactate, calcium carbonate or calcium citrate. Sittikulwitit *et al.* [11] fortified the bakery product (bread and cookies) with chicken bones extract powder and reported that the bioavailability of the calcium was higher than milk and several other calcium fortificants. Furthermore, Ketwaan *et al.* [12] prepared fried shrimp chips fortified with chicken bone extract powder and found the same concentration of calcium as available in milk, without affecting its sensory characteristics.

Due to the lactose intolerant people and poverty level of people particularly in Pakistan, they have no access to get a well balanced calcium diet to fulfill their daily requirements. As mentioned earlier that a reasonable amount of calcium is available in germ and bran portion of wheat grain but 50 % is lost during milling process. As wheat is a staple food consumed regularly, so considered as a best vehicle for fortification. Chicken bones and egg shells are a natural source of calcium. However, they are discarded as waste which pollutes the environment. The overall aim of this research was to overcome calcium deficiency in wheat with fortification by utilizing food industrial waste.

EXPERIMENTAL

Wheat (*Triticum aestivum* L.) cv. Janbaz was kindly provided by the Department of Plant Breeding and Genetics, The University of Agriculture Peshawar, Pakistan. Broilers, egg shells and $CaCO_3$ were purchased from local market in Peshawar city. All of the materials were transported to the Department of Food Science and Technology in suitable packaging materials.

Sample preparation: Wheat grains were cleaned manually and milled into flour and the whole wheat flour (WWF) was packed in polythene bags for further study. Chicken bones were boiled in water, flesh and cartilages were removed. Bones were then treated with 3 % sodium hydroxide solution to avoid the chances of microorganisms and dried at 100 °C in oven. Bones were ground into powder and named as bones extract powder (BEP) after extraction [12]. Egg shells were washed thoroughly with tape water and removed the inner membrane. After rinsing with distilled water, shells were dried and ground into powder and named as egg shell powder (ESP).

Raw materials analysis: Whole wheat flour (WWF) was analyzed for the proximate composition and minerals content. Bone extract powder and egg shell powder were analyzed for the specific minerals profiles.

Calcium fortification and preparation of bread: Whole wheat flour was fortified with bone extract powder (BEP), egg shell powder (ESP) and $CaCO_3$ as a source of calcium and formulation is shown in Table-1. Leavened bread (LB) and unleavened bread (ULB) were prepared with the addition of water and salt. In leavened breads starter was used from leftover of previous dough. Breads prepared with whole wheat flour without any fortificants were used as control. All the ingredients were mixed thoroughly for dough making. Dough was kneaded with hands for 5-10 min and was left for fermentation of 3 h. Dough (80 g) was rounded and baked for 3 min by traditional heating. After baking, breads were subjected to sensory evaluation, proximate composition and minerals profile.

Proximate analysis: Moisture, ash content, crude fat, crude protein and crude fiber were determined by the recommended

TABLE-1
FORTIFICATION OF WHOLE WHEAT FLOUR (WWF) WITH VARIOUS CALCIUM SOURCES IN DIFFERENT LEVELS (%) FOR LEAVENED AND UNLEAVENED BREAD PREPARATION

Leavened bread		Unleavened bread	
WWF (Control)	100	WWF (Control)	100
WWF (%)	BEP (%)	WWF (%)	BEP (%)
98.5	1.5	98.5	1.5
99.0	1.0	99.0	1.0
99.5	0.5	99.5	0.5
ESP		ESP	
98.5	1.5	98.5	1.5
99.0	1.0	99.0	1.0
99.5	0.5	99.5	0.5
$CaCO_3$		$CaCO_3$	
98.5	1.5	98.5	1.5
99.0	1.0	99.0	1.0
99.5	0.5	99.5	0.5

BEP = bone extract powder, ESP = egg shell powder.

method of AACC [13]. Nitrogen free extract (%) was determined by subtracting the percentages of moisture, ash, crude fat, crude fiber and crude protein from 100.

Determination of minerals: Minerals were determined by wet digestion according to the method of Jones *et al.* [14]. Sample of 0.2 g of was taken in 50 mL flask and 10 mL concentrated nitric acid was added and kept overnight. Concentrated per chloric acid (4 mL) was added to the samples and heated for digestion. Clear digested samples were cooled at room temperature, filtered with Whatman (42) and the volume was made up to the mark. Calcium, magnesium, iron, zinc, manganese and copper were measured by atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, USA). Phosphorous was determined by spectrophotometer (Model UV-1700, Shimadzu, Japan), while potassium and sodium were analyzed by flame photometer (Model PFP7, Jenway, UK).

Sensory evaluation: Sensory evaluation of the breads was carried out by the method of Larmond [15], using 9-point hedonic scale by a group of 60 panelists. Breads were evaluated for colour, taste, texture and overall acceptability, with the score 1-9, where 1 represented extremely disliked and 9 extremely liked, respectively.

Statistical analysis: Experiment was conducted in a completely randomized design (CRD) with three replicates. Data were tested by one way analysis of variance using statistical package (MSTATC). Least significant difference (LSD) was performed to determine the significant differences among the treatments (P ≤ 0.05).

RESULTS AND DISCUSSION

Proximate composition of whole wheat flour (WWF): The proximate composition of whole wheat flour was analyzed after milling and results are presented in Fig. 1. In whole wheat flour, moisture, crude protein, ash and crude fiber were found in high concentration while crude fat was found at low concentration. Our results were in agreement with the work of Riaz *et al.* [16] who concluded that whole wheat flour contained 8.83, 1.73, 14.53, 13.91, 2.71 and 71.26 % of moisture, ash, crude fat, crude protein, crude fiber and nitrogen free extract, respectively. Similar results were also observed by Wahab *et al.* [17]. A slight variation could be due to wheat cultivar, genetic makeup and climatic conditions.

Mineral composition of whole wheat flour, bone extract powder and egg shell powder: Mineral composition of whole wheat flour, bone extract powder and egg shell powder are shown in Table-2. The mineral contents of whole wheat flour were significantly (*P* ≤ 0.05) different from bone extract powder and egg shell powder. As expected, calcium contents were found lower in whole wheat flour in comparison to that of egg shell powder and bone extract powder. On the other hand, phosphorus and iron concentration were higher in whole wheat flour in comparison to egg shell powder, while lower than bone extract powder. The magnesium contents in whole wheat flour was lower than egg shell powder but higher than bone extract powder. These results were in conformity with the results reported by Suchy *et al.* [18] and Schaafsma *et al.* [19]. Wahab [20] found that chicken bone extract powder contained 2078.48, 11.30, 8665.67 and 20.91 mg/100 g of calcium, magnesium, phosphorous and iron, respectively. Our results for calcium content in egg shell powder were in close agreement with Oliveira *et al.* [21]. Hence this is expected that fortification of both bone extract powder and egg shell powder will increase the mineral contents of whole wheat flour based leavened and unleavened bread.

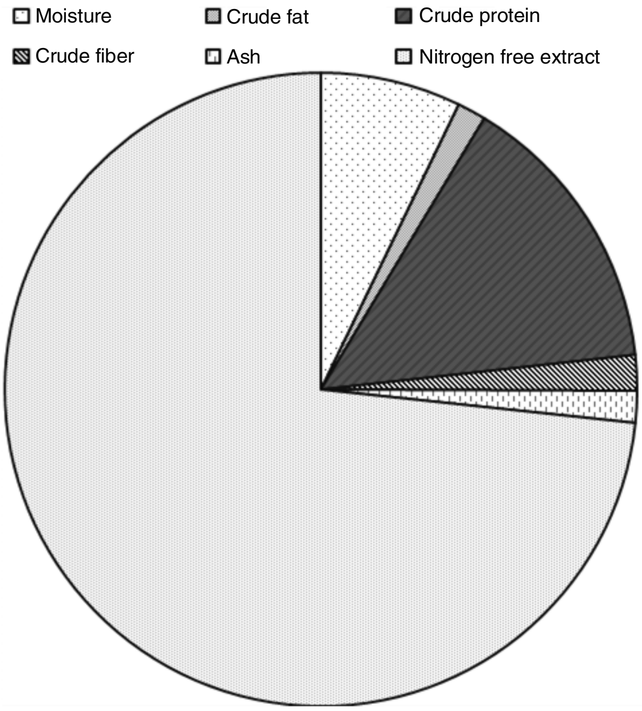

Fig. 1. Proximate composition (%) of whole wheat flour (raw sample)

Proximate composition of control and calcium fortified leavened and unleavened whole wheat flour breads

Moisture content: Statistical analysis indicated that moisture content of leavened bread (LB) and unleavened bread (ULB) fortified with bone extract powder, egg shell powder

TABLE-2
MINERALS COMPOSITION OF WHOLE WHEAT FLOUR (WWF), BONE EXTRACT POWDER (BEP) AND EGG SHELL POWDER (ESP)

Raw sample	Ca	Mg	P	K	Mn	Fe	Zn	Cu	Na
	(mg/100 g)				(ppm)				(%)
WWF	47.53 ± 0.35c	171.75 ± 0.12b	380.35 ± 0.05b	153.9 ± 0.08a	36.2 ± 1.70a	93.93 ± 0.04b	61.24 ± 0.14a	46.25 ± 0.15a	4.49 ± 0.01a
BEP	13345.13 ± 0.15b	11.67 ± 0.03c	8407.41 ± 3.74a	–	–	205.33 ± 0.65a	–	–	–
ESP	24450 ± 1.00a	365.05 ± 0.04a	91.25 ± 0.12c	–	–	20.53 ± 0.35c	–	–	–

WWF = whole wheat flour; BEP = bone extract powder; ESP = egg shell powder; Data represent the mean ± standard deviation (n = 3); a-c, characterize significant difference (*P* ≤ 0.5) with in the column.

and $CaCO_3$ were significantly ($P \leq 0.05$) decreased (Table-3). Moisture content of control leavened breads and unleavened breads was 34.42 and 33.06 %, respectively. Moisture content of whole wheat flour based breads was decreased with fortification of bone extract powder, egg shell powder and $CaCO_3$ in different levels. Highest reduction was observed in whole wheat flour based leavened breads and unleavened breads fortified with egg shell powder (1.5 %). Data also revealed that with the increasing concentration of calcium sources, moisture content of leavened breads and unleavened breads was decreased. This reduction in moisture content might be due to the fact that these calcium sources were in dry form. This statement was supported by Fik *et al.* [22] that the moisture content of the calcium carbonate fortified bread decreased with increasing dry matter content. A decrease in moisture content was found when whole wheat flour was fortified with NaFeEDTA (ferric sodium ethylene diamine tetra acetate) + $ZnSO_4$, NaFeEDTA + ZnO, elemental iron + $ZnSO_4$ and elemental iron + ZnO at different levels as compared to unfortified flour [7]. Riaz *et al.* [16] concluded that by supplementation of mung bean flour to wheat flour decreased the moisture content of supplemented breads.

Ash content: Ash content of leavened breads and unleavened breads fortified with bone extract powder, egg shell powder and $CaCO_3$ showed significance ($P \leq 0.05$) increase (Table-3). Higher ash content was observed in leavened breads when bone extract powder was fortified to whole wheat flour at the rate of 1.5 %, while, lower ash content was recorded for leavened breads prepared from whole wheat flour fortified with 0.5 % $CaCO_3$. Similarly, in case of unleavened breads maximum ash content was observed in 1.5 % bone extract powder, while minimum ash content was found in 0.5 % $CaCO_3$. Data suggested that these fortificants were higher in ash content which significantly increased the ash concentration of the fortified leavened breads and unleavened breads. With the increase of calcium levels, ash content in breads was increased. Mashayekh *et al.* [23] had prepared breads from wheat flour fortified with defatted soy flour at 3 %, 7 % and found 15 % and 33 % increased in ash content respectively, as compared to control (unfortified). Bano *et al.* [24] also prepared bread from the whole wheat flour supplemented with tartary buckwheat flour at different concentration and found an increase in ash concentration with the increase of tartary buck wheat flour.

Crude fat: A significant increase of crude fat content was found in leavened breads and unleavened breads fortified with bone extract powder, egg shell powder as compared to control (Table-3). High crude fat concentration was observed for bone extract powder (1.5 %) while low in $CaCO_3$ (1 %) in case of leavened breads. Overall, fortification of bone extract powder in all levels increased crude fat content of whole wheat flour based leavened breads and unleavened breads more than that of egg shell powder and $CaCO_3$. Our result suggested that bone extract powder has high fat concentration which increased the crude fat of leavened breads and unleavened breads. Our results were in line with Bano *et al.* [24] that high fat content in tartary buck wheat flour increased the fat content of the supplemented whole wheat flour bread. No effect of $CaCO_3$ at all concentrations on fat was found in leavened breads, while $CaCO_3$ (0.5 and 1 %) reduced the fat content of unleavened breads as compared to control. Similarly, in connection with literature, NaFeEDTA (ferric sodium ethylene diamine tetraacetate) +

TABLE-3
MEAN VALUES OF PROXIMATE COMPOSITION (%) OF CONTROL AND BONE EXTRACT POWDER, EGG SHELL POWDER AND $CaCO_3$ FORTIFIED LEAVENED AND UNLEAVENED WHOLE WHEAT FLOUR BREADS

		WWF (%)	Bone extract powder (%)			Egg shell powder (%)			$CaCO_3$ (%)		
		100	1.5	1.0	0.5	1.5	1.0	0.5	1.5	1.0	0.5
Moisture	Leavened bread	34.42 ± 0.22a	26.33 ± 0.20d	27.00 ± 0.10cd	30.35 ± 0.07b	23.90 ± 0.67e	24.88 ± 0.62e	26.09 ± 0.93d	28.01 ± 0.94c	29.68 ± 1.20b	30.69 ± 0.25b
	Unleavened bread	33.06 ± 0.11a	25.58 ± 1.10 d	26.80 ± 0.74cd	29.85 ± 0.94b	22.77 ± 0.86e	23.69 ± 0.56e	25.83 ± 0.57d	27.48 ± 0.48cd	28.22 ± 1.12c	30.82 ± 0.82b
Ash	Leavened bread	2.97 ± 0.06i	14.98 ± 0.34a	12.59 ± 0.56b	9.13 ± 0.02e	11.42 ± 0.49c	9.87 ± 0.21d	8.20 ± 0.34f	7.66 ± 0.3 f	6.32 ± 0.64g	4.89 ± 0.70h
	Unleavened bread	3.16 ± 0.08g	17.46 ± 0.30a	13.21 ± 1.00b	10.04 ± 0.94d	12.52 ± 0.41b	11.12 ± 0.75c	9.03 ± 0.66d	7.88 ± 0.60e	6.97 ± 0.55e	5.11 ± 0.28f
Fats	Leavened bread	1.73 ± 0.08f	6.26 ± 0.10a	5.14 ± 0.15b	3.81 ± 0.09c	2.34 ± 0.13d	2.18 ± 0.03d	1.98 ± 0.04e	1.79 ± 0.18ef	1.60 ± 0.12f	1.73 ± 0.10f
	Unleavened bread	2.00 ± 0.03ef	4.08 ± 0.00b	5.80 ± 0.17a	4.30 ± 0.27b	2.78 ± 0.12c	2.30 ± 0.21d	2.17 ± 0.06e	2.24 ± 0.13de	1.63 ± 0.08g	1.98 ± 0.14f
Protein	Leavened bread	12.67 ± 0.65e	18.45 ± 0.10a	17.32 ± 0.11b	15.78 ± 0.49c	16.23 ± 0.98c	15.63 ± 0.56cd	14.95 ± 0.39d	13.15 ± 0.25e	13.18 ± 0.07e	12.98 ± 0.24e
	Unleavened bread	13.03 ± 1.40f	19.16 ± 0.63a	18.04 ± 0.20b	15.91 ± 0.39de	17.15 ± 0.15c	16.33 ± 1.19d	15.47 ± 0.33e	15.07 ± 0.17e	13.11 ± 0.46f	12.25 ± 0.28f
Fiber	Leavened bread	2.03 ± 0.06f	3.07 ± 0.12a	2.84 ± 0.06b	2.66 ± 0.13c	2.82 ± 0.06b	2.60 ± 0.13c	2.53 ± 0.08d	2.45 ± 0.10d	2.21 ± 0.09e	2.15 ± 0.08e
	Unleavened bread	2.19 ± 0.05d	3.27 ± 0.14a	3.17 ± 0.09a	2.55 ± 0.07c	2.50 ± 0.08c	2.72 ± 0.14b	2.59 ± 0.07bc	2.47 ± 0.05c	2.28 ± 0.12d	2.12 ± 0.04d
NFE	Leavened bread	46.17 ± 0.11 b	30.45 ± 0.62g	34.88 ± 0.49f	38.03 ± 0.22e	43.22 ± 0.26d	44.98 ± 0.67c	46.35 ± 0.36b	46.24 ± 0.20b	47.17 ± 0.19a	46.77 ± 0.12ab
	Unleavened bread	46.09 ± 0.20b	29.57 ± 0.28g	32.92 ± 0.65f	37.18 ± 0.41e	41.62 ± 0.63d	44.20 ± 0.98c	45.28 ± 0.28b	45.96 ± 0.43b	47.53 ± 0.34a	48.05 ± 0.63a

Data represent the mean ± standard deviation (n = 3); a-h, characterize significant difference ($P \leq 0.5$) within the row; WWF = whole wheat flour.

$ZnSO_4$, NaFeEDTA + ZnO, elemental iron + $ZnSO_4$ and elemental iron + ZnO at different concentrations did not affected the fat content of fortified and unfortified wheat flour [7].

Crude protein: The statistical analysis indicated that fortification of bone extract powder and egg shell powder in various concentrations significantly ($P \leq 0.05$) increased the crude protein content of leavened breads and unleavened breads (Table-3). Highest crude protein (18.45 %) was observed for whole wheat flour leavened breads and unleavened breads samples, fortified with bone extract powder at 1.5 %, while the lowest crude protein (12.25 %) was recorded in unleavened breads fortified with 0.5 % $CaCO_3$. No significant ($P > 0.05$) effect of $CaCO_3$ (at all concentrations) was found on the protein content of the breads except in unleavened breads at 1.5 % level as compared to the control. Furthermore, protein content in control leavened breads and unleavened breads (Table-3) also decreased as compared to the protein content of the whole wheat flour (Fig. 1). This might be due to the baking process which significantly reduced the amino acid content of the bread [25]. Higher crude protein was found after fortification of whole wheat flour with bone extract powder at various concentrations as compared to egg shell powder. Protein content in both types of breads was found to be increased by increasing bone extract powder in whole wheat flour because bone extract powder is a natural source of protein and other nutrients [20]. An increase of 21.4 % and 29.1 % in protein content was observed in bread made from wheat flour fortified with 3 % and 7 % soy flour, respectively as compared to control [23].

Crude fiber: Fortification of whole wheat flour with bone extract powder, egg shell powder and $CaCO_3$ also enhanced the crude fiber content in both type of breads (Table-3). Bone extract powder (particularly 1.5 %) highly increased the crude fiber of the leavened breads and unleavened breads. An increase in crude fiber was also found with egg shell powder fortification in both leavened breads and unleavened breads, while $CaCO_3$ non-significantly increased the fiber content of unleavened breads. An increase in crude fiber content was observed by Sharma and Chauhan [26] with the addition of fenugreek flour to whole wheat flour. However, the results in this study were in contradiction with the finding of the Wahab [20] who reported a reducing trend in crude fiber content particularly in unleavened breads fortified with bone extract powder.

Nitrogen free extract (NFE): Nitrogen free extract values were significantly ($P \leq 0.05$) decreased in leavened breads and unleavened breads when fortified with bone extract powder and egg shell powder in different levels. On the other hand, $CaCO_3$ maintained almost similar results to that of control with few exceptions as shown in Table-3. Generally, nitrogen free extract was decreased with increasing levels of calcium sources in both leavened breads and unleavened breads. However, maximum reduction was found in both types of breads made from whole wheat flour fortified with bone extract powder in various concentrations. Our results were in the same trend with Riaz *et al.* [16] who fortified wheat flour with mung bean and mash bean and concluded that nitrogen free extract level decreased as mung bean and mash bean in wheat flour increased. Bano *et al.* [24] also observed nitrogen free extract reduction in bread prepared from supplemented whole wheat flour with tartary buck wheat flour.

Mineral profile of control and calcium fortified leavened and unleavened whole wheat flour breads

Calcium content: Whole wheat flour (WWF) fortified with bone extract powder, egg shell powder and $CaCO_3$ showed a significant ($P \leq 0.05$) increase in calcium concentration of leavened breads and unleavened breads (Table-4). Among all the samples, maximum calcium content (1545.3 mg/100 g) was found in whole wheat flour fortified with egg shell powder (1.5 %) in leavened breads, while in case of unleavened breads similar amount of calcium was recorded in bread fortified with bone extract powder (1.5 %). As these fortificants are naturally a rich source of calcium, Sittikulwitit *et al.* [11] hence, expectedly increased the calcium content of the breads. Romanchik-Cerpovicz and Rebecca [10] found 8.6 fold increase of calcium in calcium fortified tortillas as compared to non-fortified (control). Similarly, a high increase in calcium content was also observed in bakery products when fortified with chicken bones extract powder [11]. Kettawan *et al.* [12] fortified fried shrimp chips with different concentration of bone extract powder and found high increment in calcium amount in the product.

Phosphorous, magnesium, potassium and sodium content: Phosphorous content was found to be significantly ($P \leq 0.05$) high in leavened breads and unleavened breads fortified with bone extract powder and egg shell powder (Table-4). Maximum increase was observed for leavened breads and unleavened breads fortified with bone extract powder because chicken bones are good source of phosphorous (Table-2), as stated by Schaafsma *et al.* [19]. Similarly, Sittikulwitit *et al.* [11] and Ketwaan *et al.* [12] also reported that bone extract powder contains calcium and phosphorous at the ratio of 2:1, respectively. On the other hand, phosphorus content was reduced significantly in leavened breads and unleavened breads fortified with $CaCO_3$ as compared to control bread (Table-4). Lower phosphorous content was observed in leavened breads than unleavened breads. This reduction might be due to leavening/baking process which reduces phytate contents [27].

Mean values of magnesium content in control and calcium fortified leavened breads and unleavened breads are presented in Table-4. Highest magnesium concentration was observed in whole wheat flour based bread fortified with egg shell powder at all three levels. This is attributed to high magnesium content in egg shells (Table-2). Schaafsma *et al.* [19] reported that out of the total weight of egg shell, 96 % are calcium carbonate, 1 % magnesium carbonate, 1 % calcium phosphate and some organic substances. A slight increase of magnesium content was found in leavened breads and unleavened breads fortified with bone extract powder and $CaCO_3$ (1.5 and 1 %), while decreased at 0.5 % of these fortificants.

A significant difference of potassium content was shown in leavened breads and unleavened breads fortified with different concentrations of bone extract powder, egg shell powder and $CaCO_3$ (Table-4). Breads fortified with bone extract powder were high in potassium, particularly at 1.5 and 1 % level. While in comparison to control, potassium level of leavened breads fortified with egg shell powder (1.5 and 1 %) was non-significantly increased. In case of $CaCO_3$, potassium contents were highly increased at 1.5 and 1 % while decreased at 0.5 % in leavened breads. On the other hand, fortification of bone extract

TABLE-4
MEAN VALUES OF CALCIUM, PHOSPHOROUS, MAGNESIUM, POTASSIUM (mg/100 g), SODIUM (%), IRON, ZINC, COPPER AND MANGANESE (ppm) OF CONTROL AND CALCIUM FORTIFIED LEAVENED AND UNLEAVENED WHOLE WHEAT FLOUR BREADS

		WWF (%)	Bone extract powder (%)			Egg shell powder (%)			$CaCO_3$ (%)		
		100	1.5	1.0	0.5	1.5	1.0	0.5	1.5	1.0	0.5
Ca	Leavened bread	42.5 ± 0.02j	1465.0 ± 2.00c	952.57 ± 0.31e	541.10 ± 1.15h	1545.3 ± 0.42a	1002.5 ± 0.31d	550.27 ± 0.83g	1472.4 ± 1.85b	922.50 ± 1.90f	416.15 ± 0.98i
	Unleavened bread	40.34 ± 0.82j	1545.0 ± 3.00a	1032.5 ± 1.90d	472.83 ± 0.85h	1510.2 ± 0.16b	997.63 ± 0.55e	537.43 ± 0.80g	1450.4 ± 5.41c	937.50 ± 0.20f	430.59 ± 1.05i
P	Leavened bread	364.12 ± 0.11g	2321.5 ± 0.32a	2064.3 ± 0.97b	1755.2 ± 0.98c	559.75 ± 0.25d	462.75 ± 0.23e	383.16 ± 0.79f	296.68 ± 0.77 h	239.20 ± 0.17i	189.57 ± 0.31j
	Unleavened bread	374.03 ± 0.11g	2414.2 ± 0.15a	2111.5 ± 0.80b	1821.5 ± 1.10 c	585.48 ± 0.76d	483.33 ± 2.06e	396.37 ± 0.31f	339.19 ± 0.42h	272.25 ± 0.13i	214.23 ± 0.25j
Mg	Leavened bread	166.2 ± 0.14g	175.75 ± 0.22d	173.53 ± 0.35e	149.40 ± 0.80i	192.76 ± 0.21a	181.26 ± 0.16b	175.50 ± 0.40d	170.48 ± 0.58f	177.53 ± 1.15c	163.71 ± 0.20h
	Unleavened bread	168.23 ± 0.25g	172.24 ± 0.12d	169.40 ± 0.10f	150.47 ± 0.70i	190.49 ± 0.51a	184.23 ± 0.93b	172.47 ± 0.31d	171.40 ± 0.20e	176.23 ± 0.68c	162.53 ± 0.35h
K	Leavened bread	141.07 ± 0.94f	160.33 ± 0.93a	159.0 ± 0.07b	144.38 ± 0.06c	141.63 ± 0.57e	140.63 ± 0.59f	136.97 ± 0.11i	142.80 ± 0.19d	142.04 ± 0.06d	137.99 ± 0.04g
	Unleavened bread	137.53 ± 0.45h	159.46 ± 0.36a	157.74 ± 0.12b	143.95 ± 0.06c	142.40 ± 0.37d	140.95 ± 0.08ef	140.50 ± 0.47f	142.21 ± 0.07d	141.29 ± 0.31e	138.62 ± 0.08g
Na	Leavened bread	3.66 ± 0.01g	4.46 ± 0.01a	4.33 ± 0.01b	4.17 ± 0.03c	4.09 ± 0.01d	3.97 ± 0.00e	3.84 ± 0.05f	4.18 ± 0.01c	4.13 ± 0.01c	4.07 ± 0.01d
	Unleavened bread	3.82 ± 0.00 h	4.48 ± 0.02a	4.32 ± 0.03b	4.23 ± 0.03c	4.13 ± 0.00d	3.99 ± 0.00f	3.89 ± 0.01g	4.15 ± 0.00d	4.04 ± 0.00e	3.99 ± 0.00f
Fe	Leavened bread	43.06 ± 0.12g	61.25 ± 0.12e	50.26 ± 0.15f	31.27 ± 0.17h	86.26 ± 0.13c	75.27 ± 0.18d	53.25 ± 0.11f	91.25 ± 0.13b	96.30 ± 0.10a	33.53 ± 0.35h
	Unleavened bread	46.74 ± 0.18c	69.53 ± 0.25b	47.10 ± 0.10c	29.53 ± 0.35d	80.23 ± 0.07a	72.53 ± 0.25ab	54.47 ± 0.25c	74.68 ± 0.43ab	75.25 ± 0.14ab	35.53 ± 0.35d
Zn	Leavened bread	46.28 ± 0.07e	71.73 ± 0.25a	67.75 ± 0.23b	65.53 ± 0.75c	65.24 ± 0.11c	50.48 ± 0.65d	46.32 ± 0.22e	52.47 ± 1.36d	40.67 ± 1.53f	38.26 ± 1.05i
	Unleavened bread	49.27 ± 0.13f	70.59 ± 0.66a	68.21 ± 0.20b	66.40 ± 1.02c	67.23 ± 0.05bc	51.37 ± 0.70e	46.70 ± 1.05g	53.50 ± 0.30d	39.33 ± 1.33h	40.29 ± 0.17h
Cu	Leavened bread	33.10 ± 0.10 h	44.75 ± 0.22a	43.25 ± 0.22b	42.25 ± 0.11c	41.71 ± 0.82d	38.20 ± 1.11e	34.53 ± 0.35g	35.53 ± 0.32f	33.43 ± 0.65h	31.27 ± 0.10i
	Unleavened bread	34.99 ± 0.02e	45.17 ± 0.06a	42.76 ± 0.18b	42.99 ± 0.03b	43.05 ± 0.04b	37.25 ± 0.10c	34.79 ± 0.05e	36.24 ± 0.93d	32.74 ± 0.21f	31.79 ± 0.18g
Mn	Leavened bread	32.24 ± 0.17e	36.27 ± 0.21a	34.77 ± 0.13c	35.15 ± 0.04b	33.76 ± 0.22d	32.63 ± 0.34e	30.49 ± 0.46 hij	32.57 ± 0.31e	29.75 ± 0.26f	29.50 ± 0.30f
	Unleavened bread	30.90 ± 0.87de	35.63 ± 0.25b	35.25 ± 0.13b	37.04 ± 0.96a	34.75 ± 0.25b	32.0 ± 2.00cd	31.24 ± 0.24d	33.09 ± 0.37c	30.03 ± 0.05e	30.25 ± 0.13e

Data represent the mean ± standard deviation (n = 3); a-j, characterize significant difference ($P \leq 0.5$) within the row; WWF = whole wheat flour.

powder, egg shell powder and $CaCO_3$ in different levels considerably increased the potassium level of unleavened breads (Table-4).

The results indicated that sodium concentration seems to be effected by fortifications. An increment in sodium contents was observed for both types of bread fortified with bone extract powder, egg shell powder and $CaCO_3$. Leavened and unleavened bread fortified with bone extract powder at 1.5 % showed highest mean value of sodium *i.e.* 4.46 %, 4.48 %, respectively. Whereas 0.5 % egg shell powder fortified bread showed lowest mean value (3.84 %) of sodium in leavened breads and 3.89 % in unleavened breads (Table-4). It indicated that bone extract powder and egg shell powder greatly affected the minerals profile of the leavened breads and unleavened breads, probably due to high profile of minerals naturally available in chicken bones and egg shells [11,19].

Iron, zinc, copper and manganese content: Iron concentration of leavened and unleavened breads fortified with bone extract powder, egg shell powder and $CaCO_3$ were significantly increased at 1.5 % and 1 % while decreased with the addition of bone extract powder and $CaCO_3$ at 0.5 % (Table-4). When the level of fortificants increased, high iron concentration was observed. The possible reason here is the addition of these calcium sources especially bone extract powder (Table-2). It was obvious that high iron contents found in leavened breads as compared to unleavened breads, with few exceptions. Qazi *et al.* [27] and Bano *et al.* [24] reported a reduction in the phytic acid by the fermentation process, which resulted high amount of minerals availability. Similarly, Tripathi and Patel [28] reported an enhancement in the bioaccessibility of iron by heat process in finger millet (FM) flour fortified with ferrous fumarate in combination with EDTA, folic acid and citric acid as co-fortificants, as compared to unfortified flour bread.

Fortification of whole wheat flour with bone extract powder, egg shell powder and $CaCO_3$ affected the zinc, copper and manganese content of leavened breads and unleavened breads. Bone extract powder at all concentrations increased the zinc, copper and manganese contents in both types of bread as compared to the control breads. Egg shell powder at 1.5 and 1 % levels increased the zinc, copper and manganese contents

TABLE-5
MEAN VALUES OF COLOUR, TASTE, TEXTURE AND OVERALL ACCEPTABILITY OF CONTROL AND CALCIUM FORTIFIED LEAVENED AND UNLEAVENED WHOLE WHEAT FLOUR BREADS

Treatments (%)	Colour		Taste		Texture		Overall acceptability	
	Leavened bread	Unleavened bread	Leavened bread	Unleavened bread	Leavened bread	Unleavened bread	Leavened bread	Unleavened bread
Control (100)	7.67±0.58a	6.33±0.58d	7.33±0.58b	7.00±0.00b	6.00±0.00a	5.26±0.23c	7.11±0.19a	6.55±0.51c
BEP (1.5)	2.33±0.12g	3.14±0.10f	2.67±0.58f	3.00±1.00e	3.29±1.12c	3.49±0.18d	2.33±0.67e	2.77±0.51f
BEP (1.0)	3.52±0.07f	3.02±0.12f	3.30±0.05e	2.92±0.14e	3.88±1.35c	3.52±1.02d	2.97±0.32e	3.82±0.15e
BEP (0.5)	4.09±0.12ef	3.69±0.16e	4.90±0.31d	3.71±0.16e	5.65±0.17b	4.86±1.22c	4.03±0.11d	5.98±0.32d
ESP (1.5)	7.12±0.29c	8.24±0.24a	6.96±0.36bc	7.04±0.53b	4.54±0.94bc	4.98±1.98c	7.22±0.27a	6.81±0.45bc
ESP (1.0)	7.01±0.30c	7.40±0.06b	6.44±0.51c	5.4±1.42d	5.41±0.27b	6.20±0.20bc	6.33±0.47b	6.32±0.22c
ESP (0.5)	6.59±1.43d	5.95±0.29d	5.24±0.22d	6.06±0.33cd	6.00±1.00a	6.93±0.93b	7.02±0.47a	7.52±0.18ab
$CaCO_3$ (1.5)	7.32±0.23b	8.02±0.17a	7.29±0.15b	7.33±0.58a	5.99±0.85a	7.41±0.22ab	7.27±0.19a	8.03±0.20a
$CaCO_3$ (1.0)	6.40±0.07d	7.22±0.10bc	7.63±0.21a	7.42±0.52a	6.17±0.18a	7.73±0.14a	6.84±0.18ab	7.14±0.13b
$CaCO_3$ (0.5)	5.57±0.17e	6.85±0.11c	7.32±0.11b	6.22±0.90c	6.09±0.87a	8.24±0.09a	5.59±0.52c	7.62±0.32ab

Data represent the mean ± standard deviation (n = 3); a-g, characterize significant difference ($P \leq 0.5$) within the column; BEP = Bone extract powder, ESP = Egg shell powder

while at 0.5 %, the concentration of these mineral were not strongly affected in both breads. A slight increase in zinc and copper were detected in $CaCO_3$ at 1.5 % in both leavened breads and unleavened breads, as compared to control treatments (Table-4). A decrease of these minerals was pronounced in both leavened breads and unleavened breads as compared to the minerals content of the whole wheat flour (Table-2). This reduction is possibly due to over baking process [20].

Sensory analysis of control and calcium fortified leavened breads and unleavened breads: The mean score values of colour, taste, texture and overall acceptability for control and bone extract powder, egg shell powder and $CaCO_3$ fortified leavened breads and unleavened breads are presented in Table-5. In case of leavened breads, highest ($P \leq 0.05$) mean score (7.67) for colour was recorded in control, while the lowest (2.33) value was in 1.5 % bone extract powder. In case of unleavened breads, panelists preferred breads fortified with 1.5 % egg shell powder, while at 1 % bone extract powder was the most unlike. Breads fortified with bone extract powder at all three levels got the lowest mean score. It was also observed that as the level of bone extract powder increased in whole wheat flour, colour of the breads became darker. The breads colour was affected by mainly due to the dark colour of the bone extract powder, as Bano *et al.* [24] reported that the dark colour of the buckwheat affected the colour of the supplemented whole wheat flour based breads. Reduction in colour values might be due to the reaction between amino acid and reducing sugar as observed by Dhingra and Sood [29].

Highest ($P \leq 0.05$) mean taste score was observed in 1 % $CaCO_3$ in both leavened breads (7.63) and unleavened breads (7.42). Whereas, bone extract powder at all concentrations decreased the taste score of both types of bread as compared to control and all other treatments (Table-5). No significant difference in texture of leavened breads was found in control, egg shell powder and $CaCO_3$ fortified samples while, bone extract powder at 1.5 and 1 % considerably ($P < 0.05$) reduced the texture of leavened breads. In case of unleavened breads highest mean score was found in bread fortified with $CaCO_3$ (Table-5). Our results were in line with the work of Bano *et al.* [24].

Fortification of whole wheat flour with bone extract powder, egg shell powder and $CaCO_3$ in various levels significantly ($P < 0.05$) influenced the overall acceptability of leavened breads and unleavened breads. Highest overall acceptability value was observed in both leavened breads and unleavened breads fortified with 1.5 % $CaCO_3$, which shows the preference of panelist. Whereas, breads fortified with 1.5 % bone extract powder had lowest overall acceptability score. As the levels of all these fortificants increased, sensory attributes decreased particularly with bone extract powder (Table-5).

Overall in the sensory analysis, fortificants particularly bone extract powder, degraded the sensory attributes of breads in all aspects. Our results are in contradictory with Sittikulwitit *et al.* [11] and Ketwaan *et al.* [12] who prepared bakery products and shrimps chips and reported no difference in control and fortified samples. This could be explained that different ingredients such as sugar, shortening, whole milk *etc.* might masked/altered the organoleptic attributes of bone extract powder fortified bakery products.

Conclusion

As calcium was the main concern of the current study and calcium content of leavened breads and unleavened breads increased significantly. These sources are the best among the all other available alternatives of calcium. It will help to increase the amount of calcium particularly in Asian diet. However, further work is needed on the bioavailability of calcium and phytic acid content of both leavened and unleavened breads.

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