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Physicochemical Characteristics of Whole Wheat Flour Fortified with Four Different Levels of Tricalcium Phosphate

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ABSTRACT

Introduction: Dietary calcium intakes have been reported to be lower than recommended intakes in women and adolescents. This essential nutrient has not received adequate attention at the population level. Food fortification, particularly of staples can help bridge the gap between requirement and customary intakes, without altering diet patterns.

Aim/Objectives: This study aimed to fortify wheat flour to increase calcium content, using four levels of Tri Calcium Phosphate (TCP), to determine the optimum level, based on physicochemical characteristics.

Methods: Wheat flour was fortified with four levels of TCP such that 150g of flour would provide 200mg, 300 mg, 400 mg or 500 mg. Physicochemical properties (Water absorption capacity, swelling power, swelling volume, solubility index, bulk density, solvent retention capacity, particle size) were analyzed in fortified and unfortified flour samples.

Results: Fortified flours had lower water absorption capacity than unfortified wheat flour ($p<0.000$). The solubility index ($p<0.000$) for fortified flours was 1-5% lower than the unfortified flour. Bulk density for all flours was 0.40- 0.42 g/ml. Particle size ($p<0.000$) and water retention increased with increasing amount of TCP. Overall, the physicochemical characteristics of the fortified samples did not differ from the unfortified wheat flour. Thus, the fortified wheat flour could be used in various processed foods.

Conclusions: This is one of the few studies to have attempted fortifying wheat flour to provide about 50% of the EAR from 150g of wheat flour. The study showed that TCP addition did not adversely affect physicochemical characteristics, making it a suitable fortificant for wheat flour.

Key Words: Tricalcium phosphate, Particle size, Solvent retention capacity, Swelling volume, Water absorption capacity, Calcium fortification

INTRODUCTION

Calcium is involved in vascular contraction and vasodilation, muscle functions, nerve transmission, cell-signaling pathways and hormonal secretion.[1] The 5th National Family and Health Survey in 2015-16,[2] indicated that calcium adequacy was low,particularly for undernourished girls and women.[3] Sanwalkaand co-authors[4] reported that median calcium intakes of rural adolescent boys and girls from Pune were substantially low. Patil and coworkers[5] indicated that more than two-thirds of adolescent girls in Maharashtra's Konkan region were calcium deficient. Similarly, in Karnataka, Gupta et al.,[6] found that the mean calcium intakes were considerably lower than the estimated average requirements. The National Nutrition Monitoring Bureau (NNMB)[7] data for urban and rural India showed that the intakes were 402 and 331mg/consumption unit, respectively. These intakes are approximately half of the Estimated Average Requirements (EAR) 800mg recommended by the Indian Council of Medical Research -National Institute of Nutrition (ICMR- NIN).[8]

Cormick and colleagues [9] noted that pregnant women in low-middle-income nations consumed less calcium (647.6 mg/day) than women in high income countries (943 mg/

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day). The NNMB report[7] indicated that calcium intakes of pregnant and lactating women were approximately 400 mg, much lower than the presently recommended intake by the ICMR- NIN.[8]

The Government of India has various schemes and programs to combat iron and folate deficiencies, through food fortification, and micronutrient supplementation.[10] The Ministry of Health and Family Welfare, Government of India issued guidelines[11] in 2014 for pregnant women to be given 1g supplemental calcium daily, in two divided doses. However, there has been no concerted effort to improve the calcium intakes of the general population in the country, despite intakes being inadequate.

This highlights the need for food-based strategies targeting the entire population rather than individuals by age or physiological state. Food fortification has been successfully used in different parts of the world. Presently, wheat flour (*atta)* fortified with iron, folic acid and vitamin B12 is permitted by Food Safety and Standards Regulations [12] in India, but wheat flour fortified with calcium is not widely available. In light of the reports about the inadequate calcium intake, we studied calcium fortification of wheat flour as a vehicle, because wheat is a staple cereal. Therefore, we examined the feasibility of using TCP as a fortificant to enhance the calcium content of wheat flour. We hypothesized that TCP addition would not adversely influence the physicochemical characteristics of wheat flour. We aimed to determine whether 50% of the EAR for calcium can be met through fortification with TCP.

MATERIALS AND METHODS

Procurement of wheat flour**:** Wheat flour (MP Sehore variety) was purchased from a commercial miller (M/s Navneet Provisions, Mumbai).

Choice of fortificant: FSSAI permits any of the following salts as a fortificant: calcium carbonate, calcium chloride, calcium citrate, calcium phosphate monobasic, calcium phosphate dibasic, and calcium phosphate tribasic. [13] Tricalcium Phosphate (TCP) was selected as the fortificant based on the cost; it is tasteless and odorless, does not alter taste when added to a food, is stable in air and does not cause any gastrointestinal discomfort. The amounts added were within the FSSAI regulation that per kilogram of *atta,* the nutrient level should not be more than 1500 mg. Tricalcium phosphate was purchased from M/s Lyons Chemicals, Baroda, Gujarat.

Level of fortification: The amount of TCP to be added was based on the EAR for adult nonpregnant and pregnant women. It was estimated that about 150g of flour/day, through 5 chapatis/rotis (one chapati containing 30g wheat flour), would provide approximately 50% (400 mg) of the EAR of 800mg. Four levels of fortification were studied: 133 mg/100g of flour, 200 mg/100g of flour, 267mg/100 g of flour, 333 mg/100g of flour. (Figure 1)

Fortification of the flours: Mixing of TCP was blended with wheat flour and packaged flours at M/s Srinam Foods, Mumbai. Each flour blend was prepared separately. To 40 kg ± 1g of wheat flour, the required amount of TCP (accurate to 0.001 g) was added and mixed thoroughly. The flours were filled in pouches of 1 kg capacity [300 mm X195 mm (12-micron polyester, 9-micron foil and 75-gauge polyester)] that were heat-sealed. Samples were analyzed for the following physicochemical characteristics: water absorption capacity, swelling power, swelling volume and solubility index, bulk density, solvent retention capacity (SRC) and particle size.

The samples were analysed in triplicate. All chemicals used were of analytical grade (AR or GR).

Sampling of Flours for analysis: Three samples (50 g each) was obtained by the quartering method and stored in acid-washed air tight plastic containers, until analyses.

Physicochemical Characteristics

Water Absorption Capacity (WAC) was determined by the method described by Amandikwa et.al., [14] using 1± 0.01 g of the sample. The WAC was expressed as grams of water absorbed per gram of flour sample.

Swelling power, swelling volume and solubility index were determined on 0.25 g flour by the method described by Ee KY et al., [15] Swelling power and solubility were calculated as follows:

Swelling Power = SP/ 1g- DR

Solubility: DR(g)/1g x 100%

Bulk density was determined by the gravimetric method described by Amandikwa et.al., [14] with 10± 0.01 g of the flour. Loose Bulk Density (LBD)and Packed Bulk Density (PBD) were calculated.

Solvent Retention Capacity (SRC) was determined on 1 ± 0.01 g of sample by the procedure of the American Association for Clinical Chemistry (AACC) 56-11.02, as described by Ee KY et al.,[15]. Four solvents were used: 5% (w/w) lactic acid solution, 5% (w/w) sodium carbonate solution, 50% (w/w) sucrose solution and distilled water. Percent SRC was calculated.

Particle Size (PS) distribution of the flours was determined according to the method used by Oladunmoye et.al.,[16]. using a set of two test sieves (Jayant Scientific Ind.) with mesh size of 150 (BSS 150)- and 100(BSS 100) microns, respectively. For each measurement, 100g ± 0.01g of sample was used. PS was expressed as the percentage of the initial weight of the flour.

Statistical Analysis

Data was analyzed using SPSS version 22.0. For comparison between flours, analysis of variance and post-hoc Bonferroni tests were applied. $p \leq 0.05$ was considered significant.

RESULTS

In the present study, wheat flour was selected for calcium fortification, as it is a staple food in numerous countries, as well as being a major ingredient in numerous food products, some with a long shelf life. The flour was fortified with four levels of tricalcium phosphate. These flours were compared with the control (unfortified) wheat flour for water absorption capacity, solvent retention capacity, swelling power, swelling volume, solubility index, bulk density, and particle size.

Water Absorption Capacity (WAC): Addition of TCP significantly increased the WAC of the fortified flour samples (Table 1). The WAC of the fortified flours was approximately 1g more than that of the control flour, representing an increase of 66-78%. However, increasing levels of TCP did not alter WAC much.

Solubility Index**:** Water solubility index is usually studied for a new flour or flour composite. It indicates the amount of polysaccharides present in the flour that diffuse or are released upon addition of excess water or form fragments due to addition of excess water. [17,18, 19] In the calcium-fortified flour samples, the solubility index was lower (1-5%) compared to the control wheat flour. Thus, addition of TCP as a fortificant to wheat flour did not significantly affect the water solubility of the fortified flours (Table 1).

Swelling volume and swelling power: These characteristics were not affected by TCP addition, regardless of the amount of fortificant added (Table 1).

Bulk density: Both the loose and packed bulk densities of the wheat flour were not influenced by the addition of the fortificant (Table 2). The loose bulk density for the unfortified flour was 0.42 g/ml and those of the fortified variants were in a small range of values from 0.40 - 0.42 g/ml.

Solvent Retention Capacity: Four solvents [water, sucrose, sodium carbonate, lactic acid (LA)] were used to assess SRC. Three solutions were used: 50% sucrose, 5% sodium carbonate, 5% LA and water as the standard SRC Solvent, as the water SRC value is related to the overall WHC contributed by the flour functional components. SRC using water was higher for all fortified flours than the unfortified flour (Table 3).All four solvent SRC values differed significantly between the four fortified variants.

The water and LA SRCs increased with increasing amount of TCP. The SRC for the 50% sucrose solution was highest for the unfortified flour followed by 50% LA, 50% sodium carbonate and lastly water.

Particle size: Particle size of the unfortified and four fortified flours was studied using two mesh sizes – 150 and 105microns to represent the coarser and finer fractions, respectively. The particle sizes of flour samples containing 300 and 400 mg calcium in 150 g of flour were significantly higher than the flour containing 500 mg TCP and the unfortified flour (Table 4). With mesh size 105 microns, the percent flour retained on the sieve varied between 10.6-18.7% for the fortified variants, whereas 14.6% of unfortified flour was retained. This was in the same range as the fortified variants (Table 4). With both the sieve sizes, the flour samples containing 300mg of TCP exhibited the highest retention followed by the flour containing 400 mg calcium per 150 gm of flour.

DISCUSSION

Physicochemical properties of the flours influence the physical and chemical attributes of the food during processing. Wheat flour has unique functional characteristics making it a useful ingredient in food manufacture. However, it can present challenges while baking food products, particularly in terms of functionality and specific quality characteristics.

Water absorption capacity helps determine the optimum amount of water that can be added to the flour to make a dough of the desired consistency.[16, 17] Higher WAC enhances baking quality and helps maintain the freshness of bakery items e.g. cakes, bread.[16] A high WAC is favorable for using the flour as a thickener. In the present study, the TCP-fortified flour had a higher WAC, indicating that TCP flour is useful for bakery products.

Janve and Singhal [18] found that calcium fortification of rice increased the water absorption index. Similar trends were observed by Codină and coworkers [20] for wheat flour dough and Khan et al., [21] for bread flour fortified with calcium extracted from eggshells. Codină and coworkers [20] reported that addition of calcium ions from lactate and gluconate salts boosts interactions with the starch and proteins present. Calcium ions may favour increase in the solubility and hydration capacity of wheat flour proteins. The increase in the WAC of fortified flours has been attributed to this interaction of calcium ions with starch granules and starch destabilization causing an increase in the space between the amylopectin chains of the damaged starch, eventually leading to increased absorption of water in these granules.[22] Miller and Hoseney[23] stated that anions decreased the electrostatic repulsion between proteins, allowing them to form a more compact dough.

Swelling power reflects the water-holding capacity of starch granules and is important for various products like noodles. The swelling power of whole meal wheat flour was found to correlate positively with the eating quality of noodles.[24] When wheat starch granules are heated with water, they swell up and the volume increases. The swelling capacity of flours depends on their particle size, the variety, the processing method or unit operations[25], and increases with increasing starch content.[17,24]

Oladunmoye et al.,[16] and Bhat et al.,[19] reported bulk density of whole wheat flour as 0.49-0.51g/ml. In our study, unfortified flour's packed bulk density was 0.56 g/ml. The fortified samples had similar values, between 0.51 – 0.61 g/ml. Thus, fortification of wheat flour with different amounts of TCP did not significantly influence bulk density of the flours, indicating that fortified wheat flour can be easily used for products like complementary foods.

When excess water is added to flour, the linked polymeric materials i.e. gluten, starch and pentosans in the flour swell to varying degrees, reflected by the change in the volume/ weight. This helps to assess the functional contribution of the flour components in various products and the overall quality, the level of damaged starch, the swelling behavior of the functional flour polymers i.e. gluten, gliadins, glutenin, and pentosans.[26, 27] It reflects the flour's ability to imbibe water during mixing, retain it within a matrix/ gel, undergo hydration performance and the ability to release that water during the baking process and influence the quality of the finished product. In the present study, the water and LA SRCs increased with increasing amount of TCP.

Values for water SRC ≤ 51g/100g, sodium carbonate SRC ≤ 61g/100g, sucrose≤ 89g/100g or a LA SRC of 87g/100g are suitable for cookies. For spongy products and good dough properties, the required SRC values are: water SRC ≤ 57g/100g, sodium carbonate SRC ≤ 72g/100g, sucrose SRC ≤ 96g/100g and LA SRC ≥ 100g/100g.[30, 31] In the present study, the LA SRC was> 100g/100g, indicating that the four levels of TCP fortificant used, gave flours having good dough characteristics, suitable for bread-making.[32]

The particle size in a flour determines the physical and chemical properties of the flour, water absorption/hydration capacity, solubility, swelling properties, functionality of the gluten network, thereby affecting the dough properties and the final product quality.[33,34] The smaller the flour particle, the better will be the WRC and dough characteristics.[35] Finer particles are more suitable for bread making in terms of extensibility, stability, porosity, texture and compactness. Similarly, Bassi and co-investigators[36] observed that smaller flour particle size was associated with lower contents of ash, fat, fibre, phytic acid, antioxidant activity and total phenolic content of chapatis. Also, pasting properties and other functional properties important for chapati-making qualities were improved.In the present study, the amount of TCP added did not influence the particle size indicating that it will not negatively influence the product properties. The present study results indicate that it would be worthwhile to study the functionality of calcium-fortified wheat flour in various popular, commonly consumed food products.

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Conflict of Interest: None

Authors' Contribution: SA Udipi and Jagmeet Madan designed the entire study and were the Principal Investigators, SA Udipi with Pranali Pangerkar and Shreya Warrier identified all the tests conducted. Pranali Pangerkar, Shreya Warrier and Nidhi Randive were responsible for standardizing the methodology and performing all tests. Anuradha S with Prakash Rao was responsible for coordinating the study, procurement of chemicals and forticant. wheat flour, supervising the fortification and packaging of samples. Shreya Warrier, Pranali Pangerkar and SA Udipi drafted the manuscript which was edited and finalized by all authors.

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Table 1: Water Absorption Capacity, Solubility Index, Solubility Power and Swelling Volume of Unfortified and Fortified Wheat Flour containing different amounts of TCP)

Characteristics	Mg of TCP added /100g of wheat)					
	0	133	200	267	333	F, p Value
Water Absorption Capacity (WAC) ml/g	1.37±0.16 1.16-1.50	2.37±0.25 2.16-2.66	2.44±0.25 2.16-2.66	2.28±0.08 2.16-2.33	2.32±0.03 1.98-2.59	25.284, 0.000
Solubility Index (%)	1.93±0.22 1.74-2.23	1.85±0.19 1.74-2.14	1.91±0.31 1.67-2.35	1.83±0.18 1.67-2.05	1.88±0.17 1.66-2.03	0.137, 0.966
Swelling Power (g/g)	1.85±0.22 1.68-2.15	1.79±0.21 1.64-2.11	1.86±0.32 1.61-2.31	1.78±0.19 1.61-2.01	1.83±0.17 1.61-1.99	0.105, 0.979
Swelling Volume	2.25±0.22 2.0-2.50	2.21±0.37 1.83-2.67	2.04±0.53 1.50-2.50	1.94±0.31 1.60-2.33	2.18±0.24 1.90-2.33	0.550, 0.702

Table 2: Loose Bulk Density and Packed Bulk Density of Unfortified and Fortified Wheat Flour containing different amounts of TCP

	(Mg of TCP added /100g of wheat)					
Characteristics	0	133	200	267	333	F, p value
Loose Bulk Density (LBD)	0.42±0.006 0.41-0.42	0.40±0.016 0.38-0.41	0.42±0.004 0.42-0.43	0.41±0.010 0.39-0.41	0.41±0.211 0.38-0.43	1.089, 0.397
Packed Bulk Density (PBD)	0.56±0.72 0.58-0.69	0.52±0.73 0.53-0.69	0.55±0.59 0.56-0.58	0.51±0.70 0.53-0.67	0.61±0.08 0.52-0.68	0.814, 0.535

Table 3: Solvent Retention Capacity of Unfortified and Fortified Wheat Flour containing different amounts of TCP

	(Mg of TCP added /100g of wheat)					
Solvent	0	133	200	267	333	F, p Value
Distilled Water (%)	149.5±4.22 145.4-155.0	135.8±2.9 132.2-139.2	141.9±2.9 139.4-145.3	150.4±2.0 148.8-153.3	171.3±1.8 169.3-173.6	86.376, 0.000
50% w/w Sucrose Solution (%)	212.3±2.9 208.3-215.3	204.4±4.3 200.6-210.0	190.6±3.7 186.3-195.3	208.5±2.2 205.3-210.3	226.3±3,77 221.7-230.7	55.516, 0.000
50% w/w Sodium Carbonate soln. (%)	189.4±2.8 185.6-192.2	173.2±2.3 170.3-175.2	202.2±3.5 198.2-205.3	190.1±1.8 188.3-192.4	195.6±2.7 192.7-199.0	64.831, 0.000
50% w/w Lactic Acid Soln. (%)	194.5±3.5 190.3-198.1	170.8±1.6 169.3-173.1	172.9±2.1 170.3-175.3	184.8±2.1 182.7-187.3	184.6±9.2 180.1-187.5	54.850, 0.000

Table 4:Particle Size: Unfortified and Flours Containing Different Amounts of TCP

Characteristics	(Mg of TCP added /100g of wheat)					F, p value
	0	133	200	267	333	
Sieve Size: (BSS- 100; Microns: 150)						
Mean Sample Weight	100.0±0.04 100.0-100.1	100.1±0.08 100.0-100.2	100.1±0.06 100.0-100.1	100.0±0.04 100.0-100.2	100.1±0.06 100.0-100.2	0.658, 0.630
% Sample after sieving	84.3±1.08 83.2-85.3	84.7±1.21 83.6-85.9	79.8±1.09 78.6-81.1	82.3±0.57 81.8-82.9	84.5±1.22 82.6-85.3	15.146, 0.000
%Sample retained	15.7±1.08 14.7-16.9	15.3±1.21 14.1-16.4	20.2±1.09 19.0-21.4	17.7±0.57 17.1-18.3	15.5±1.22 14.8-17.4	15.146, 0.000
Sieve Size: (BSS- 150; Microns: 105)						
Mean Sample Weight	84.3±1.1 83.2-85.4	84.7±1.3 83.6-86.0	79.9±1.1 78.7-81.0	82.3±0.6 81.8-82.9	84.5±1.2 82.7-85.3	14.726, 0.000
% Sample after sieving	85.4±0.9 84.3 -86.4	85.8±1.5 83.5-86.7	83.3±2.2 81.3-86.0	82.8±6.3 73.5-86.7	85.7±3.2 82.2-89.4	0.705, 0.601
% Sample retained	14.6±0.9 13.6-15.7	14.2±1.5 13.3-16.5	16.7±2.2 13.9-18.7	17.2±2.2 13.1-26.5	14.3±3.2 10.6-17.8	0.705, 0.601

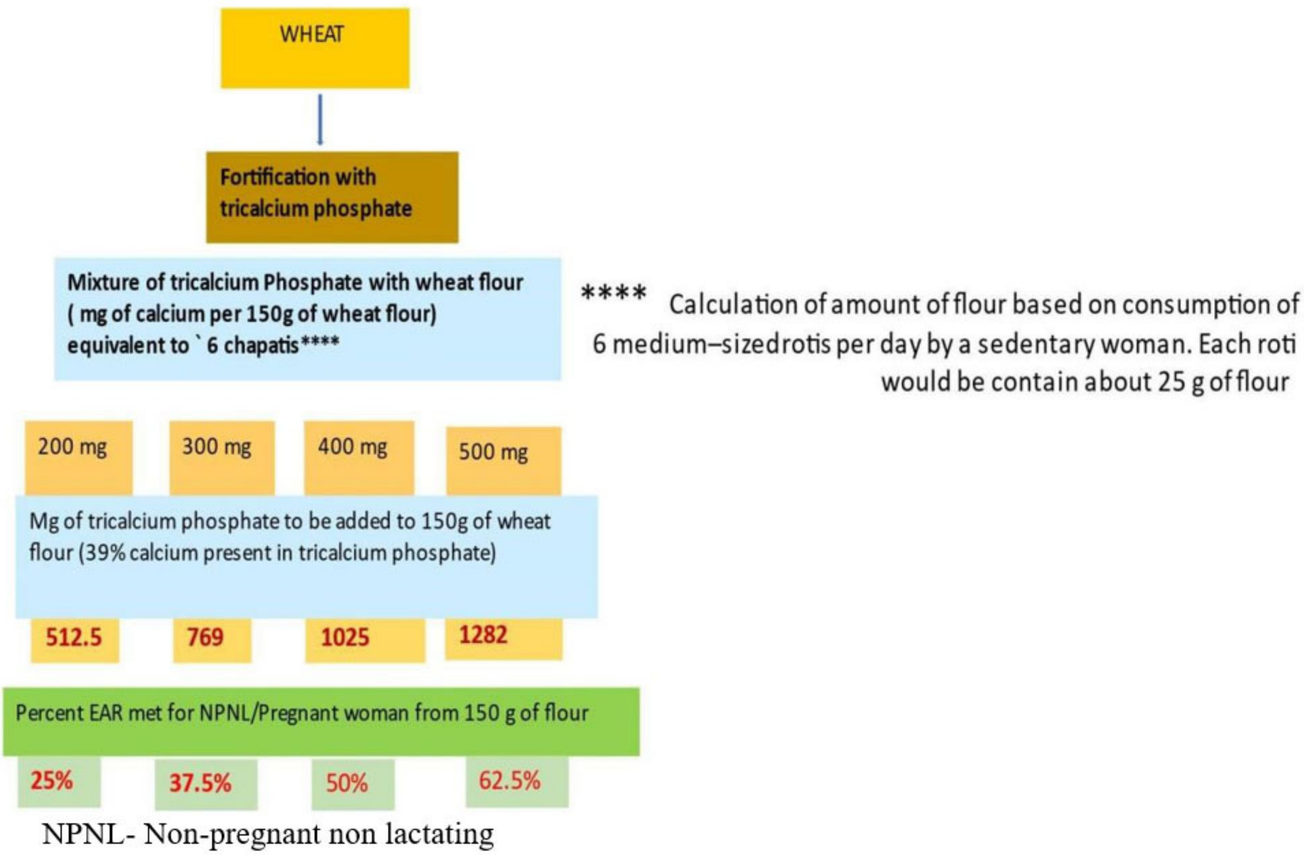

Figure 1: Amount of tricalcium phosphate (four levels) used for fortification of wheat flour.