

A Review on Requirements for Vitamin D Fortification in Pakistan

Rabail Javed¹, Shagufta Naz², Faiza Saleem², Muhammad Aasim¹, Rabia Zafar³
PHRC, National Health Research Complex¹, Sheikh Zayed Medical Complex, Department of Biotechnology, Lahore College for Women University², Forman Christian College³, Lahore.

Abstract

Despite the fact that Pakistan is a country situated near equator and has plenty of sunshine, it still has major population deficient in vitamin D3. The majority of food opted by general public is not sufficient source of vitamin D. For the reason vitamin D fortification is a compulsory requirement for healthy living. Developed countries like United Kingdom and Canada have overall improved their population vitamin D status by fortifying foods with vitamin D. For applying a well maintained vitamin D food fortification strategy in Pakistan, it is necessary to review various methods, type of food and their fortification results implemented all over the world. This review investigates all such prospective approaches which could be used for the fortification of foods in Pakistan. Looking at the socio economic status the majority of Pakistani residents, vitamin D fortification of staple foods such as whole grains, flour and rice by spray methods might serve as viable approaches in targeting majority of Pakistani population to ingest and improve their vitamin D status.

Key words: Vitamin D, food fortification, cholecalciferol.

Introduction

Vitamin D is a seco-steroid which is critical for intestinal calcium absorption. The role of vitamin D not only helps in maintaining normal blood glucose concentrations, cell differentiation but also regulates secretions of hormones and cellular growth. It prevents diseases as diabetes, cancer, cardiovascular diseases, auto immune and psychological disorders and also improves mental health.¹

Vitamin D is divided into two major types naming cholecalciferol (D3) and ergocalciferol (D2). Cholecalciferol is synthesized in the dermal layers of skin after sunlight exposure and from dietary sources of few foods including liver oils and fatty fishes especially tuna and salmon. Mushrooms,

beef liver, egg yolk and dairy products are also some sources of vitamin D3 to a lesser extent.²⁻⁴ It is important to know that some mushrooms escalate vitamin D levels under sunlight.

Ergocalciferol mostly originates from plant sources. It can also be taken through supplements. Vitamin D is metabolized in the liver to circulating 25-hydroxyvitamin D.⁵⁻⁸ Vitamin D is influenced by several factors including seasonal variations, skin colors and duration of sun exposure. It is known that sun light intensity causes seasonal disparities in vitamin D levels.⁹ Studies elaborates that there is less production of vitamin D in dark skin people than other racial groups due to the fact that pigmentation (melanin) blocks UV rays to enter in the skin and reduces vitamin D3 production in the skin.¹⁰⁻¹²

Deficiencies in micronutrients are more prevalent in countries where poverty rises at alarming scale. Statistics provided by world economics elaborates developing countries from south of the world to be countries with lack of resources and per capita income.¹³ Pakistan is one such country with high poverty rate. It has been calculated by Human Development Index that almost 60.3% of Pakistani residents are earning 100 Rs per day in comparison to 24.9% and 49.6% in India and in Bangladesh respectively.¹⁴

A nutritional Survey in Pakistan reveals that ignorable efforts have been done for improving micronutrient deficiencies in Pakistani population

Corresponding Author:

Rabail Javed

PHRC, National Health Research Complex
Sheikh Zayed Medical Complex, Lahore.
Email: rabailjaveed@hotmail.com

Received: 13 April 2017, **Accepted:** 12 December 2017,

Published: 28 June 2018

Authors Contribution

RJ & SN conceptualized the project. RJ also did the data collection, drafting revision and writing of the manuscript. FS & RZ did the literature search. Statistical analysis was done by MA.

especially among women and children. The exception is improvement in iodized salt usage and mega reductions in urinary iodine deficiency rates.⁷

It is estimated that pregnant females are highly deficient of vitamin D3 (70%). Children are also prone to vitamin D3 deficiency up to an alarming level (40%). Socio-economically deprived families cannot afford to buy food rich in vitamin D, resulting in persistently high rates of stunting (44%) and wasting (15%) among children born in such families. If the situation is not properly addressed this could act as a major bottle neck in tackling child survival in Pakistan.

Food fortification is an efficient and practical approach in supplementing food with vitamin D and fighting against diseases related to its deficiency. The process of food fortification is widely applied in many developed countries UK, USA and Canada. The earliest food fortification was done in 4000 BC by adding iron fillings to sweet wine.¹⁵⁻¹⁶

Although fortification of food provides a rapid and cheaper way of increasing vitamin D intake, however there are more chances of vitamin D intoxicity (if high level of fortified food is taken). For the purpose proper dose fortification is required. The specificity of food with vitamin D also plays an important role during fortification and only specific food should be targeted for food fortification. Every country has their own standard legislations and quality control for fortification.

In countries like America, people are dependent on fortified vitamin D diet for their healthy living. In early nineties vitamin D fortification program was initiated in USA to fight against calcium deficiency diseases as rickets.² There is an utmost need of simpler and efficient new methods for estimation of vitamin D in foods.¹⁷

Pakistan is a developing country and among those ten countries which have very low capita income. People of this country cannot afford food rich in vitamin D as they are expensive then routine foods they generally eat like grains, pulses and vegetables. Consequently there is dire requirement for fortification of less costly foods and food products with vitamin D to a level which will not only built sufficient levels of vitamin D in them and also not intoxify with excessive intake.

The goal of this review is to emphasize the importance of vitamin D food fortification and to discuss different methods, pros and cons which should be considered before opting any strategy for vitamin D food fortification in Pakistan. It will also highlight on targeted fortification strategies which can best meet vitamin D nutritional requirements for children.

Optimal vitamin D intake

According to the “Endocrine Society’s Clinical Practice Guidelines”, the lower sufficient limit for 25(OH) vitamin D serum is given as 30 ng/ml.¹⁸⁻²⁰ Those who do not have a sufficient level of vitamin D are recommended to take 600 IU/day for all individual between the age of 1–70 years.¹⁶ Food fortification can add extra vitamin D up to 1000–4000 IU/day.¹⁶ In countries like Pakistan and India most of the population is insufficient in Vitamin D serum levels and need a better fortification plan as even vitamin D sources like milk also becomes unaffordable by majority of population.¹⁶

Vitamin D fortification in Canada and USA and its impact

Vitamin D can be threatening for its cellular toxicity if administered in high dose. In countries like Canada and United States there are special guidelines for vitamin D fortification and a lawful intake is imposed by regulatory bodies for both genders and all age groups.²¹

Canada has opted compulsory program of food fortification through Canadian Food and Drug Regulations.²² Many times there is a dire need by public for nutritional supplementation. In such conditions food fortification becomes a mandatory action in Canada. In Canada vitamin D food fortification is allowed not only in milk but also in margarine. The fluid milk contains vitamin D “not less than 300 IU of vitamin D for daily intake and not more than 400 IU of vitamin D” considering daily total energy intake as < 2500 kcal.^{23,24} This provides up to 44% recommended vitamin D considering a daily serving size of 250-ml. Vitamin D fortification is also carried out in evaporated milk, powdered and goat milk. Margarines used in Canada are vitamin D fortified (530 IU/100 g).²⁵ Some formulated liquid diets products like eggs, meal replacement and nutritional supplements are also acceptable for fortification of vitamin D in Canada. However cheese and industrial milk are not acceptable for fortification.²⁶ In United States the fortification of vitamin D is optional unlike Canada.

Irradiation by ergocalciferol and cholecalciferol resins of vitamin D2, D3 are produced. They can be used as vitamin D supplements in the food grouping as shown in Table.

Fortification in India and its impact

Currently fortification of vitamin D in India has not been established upto mark. There are reviews and studies to support different

Table: Vitamin D fortification in the United States and Canada: current status and data needs.⁴

Food Category Products	Status of Fortification	Level of Fortification Allowed (Max)	Fortified Products Estimate	Regular Fortification Level Of Products
Breakfast cereals	Optional	350 IU/100 g	Most	40–140 IU (10–35% DV)
Rice(enriched)	Optional	90 IU/100 g	None	None
Corn meal (enriched)	Optional	90 IU/100 g	None	None
Noodle (enriched)	Optional	90 IU/100 g	None	None
Macroni (enriched)	Optional	90 IU/100 g	Very few	40 IU/252 g (10% DV)
Milk Fluid	Optional	42 IU/100 g	All	400 IU/quart
Milk (Acidified)	Optional	42 IU/100 g	All	400 IU/quart
Milk (cultured)	Optional	42 IU/100 g	All	400 IU/quart
Milk (concentrated)	Optional	42 IU/100 g	All	400 IU/quart
Milk (non fat dried and with Vit A+D)	Required	42 IU/100 g	All	400 IU/quart
Milk (Evaporated)	Required	42 IU/100 g	All	400 IU/quart
Milk (whole dry)	Optional	42 IU/100 g	All	400 IU/quart
Plain Yogurt	Optional	89 IU/100 g	Few	40–80 IU/RACC
Yogurt(low fat)	Optional	89 IU/100 g	Few	40–80 IU/RACC
Yogurt (non fat)	Optional	89 IU/100 g	Few	40–80 IU/RACC
Margarine	Optional	331 IU/100 g	Few	40–140 IU/RACC
Drinks and fruits (enriched with calcium) ³	Optional	100 IU/RACC	NA	100 IU/RACC

- RACC, Reference amount for custom consumption
- NA, not appropriate

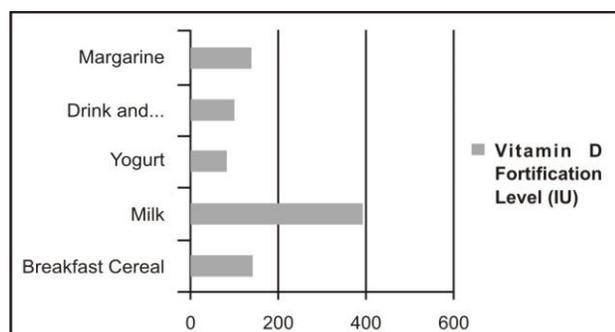


Figure: Vitamin D Fortification level in United States and Canada

fortification strategies. One of them uses calcium fortified laddo in toddlers for their improved total

body less head bone mineral content while another emphasizes on milk supplementation in 10-14 years children for better bone development. The children who were receiving milk/calcium in combination with vitamin D were having higher percentage value than the deprived group. An increase of 35% against 28% was observed in controls eating calcium laddo with vitamin D supplement. Similarly in the other study the mean percentage of change in 25(OH)D level was 177.29%. than group 5.25%.^{27,28} Companies like Amul and Kellogs breakfast cereal are providing fortification but have many limitations.²⁹ It is evident from facts that India is struggling for initiating a vitamin D fortification program and providing knowledge to pharmaceu- tics and general public for awareness about benefits of using fortified food.

India has already marketed vitamin D2 and has proven its efficacy in anti-ricket properties.³⁰ A study from Delhi India reports that almost all the pharmacists are unaware of vitamin D2 supplementation or fortification and recommends only vitamin D3 supplements in injection or tablet form.²⁹ Studies from India suggest that to attain sufficiency, > 1000 IU/day fortification may be required which is far above than the recommended levels of Canada and USA. The reason behind is high deprivation of vitamin D in general public not only in food but also in skin production (high melanin blocks UV light to activate vitamin D3 production in skin).¹⁰

Process of fortification for vitamin D in other countries verses Pakistan

It is important to review fortification techniques used in different countries especially India which is Pakistan's primary neighbor, in order to design any valid food fortification strategy for Pakistani public.

Fortification as of dairy products, cereals, bread, rice/ rice flour and juices even salt or sugar are not only adapted in India but are mandatory in Canada and USA.³¹

The first strategy in building a fortification protocol involves a widely and regularly consumed staple food and its commercial availability through all seasons.³¹⁻³⁴

The protocol should permit a nutrient premix which would relatively be easy and low priced technology upto its best quality assurance.

Vitamin D stability and storage in food items is a major concern while designing food fortification technique.³¹⁻³⁴

Vitamin D stability is affected not only by UV light but also oxygen in air and acids or acidific affects.³⁰ Vitamin D is steady at 4–8°C, sub-0 temperatures and suitable for cooking up to 200 °C. The stability is unaffected at 25°C for weeks although heat transformation can start between temperatures like 110 or 170 °C (temperature and time of heating are inversely proportional to each other).³⁵

Studies from India reveals that vitamin D3 shows greater than 90% steadiness in fortified products. These food includes "Short period processing at high temperature of 2% milk, processing at ultra raised temperatures then heating milk for almost 1 to 2 seconds at >135 °C).

Observing the strategies used all over the world, a list of vitamin D food fortification can be adapted in Pakistan including dairy fortification, baby milk formulas, sugar etc. However affordable staple food in Pakistan includes rice, roti, flour,

white flour and bread. India is the only country to support supplementation of cereals.³⁵

Various micronutrients can be safely added by food spraying with vitamin D. Vitamin D is susceptible to moisture. Flour is a less moisture cereal powder and is best for fortification and stability of vitamin D. It has higher vitamin D shelf life when compared with other cereals for vitamin D fortification.³⁵

Optimal requirements of vitamin D or fortification in Pakistan

Vitamin D fortification levels differ with different population demand. The population includes children, pregnant ladies, lactating mothers, adults and senior citizens.

World Health Organization provides guidelines for food fortification.³⁶ The vitamin D is not only required to maintain normal serum 25(OH)D levels (>30 ng/mL) but for also maintaining them between 30 and 100 ng/mL. Further strategies to avoid in-toxication (>100ng/ml) of vitamin D by high intake of fortified food is also required. Recommended Dietary Allowance permits 4000IU/day intake of vitamin D.³⁶

For developing vitamin D fortification strategy in Pakistan it is important to conduct a study on quality of diet and estimation of intake on mega scale and amount of sun light which is affecting in vitamin D production in skin. In Pakistan pilot studies have been conducted on nutritional intake of vitamin D on daily basis and sunlight exposure and vitamin D production in asymptomatic as well as nutritionally deprived group.^{37,38}

How to achieve required serum levels by fortified foods in Pakistan

Institute of Medicines (USA) recommends a daily intake of 400 IU vitamin D for general public except infants (600 to 800 IU). There is a lack of an international consensus on vitamin D fortification levels. Every country has adapted their own calculations. In USA the mean of individual intake of vitamin D is 11 µg/day (440 IU/day) which is coming from fortified foods ranging 120–1000 IU/day. This increases the vitamin D (25(OH)) levels upto 7.7 ng/ml, relating to 0.5 ng/ml uptake in vitamin D levels for each 40 IU intake.

The North American diet is mainly high in meat content dairy products and mushrooms which are main sources of vitamin and contributes 5 µg (200 IU).³⁹ The Pakistani diet must add a significantly lower amount of vitamin D as consisting of vegetables, pulses and low quality meat.

For estimating the fortification requirement of vitamin D in Pakistani population, the best model

is India. India shares same climatic changes and nutritional habits as in Pakistan. Assuming levels of vitamin D in general population of Indo-Pak to be 10ng/ml and required to be ≥ 30 ng/mL leaves a gap of 20 ng/ml which should be fulfilled by vitamin D food fortification. It is known that serum levels of 25(OH) vitamin D escalates 0.5 ng/ml for each 40 IU/day intake.³⁹ This would in turn provide us a requirement of 1600 IU/day or in other words 40 μ g/day intake Pakistani populations.

Guidelines on food fortification with vitamin D

Worldwide there are enormous differences in food fortification and its regulation approaches due to difference in demand of micronutrients such as vitamin D. In many industrialized countries, it is the responsibility of law to assign rules of food fortification, however this is not the case in developing countries like Pakistan.

Rice fortification methods

Hot extrusion

Dough is made by rice flour, which is called as a fortificant mix. Water is then passed through an extruder which cuts it into grains resembling rice. A high degree temperature (70–110 °C) is involved in this process resulting in fully / semi cooked rice that have similar transparency as of regular rice. Finally this rice is mixed with polish rice and marketed.⁴⁰

Cold extrusion

Imitative rice are manufactured and passed with rice made dough, a fortification mixture and water is further added through a simple pasta press. No heat production is involved in this process. A relatively low temperature below 70°C is required consequently uncooked, opaque grain are produced which are easier to differentiate from regular rice. This rice is then mixed with polish rice and marketed.⁴⁰

Coating and dusting

Fortified mixture is combined with component like gums and waxes. This is then sprayed over the surface of grain kernels in rice-premix which is mixed and blended with retail rice and polished rice.⁴⁰

Biofortification

Biofortification is a process for improving nutritional quality of food crops through agronomic practices and modern technologies. It differs from conventional fortification as it aims to raise nutritional levels in crops than increasing nutritional level by manual practices during crop processing. It

seems to be the best approach for Pakistani population where supplementation and fortification activities are difficult to implement.⁴¹

Biofortification of staple foods

Increasing nutrient quality of staple foods by breeding or genetic means is another approach of fortification. Scientist have proved that not only different cereals, legumes are selected for biofortification due to their iron content but many varieties of vegetables like sweet potatoes and carrots seems potent for fortification due to high β -carotene levels and similarly maizes due to low phytate amount in them which improves the absorption of iron and zinc.⁴²⁻⁴⁴

Bio-fortification can be used effectively to alleviate malnutrition. Inter-Agency Report to the G20 on Food Price Volatility (FAO, 2013) and Copenhagen Consensus (2012) had proved that consuming staple food crop for biofortification is the most excellent agricultural choice across stratified income groups.⁴

Wheat varieties in Pakistan for biofortification

AARI Faisalabad wheat Institute has released 79 wheat varieties for general cultivation to the farmers of the province including, Mexipak-65, Chenab-70, Blue silver, Yecora-70, Lyallpur-73, SA-42, Pak-81, Punjab-85, Chakwal-86, Inqilab-91, Bhakhar-2002, Seher-06, Faisalabad-08, Lasani-08, Chakwal-50, BARS-09, AARI-11 and Millat-11. These all varieties are stress tolerant and suited for biofortification. Out of all of these Galaxy-13 has given best yield in 2013 and can be opted for biofortification.⁴⁶ Sweet potatoes may also be a good choice for biofortification in Pakistan through genetic engineering as it is easy accessible and low in cost.

Limitations

Unfortunately there is no study on bio-fortification methods for vitamin D in Pakistan as fortified vitamin D milk and cereals are imported in this country. There is an utmost need for testing different biofortification methods for vitamin D which might be as successful as transgenic wheat varieties with enhanced micronutrients in them.

Conclusion

The deficiency of vitamin D is rising like an epidemics in Pakistan (>80%).³⁸ It has resulted in premature births, neonatal and child bone health issues, cancer, cardiovascular diseases and many other diseases. Our country cannot afford to lose

more lives. There is an emergent need for vitamin D fortification through above mentioned protocols. This could serve as a better way towards increasing vitamin D level upto normal levels and proceeding towards better future of Pakistan.

Much work is required in the context of vitamin D fortification for proving the efficacy and effectiveness of foods. There are many concerns regarding fortified food safety, cost and environmental impact which should be kept in prime consideration.

Conflict of interest: None declared.

References

- DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; 80 (6 Suppl): 1689–96.
- Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010.
- Mattila PH, Piironen VI, Uusi-Rauva EJ, Koivistoinen PE. Vitamin D contents in edible mushrooms. *J Agric Food Chem* 1994; 42: 2449-53.
- Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr* 2004; 80(6 Suppl): 1710-6.
- Cannell JJ, Hollis BW, Zasloff M, Heaney RP. Diagnosis and treatment of vitamin D deficiency. *Expert Opin Pharmacother* 2008; 9(1): 107–18.
- Holick MF. Vitamin D: A millenium perspective. *J Cell Biochem* 2003; 88(2): 296-307.
- Khan FA. Agha khan university, Karachi. Final Pakistan food fortification scoping study April 2014. (Accessed on 15th May 2016) Available from URL: https://assets.publishing.service.gov.uk/media/57a089b5e5274a31e0000214/Final_Pakistan-Food-Fortification-scoping-study_redacted-public-proofed-april-2014.pdf.
- Holick MF. Deficiency of sunlight and vitamin D. *BMJ* 2008; 336(7657): 1318–9.
- Rajakumar K, Greenspan SL, Thomas SB, Holick MF. SOLAR ultraviolet radiation and vitamin D: a historical perspective. *AmJ Public Health*. 2007; 97(10):1746-54.
- Loomis WF. Skin-pigment regulation of vitamin-D biosynthesis in man. *Science* 1967; 157(3788): 501-6.
- Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* 1982; 1(8263): 74-6.
- Harris SS, Soteriades E, Coolidge JA, Mudgal S, Dawson-Hughes B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. *J Clin Endocrinol Metab* 2000; 85: 4125-30.
- Basic, major and common characteristics of developing countries like Pakistan. (Accessed on 20th May 2018) Available from URL:<http://ahsankhaneco.blogspot.com/2012/04/basi-c-major-and-common-characteristics>
- "Human Development Report 2009 - Population living below \$1.25 a day (%)".(Accessed on 20th May 2018) Available from URL:<http://hdr.undp.org/en/content/population-living-below-125-ppp-day>.
- Richardson DP. Food fortification. *Proc Nutr Soc* 1990; 49: 39-50.
- Panda AK, Mishra S, Mohapatra SK. Iron in ayurvedic medicine. *J Adv Dev Res* 2011; 2: 287–93.
- Byrdwell WC, DeVries J, Exler J, Harnly JM, Holden JM, Holick MF, et al. Analyzing vitamin D in foods and supplements: methodologic challenges. *Am J Clin Nutr* 2008; 88: 554S-7S.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96: 1911-30.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab* 2012; 97: 1153-8.
- Heaney RP, Holick MF. Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res* 2011; 26: 455-7.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Vitamin D. In: Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press, 1997: 250-87.
- Health Canada. Consolidation of the Food and Drugs Act and the Food and Drug Regulations. (Accessed on 16th December 2003) Available from URL: http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/e_index.html
- Health Canada. Food & Drug Act B.24.102. (Accessed on 16th December 2003) Available from URL:http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/pdf/e_d-text-2.pdf
- Health Canada. Food & Drug Act B.08.003. (Accessed on 16th December 2003) Available from URL: http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/pdf/e_b-text-1.pdf
- Health Canada. Food & Drug Act B.09.016. (Accessed on 16th December 2003) Available from URL:http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/pdf/e_b-text-1.pdf
- Health Canada. Food & Drug Act B.01.404. (Accessed on 16th December 2003) Available from URL: http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/pdf/e_b-text-1.pdf
- Ekbote VH, Khadilkar AV, Chiplonkar SA, Hanumante NM, Khadilkar VV, Mughal MZ. A pilot randomized

- controlled trial of oral calcium and vitamin D supplementation using fortified laddoos in underprivileged Indian toddlers. *Eur J Clin Nutr* 2011; 65: 440-6.
28. Khadgawat R, Marwaha RK, Garg MK, Ramot R, Oberoi AK, Sreenivas V, et al. Impact of vitamin D fortified milk supplementation on vitamin D status of healthy school children aged 10–14 years. *Osteoporos Int* 2013; 24: 2335-43.
 29. G R, Gupta A. Vitamin D deficiency in india: Prevalence, causalities and interventions. *Nutrients* 2014; 6: 729-75.
 30. Thacher TD, Fischer PR, Obadofin MO, Levine MA, Singh RJ, Pettifor JM. Comparison of metabolism of vitamins D2 and D3 in children with nutritional rickets. *J Bone Miner Res* 2010; 25: 1988-95.
 31. Hanson AL, Metzger LE. Evaluation of increased vitamin D fortification in high-temperature, short-time-processed 2% milk, uht-processed 2% fat chocolate milk, and low-fat strawberry yogurt. *J Dairy Sci* 2010; 93: 801-07.
 32. Upreti P, Mistry VV, Warthesen JJ. Estimation and fortification of vitamin D3 in pasteurized process cheese. *J Dairy Sci* 2002; 85: 3173-81.
 33. Ganesan B, Brothersen C, McMahon DJ. Fortification of cheddar cheese with vitamin D does not alter cheese flavor perception. *J Dairy Sci* 2011; 94: 3708-14.
 34. Gregory JF. Chemical changes of vitamins during food processing. In: Richardson T., Finley J.W., editors. *Chemical Changes in Food during Processing Basic Symposium Series*. Van Nostrand Reinhold Company Inc.; New York, NY, USA: 1985. (Accessed on 11th November 2011) Available from URL: http://link.springer.com/chapter/10.1007/978-1-4613-2265-8_17#page-1.
 35. Pelc B, Marshall DH. Thermal transformation of cholecalciferol between 100–170 degrees C. *Steroids* 1978; 31: 23-9.
 36. World Health Organization. Food and Agricultural Organization of the United Nations. In: *Guidelines on Food Fortification with Micronutrients*. Allen LH, Benoist B, Dary O, Hurrell R, editors. WHO; Geneva, Switzerland: 2006.
 37. Javed, R, Malik SY, Yaqub S, Ghafoor F, Asim M. Levels of 25-OH Vitamin D in Healthy Asymptomatic Adults: Pilot Study. *Pak J Med Res* 2012; 51(3): 82-6.
 38. Javed, R, Ghafoor F. A Review of Vitamin D in Pakistani Population. *Pak J Med Res* 2016. 55(2): 55-9.
 39. Black LJ, Seamans KM, Cashman KD, Kiely M. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *J Nutr* 2012; 142: 1102-8.
 40. Ritu G1, Gupta A. Fortification of Foods with Vitamin D in India. *Nutrients* 2014; 6(9): 3601-23.
 41. Biofortification of staple crops. (Accessed on 20th May 2018) Available from URL:<http://www.who.int/elena/titles/biofortification/en/>
 42. Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, et al. Golden Rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J Nutr* 2002; 132(3): 506S-10S.
 43. Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, et al. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 2000; 287: 303-5. .
 44. Lucca P, Hurrell R, Potrykus I. Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr* 2002; 21 (3 Suppl): 184-90.
 45. Biofortification: High zinc wheat programme – The potential agricultural options for alleviating malnutrition in Pakistan. *Int J Food Allied Sci* 2015; 1(1):36-9.
 46. Wheat Research Institute, Faisalabad. Annual program of research work Rabi 2015-16. (Accessed on 25th June 2018) Available from URL: https://www.google.com/url?sa=t&source=web&rct=j&url=https://aari.punjab.gov.pk/system/files/APRW%2520Wheat%25202015-16.pdf&ved=2ahUKEwilkf7IzfPbAhWNGuwKHWKQDCwQFjABegQIBRAB&usq=AOvVaw1sEwUzjSziTDkoE_bmwvt2