

## Fermented Dairy Products as Zinc Fortification Vehicle

Santosh Kumar Mishra<sup>\*1</sup>, Ramandeep Kaur<sup>1</sup> and K.K. Mishra<sup>2</sup>

<sup>1</sup>College of Dairy Science and Technology, GADVASU, Ludhiana-141004, Punjab, India

<sup>2</sup>College of Veterinary Science and A.H. (NDVSVU), Rewa, M.P., India

\*Corresponding author: skmishra84@gmail.com

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### Abstract

Zinc is second most abundant trace mineral in our body after iron. In recent years zinc is gaining attention, as its deficiency is widespread across the world. Most foods are low in zinc content therefore fortification is very stable long term approach to control zinc deficiency. Fermented milk products consumption has increased drastically in recent time due to their functional properties and high nutritive values. Fermented products having certain specific ingredients with specific health benefits could be of potential interest. Milk fermented products are very effective vehicles for zinc fortification due to their popularity in many ethical population including vegetarians who do not consume meat but they consume fermented dairy products. Zinc fortification is a fruitful strategy in order to prevent zinc deficiency especially for risk groups such as elderly, vegetarians, lactating women and children. Fermented milk foods can be served as better vehicle for zinc fortification than cereals and other plant based sources.

**Keywords:** Fermented foods; Fortification; Zinc

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Zinc is second most abundant trace mineral in our body after iron. The adult human contains 2–3 g of zinc (Maret and Sandstead, 2006). In recent years zinc is gaining attention, as its deficiency is widespread across the world. Zinc is classified as a group IIB post-transition metal which has atomic weight 65.37. It exists in divalent state ( $Zn^{2+}$ ) in biological system. As it is in  $Zn^{2+}$  state, it does not cause oxidative damage in living organisms (Shrimpton and Shankar, 2008).

Zinc is widely distributed throughout all body tissues, bones, muscles and fluids with relatively high concentration 85% in bones and muscles, 11% in skin and liver. Zinc is also essential for basic metabolic processes and cellular metabolism including catalytic, structural and regulatory functions as well as plays an important role in the immune system (Saunders *et al.* 2013; Shrimpton and Shankar, 2008). It is used in protein and nucleic acid synthesis, neurogenesis, synaptogenesis, neuronal growth,

neurotransmission for manufacturing and action of insulin and as + catalytic ion in the cytoplasm of cells. Zinc participates in the reaction at active site or gives structural strength to the enzyme, as component of enzyme known as metalloenzyme.

Recommended Dietary Allowance (RDA) is defined as the average daily dietary intake level that is sufficient to meet the nutrient requirement of all healthy individuals in particular age group and gender. According to RDA amount of zinc is 15 mg for a male adult. However, pregnant and lactating women require more zinc than others they may need as much as 19 mg a day. The average elderly person's intake is only about 9 mg a day (Bhowmik *et al.* 2010). The tolerable upper limit (UL) of zinc is 40 mg/day (Otten *et al.* 2006). It is important that the fortified food should be consumed less than UL by consumer (Allen *et al.* 2006).

### **Fortification and its need**

Most foods are low in zinc content therefore fortification is very stable long term approach to control zinc deficiency. Food fortification is a process of addition of essential components such as trace elements, vitamins, protein and other components into food whether it is present in food naturally or not for the purpose of preventing or correcting deficiency of these essential nutrients in the population or specific population. Food fortification is a more cost-effective and successful strategy to overcome micronutrient malnutrition than supplementation. Where micronutrient deficiency is widely distributed in a population and dietary modification is difficult to achieve, food fortification is an appropriate alternative (Roohani *et al.* 2013).

As defined by the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (FAO, 2006), fortification refers to “the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food irrespective of whether the nutrients were originally in the food before processing or not, so as to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk to health”, whereas enrichment is defined as “synonymous with fortification and refers to the addition of micronutrients to a food which are lost during processing”.

### **Fortification and its health benefits**

Health benefits of fortification in public are:

1. Prevents or minimizes the risk of micronutrient occurrence in population or specific population.
2. Correctness of demonstrated deficiency in micronutrients in population or specific population.
3. Improvement in nutritional status and dietary intake, as result of change in dietary habits.
4. Beneficial effect with maintaining and improving health.

### **Types of Fortification**

**Mass fortification:** Mass fortification is addition of one or more macro-nutrients to food consume by general public. It is the best strategy when large number of population has an unaccepted risk in term of being or becoming deficient in particular nutrients. This type of fortification is instigated, mandated and regulated by the government. Some examples of these fortified foods are cereal, condiments, milk.

**Targeted fortification:** Targeted fortification is fortification in which fortification is done for specific group of population by increasing the intake of particular group rather than of whole population. Examples of this type of fortification are complementary food for infants, special biscuits for children, pregnant ladies and blending food for feeding refugees.

**Market driven fortification:** In this type of fortification food manufacturer takes business initiative to add one or more macro-nutritive to processed food. This type of fortification takes place within set government regulation. It also plays an important role in improving public health by reducing the risk of macro-deficiency. Examples are minerals (iron, calcium, zinc) and vitamins (vitamins A, vitamins B). The public health impact of market-driven food interventions is limited in most developing countries. Market driven fortification is widely used in industrialized countries.

**Household and community fortification:** This approach is combination of supplementation and fortification. It is still at experimental level. One of such approach involves the addition of nutrients available in sachets, to small amount of flour during milling. Micro-nutrient based powder is a related expensive way to increase nutrients intake resulting in improving local food for infant or small children where mass fortification is not possible. But it is more expensive than mass fortification.

### **Zinc Deficiency**

According to World Health Organization major contributor of several diseases in developing

**Table 1:** Global Zinc deficiency estimation

| Regions   | West Europe | North America | East Europe | China | South-east Asia | North-East Africa | South Asia | World |
|-----------|-------------|---------------|-------------|-------|-----------------|-------------------|------------|-------|
| Zn Mg/d   | 3.2         | 2.9           | 2.1         | 1.5   | 1.1             | 1.0               | 0.8        | 1.5   |
| % at risk | 8.0         | 0.9           | 12.8        | 21.4  | 71.2            | 73.5              | 95.4       | 48.9  |

country is the zinc deficiency with high mortality rate (WHO, 2006). Global zinc deficiency estimation was recorded by WHO in 2002 is mentioned in Table 1. Zinc deficiency affects one third population of world. Based on data from Agriculture Food Balance sheets the International Zinc Nutrition Consultative Group (IZNCG) has estimated that there are 176 countries under the risk of zinc deficiency. In 1860s the importance of zinc was first recognized for the growth of plant.

The lack of zinc in the human body causes loss of appetite, smell and taste failure and other symptoms; results in decreased immunity, arteriosclerosis and anemia (Bhowmik *et al.* 2010). Zinc plays a crucial and unique role in normal development and function of cells mediating innate immunity, NK cells and neutrophils. Various studies verified that there is increase in the susceptibility toward various pathogens due to zinc deficiency and affects non-specific and specific immunity (Ong *et al.* 2014). Various consequences of zinc deficiency are mentioned in Table 1 (Salgueiro *et al.* 2000).

**Table 2:** Zinc deficiency consequences

|                              |                          |
|------------------------------|--------------------------|
| Hyperammonaemia              | Abnormal dark adaptation |
| Hipogonadism                 | Weight loss              |
| Oligospermia                 | Growth retardation       |
| Emotional disorders          | Delayed wound healing    |
| Anorexia                     | Intercurrent Infections  |
| Neural tube defects of fetus | Taste abnormalities      |
| Diarrhea                     | Alopecia                 |
| Increased abortion risk      | Altered immune response  |

Zinc has a critical role in the prevention of free radical-induced injury during inflammatory processes due to its ability to act as an anti-oxidant and stabilize membranes (Ananda, 2008). It plays an

indispensable role in males and females reproduction. Zinc deficiency also alters protein synthesis along with the nature of RNA polymerase. Hence, it leads to suppress the plasma protein levels, cellular immunity, fertility, wound healing, hair growth and growth in the absence of zinc (Jeejeebhoy, 2009). It also plays important role in normal pregnancy outcome and child growth, immune functioning. It also causes diseases that increase intestinal loss of zinc and impair intestinal absorption, inflammation bowel diseases, hemolytic anemia like sickle cell diseases (Swe *et al.* 2011). Studies in rats suggested that deficiency of zinc leads to precancerous esophageal epithelial hyperkeratosis, acanthosis, parakeratosis and hyperplasia (Zapaterini *et al.* 2012). Pregnant women, the lack of zinc may lead to fetal brain cells decreases, and affect their mental development. Children's lack of zinc will hinder their normal growth and development, detrimental to intellectual development and reproductive system health. A zinc-deficient adult males, there may lead to prostatic hyperplasia, reducing the reproductive function of the system and affect fertility Other indication of zinc deficiency involves diarrhea, high rate infections, loss of tastes, night blindness, eczema and alopecia. Principle factors for zinc deficiency are diet low in zinc content, high content of phytate, genetic disorders like acrodermatitis, enteropathica, sickle-cell anaemia, malabsorption disorders (Ahmed *et al.* 2012).

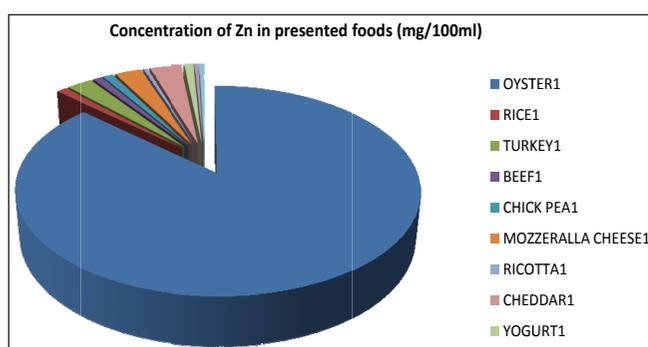
#### Excess Zinc intake

Zinc has potential to interact with many biological functions and adverse them as it required to performs numerous essential functions in our body. There may be increase in zinc concentration when exposed to high amount of zinc. Acute toxicity due high doses of

zinc occurs only in unusual circumstances. The high concentration of Zn in water or beverages could be toxic to humans, causing nausea, abdominal cramps, vomiting, tenesmus, and diarrhea with or without bleeding (Maret and Sandstead, 2006).

### Sources of Zinc

Zinc is widely distributed in different foods but different food would have different bioavailability. Oysters, red meat, flesh of mammal, fowl and fish serve as the rich sources of zinc (Fig. 1).



**Fig. 1:** Concentration of zinc in various foods (Raynal *et al.* 2008)

Legumes and cereals are the moderate source of zinc but contain high amount of phytates. Zinc from animal source is more likely to absorb than from plant origin reason being legumes, cereal grains and nuts are rich in phytate. This is the major concern for those who only depend on plant based foods. In plant seeds Phytate acts as a primary storage form of both phosphate and inositol that (Kumar *et al.* 2010). During fermentation these fermenting organisms produces phytase which breaks the phytates. Milk and most milk products are generally low in zinc content (King and Cousins, 2005). Cheese contains high amount of zinc in comparison with other milk products. In contrast to fruits, vegetable are low source of zinc although green leafy vegetable have fairly high zinc content but there is huge variation in its bioavailability. Zinc bioavailability been proposed as a predictor of the molar ratio of phytate to zinc in the diet and ratios greater than 15 have been connected with suboptimal zinc status. In order to minimize the risk of deficiency of zinc, dietary diversification

is recommended, including the introduction of food processing techniques, like fermentation, that will reduces the zinc-chelating potency of phytate and the more consumption of foods from animal sources.

### Zinc Fortification

It is estimated that approximately 2 billion people in the world are at risk of inadequate zinc intake (Song *et al.* 2010). Therefore, zinc fortification is a fruitful strategy in order to prevent zinc deficiency especially for risk groups such as elderly, vegetarians, lactating women and children. Till now study on zinc fortification clearly depicts that zinc fortification increases dietary intake of zinc and total absorption of zinc (Hess and Brown, 2009).

The important factors that will effect on fortification to be successful are:

1. Selection of food to be fortified.
2. Preparation methods of the food vehicle.
3. Cost.
4. Resistance toward inhibitory components like phytic acid, tannins, fiber, selenium and calcium of zinc absorption.
5. Stable, acceptable with no change in its sensory characteristics.
6. Adequate level of fortification agent.
7. Must not undergo change in texture, color and appearance.

Zinc is suitable for food fortification, as zinc salts like zinc sulphate, zinc oxide, zinc gluconate, zinc chloride and zinc stearate are white or colorless and having varying solubility. These salts have also attained Generally Recognized as Safe (GRAS) status from American Food and Drug Administration (FDA) (Table 3). The food fortified with zinc oxide and zinc sulphate shows good absorption in foods (Hess and Brown, 2009).

Zinc sulfate and Zinc gluconate are also used as authorized source of zinc in foods and food supplements by European Regulation (EC) N.

**Table 3:** Zinc compounds attained GRAS status

| Different Salts | Property   | References   |
|-----------------|--|--|
| Zinc oxide      | <ul style="list-style-type: none"> <li>❖ insoluble in basic pH</li> <li>❖ poorly absorbed</li> <li>❖ precipitates in liquid foods</li> <li>❖ it does not change organoleptic character of food</li> </ul>                        | Salgueiro <i>et al.</i> 2002; Salgueiro <i>et al.</i> 2000 |
| Zinc sulfate    | <ul style="list-style-type: none"> <li>❖ great solubility</li> <li>❖ even at neutral pH</li> <li>❖ has better absorbance in the human body than zinc oxide.</li> <li>❖ has sensitive to inhibitors</li> </ul>                    | Čmejlová <i>et al.</i> 2009                                |
| Zinc acetate    | <ul style="list-style-type: none"> <li>❖ absorbed at low pH</li> </ul>   | Salgueiro <i>et al.</i> 2002                               |
| Zinc citrate    | <ul style="list-style-type: none"> <li>❖ is well absorbed</li> <li>❖ has an undesired taste</li> </ul>   | Salgueiro <i>et al.</i> 2002                               |
| Zinc gluconate  | <ul style="list-style-type: none"> <li>❖ a high bioavailability</li> <li>❖ no negative effects on the sensory properties</li> <li>❖ same bioavailability, toxic effect on rats and metabolic behavior as zinc sulfate</li> </ul> | Boccio and Monteiro, 2004<br>Salgueiro <i>et al.</i> 2002  |

1925/2006. Since 1960, these two salts have been used as fortification agent in United States (Whittaker, 1998).

#### Fermented Dairy product as Fortification vehicle

Milk serves as complete and balanced source of dietary need for humans. Modern consumers are more interested in healthy food rather than being flavorful. Consumers expected that the food they eat should be healthy and able to prevent diseases. Fermented milk products consumption has increased drastically in recent time due to their functional properties and high nutritive values. Fermented products produce contain specific ingredients that would specific health benefits could be of potential interest. Milk fermented products are very effective vehicles for zinc fortification due its popularity in many ethical population including vegetarians who do not consume meat but they consume fermented dairy products. Milk Fermented foods can be served as better vehicle for zinc fortification than cereals and other plant based sources. Cereal and legumes are rich in phytate content. Phytate is phytic acid (myo-

inositol hexaphosphate) made up of inositol ring with six phosphate ester groups that are associated with salts like magnesium, calcium phytate. But myo-inositol phosphate has less than five phosphate groups do not have any adverse affect on the absorption (Lönnerda *et al.* 1989). Phytate is principle storage source of phosphorus in legumes, oleaginous seeds and cereals. In most of the cereals phytate content is confined to bran but in case of maize is located in germ (Kumar *et al.* 2010). The phytate content in cereal varies from 0.06% to 2.22% (Reddy *et al.* 2002). International Zinc Nutrition Consultative Group has recommended that the phytate: zinc ratio is used to describe the negative effect of phytate on zinc bioavailability. Hambidge and coworkers have established negative relationship between dietary phytate and zinc absorption (Hambidge *et al.* 2005).

Impact of zinc on different components of dairy products: Milk proteins consists of casein protein ( $\alpha_{s1}$  casein,  $\alpha_{s2}$  casein,  $\beta$  casein,  $\gamma$  casein,  $\kappa$  casein), whey protein ( $\beta$  lactoglobulin,  $\alpha$  lactoalbumin), lactoferrin, immunoglobulin (IgG<sub>1</sub>, IgG<sub>2</sub>, IgA, IgM), serum albumins) and milk fat globule membrane

proteins (Livney, 2010). Casein is major protein in milk constitutes 80% of milk protein. Casein micelles are made of thousands of casein subunits in conjunction with calcium phosphates. These micelles are held together via electrostatic interactions and hydrophobic interactions (Hutkins, 2006). It was observed that that 32% of the zinc in bovine skim milk was directly associated to caseins, while about 63% was bound to colloidal calcium phosphate and approximately 5% was associated with citrate. During the digestion of casein the casein phosphopeptides are formed that may have different affect on zinc absorption than casein. These phosphophate contains small peptide having phosphorylated threonine and serine residues. These phosphoserine residues appeared to be the primary Zn-binding sites in caseins and other potential binding sites are Histidine and carboxylic group (Philippe *et al.* 2005). Lönnerdal (2000) reported that casein present in milk had negative effect on zinc absorption because partially degraded casein subunits carry negative charge and thereby, binding to zinc and decreases bioavailability (Drago and Valencia, 2006). Other factors that effect on zinc absorption are reducing the pH that may change the ionization state of the phosphoserine and histidine residues leads to decrease in Zn binding. There is also small decrease in Zn binding because increasing ionic strength is may be due to the changes in the activity of Zn.

Calcium, potassium, sodium, phosphate, magnesium and the trace minerals like iron and zinc are the main sources of mineral in milk. The calcium and iron at higher concentrations interfere with zinc absorption (Salguiero *et al.* 2000). The several studies have shown that during the low intake of iron and iron fortification does not affect so much zinc absorption (Institute of Medicine, 2001; Allen *et al.* 2006). Recommended addition rate of zinc and iron is 1:1 molar ratio (Salgueiro *et al.* 2002).

Yogurt is a coalgulated milk product that has been started in the Middle East and spread all over the world. Yogurt has gain worldwide popularity particularly among children and woman as sack food or luncheon due its safe and nutritious image. Yogurt

market has dramatically increased from \$9.7 billion in 2005 to \$15.4 billion in 2010 (Heller, 2011). Yogurt is defined as coalgulated milk product by action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* from milk and milk products. Yogurt is excellent source of calcium, phosphorous, proteins, thiamine, vitamin B<sub>12</sub>, folate, niacin, magnesium fermented milk products, which are of great importance and include certain specific proteins, vitamins, bioactive peptides, organic acids and oligosaccharides (Joon *et al.* 2017; Kaur *et al.* 2017; Joon *et al.* 2018) as compare to milk. Yogurt is well known for its high digestibility and bioavailability of nutrients. It is also has several health benefits like it recommended to lactose intolerance people, weight control, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, anti-carcinogenic effect (Perdigon *et al.* 2002), lowering of blood cholesterol, anti tumor and aids in immune function (McKinley, 2005).

Moreover, now in market yogurt is available in different types of varieties on the basis of texture (liquid, set and stirred yogurt), flavor (natural, fruit, cereal, chocolate) and fat content (high fat, low fat and fat free) (Modhu *et al.* 2016). Therefore zinc fortification of yogurt can be very effective in preventing and controlling zinc deficiency due to its worldwide consumption and very high digestibility and high nutritive value.

Cheese is another fermented product which can be used as useful source for fortification. Its consumption has been increased drastically in last decades. Cheese consumption around the world is given below (IDF, ZMB, FAPRI, CNIEL, PM FOOD & DAIRY CONSULTING).

**Table 4:** Cheese consumption around the world

|               | 2000  | 2012  | 2000-2012 | 2020  | 2012-2020 |
|---------------|-------|-------|-----------|-------|-----------|
| Europe        | 7,502 | 8,870 | 18%       | 9,629 | 9%        |
| North America | 4,390 | 5,557 | 27%       | 6,950 | 25%       |
| South America | 918   | 1,642 | 78%       | 2,041 | 24%       |

|                        |       |       |     |       |     |
|------------------------|-------|-------|-----|-------|-----|
| Asia                   | 557   | 1,023 | 84% | 1,672 | 63% |
| Middle East and Africa | 1,108 | 1,840 | 66% | 2,676 | 45% |

Cheese is rich in bioactive peptides proteins, amino acids, fat, fatty acids, vitamins and minerals. It is also available in different types Fresh Cheese (Feta, cottage, cream), Semi-soft Ripened cheese (Edam, St. Nectaire, Pont L'Eveque), Soft Ripened (Camembert, Brie), Hard cheese (Cheddar, Parmigiano-Reggiano), Processed cheese (American, Velveeta). Recent research by Ahraman and Ustunol suggested that zinc content in zinc fortified cheese was 5 times more than control cheese. Zinc fortified cheese has slightly higher fat and ash content than control cheese but both have relatively similar moisture content. Therefore cheese can serve as great source for fortification.

#### Zinc fortification in staple food

Zinc fortification in cereal food staples such as wheat flour, maize flour, or rice is not yet widely accomplished, but many new flour fortification programs are being started. Currently, there are four countries— Indonesia, Mexico, Jordan and South Africa in which zinc fortification of wheat flour is mandatory. Thirteen countries (Azerbaijan, Tajikistan, Tanzania, Uganda, Uzbekistan, Vietnam, Zambia, Kazakhstan, Kenya, Kyrgyzstan, Lesotho, Mexico, Mongolia and Palestine) which have included wheat flour fortification programs of zinc voluntary. In developing countries staple food such as cereal, corn and vegetables are very useful and safe technique for enhancing the status of zinc among various groups of population (Cakmak, 2008; McDonald *et al.* 2008; Wissuwa *et al.* 2008). But there are number of factors that influence the bioavailability of zinc intake. Study conducted in human reveals two of these components exert significant impact on zinc absorption the amount of myo-inositol phosphate that is consumed with meal (Lönnerdal, 2000). The phytate content in legumes ranges from 0.17% to 9.15% distributed uniformly all over cotyledons. Roots, tubers and leafy vegetables and fruits have

low phytate content. Zinc, iron and calcium being the Phytic acid chelates metal ion leads to formation insoluble complex in gastrointestinal tract (Egli *et al.* 2004). This insoluble complex cannot be dissolved or absorbed due to absence of phytase enzymes in intestine. *In vivo* radioactive and stable isotope studies, the inhibitory effect of phytate on mineral absorption has been confirmed in which fractional absorption of iron, zinc and calcium has been reported that from the diets with a high content of phytate than from diets with a lower phytate content (Egli *et al.* 2004, Hambidge *et al.* 2005). Other factors affecting the zinc absorption are the age of consumer because children (up to 14 years) have approximately one third of the zinc absorption per day that of adults. Zinc content present in food itself affects the absorption of zinc. Besides phytate, the other factor that affects zinc absorption is the quantity of zinc ingested. As the amount of ingested zinc increases, so the efficiency of zinc absorption decreases. In general, the efficiency of zinc absorption from a diet ranges from about 15% to 35%, depending largely on the amount of zinc consumed and the presence of dietary phytate (Hambidge *et al.* 2010). The different type of protein (animal protein, milk protein, plant protein) present in food has influence on zinc bioavailability.

#### CONCLUSION

Today functional foods are typically marketed to large groups of the total population. In India iron and zinc deficiency is recognized as the major cause of anemia in tribal communities. Milk fermented products can be used as a carrier to transport multi-micronutrient to this deficient tribal society. Most of the evidence supporting the utility of micro nutrient supplementation has derived from clinical trials with very few attempts to deliver these supplements in large-scale interventions. Milk and dairy products are frequently consumed by populations and are considered as the ideal carriers in food fortification programs. However, these products are low in zinc. Therefore, it is estimated that fortification of these foods with a proper zinc salt is an effective and economic strategy to prevent zinc deficiencies.

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