Technews

National Dairy Development Board

For Efficient Dairying

July- September 2017 **Issue No. 93**

Fortification of Milk and Milk Products

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue **"Fortification of Milk and Milk Products"** It may be understood that the information given here is by no means complete.

In this issue:

- Introduction
- Vitamins in Milk and Milk products
- Stability of vitamins during Processing and storage
- Minerals in Milk and Milk products
- Fortification of Milk and Milk products
- References

INTRODUCTION

Milk is a nutritionally complete food. It is a complex biological fluid consisting of fats, proteins, minerals, vitamins, enzymes, and carbohydrate and is a good source of the daily vitamin requirement of an adult person. It contains the fat-soluble vitamins A, D, E, and K, and the water soluble B-group vitamins B_1, B_2, niacin, biotin, panthothenic acid, B_6, folate and B_{12}, and ascorbic acid (vitamin C). The soluble and colloidal phases of milk contain varying amounts of different salts (approximately 20 trace elements are found in milk, including copper, iron, silicon, zinc, and iodine). The mineral (ash) content of milk is approximately 0.7–0.8%. Some trace minerals are essential for health, e.g., iron, zinc copper, and manganese, whilst others can be toxic, e.g., aluminum, mercury, arsenic, cadmium, and lead. Deficiencies or excesses in these minerals can result in a variety of physiological symptoms. The amount of each vitamin and salts varies with stage of lactation and the animal's diet and health etc.

Processing methods have the potential to alter the stability of vitamins in milk. From a technological point of view, a number of physical and chemical factors may negatively affect nutrients' stability, either naturally present or added to food. Vitamins are sensitive to heat, light, and humidity, as well as oxidizing and reducing agents. Nutrients present in or added to liquid milk though fairly stable, are heat labile and susceptible to denaturation during processing; especially the vitamins. The nutrients lost during processing and storage can be replaced through fortification. The use of stabilized, encapsulated forms of vitamins has greatly improved the resistance of vitamins to severe processing and storage conditions.

Micronutrients in Milk and Milk products

1. Fat Soluble vitamins

The fat-soluble vitamins are retinol (vitamin A), calciferols (vitamin D), tocopherols (and related compounds, vitamin E) and phylloquinone (and related compounds, vitamin K).

Vitamin A (retinol) is the parent of a range of compounds known as retinoids, which possess the biological activity of vitamin A. In general, animal foods provide preformed vitamin A as retinyl esters. While plant foods provide precursors of vitamin A, i.e. carotenoids. Only carotenoids with a β-ionone ring (e.g. p-carotene) can serve as vitamin A precursors.

Vitamin A has a number of roles in the body: it is involved in the vision process, in cell differentiation, in growth and bone remodeling and in the immune system. Vitamin A deficiency (1665 IU/day) results in night blindness, xerophthalmia (progressive blindness caused by drying of the cornea of the eye), keratinization (accumulation of keratin in digestive, respiratory and urogenital tract tissues) and finally exhaustion and death. At excessive intake levels (>50,000 IU/day), vitamin A is toxic.

The major dietary sources of retinol are dairy products, eggs and liver, while important sources of β-carotene are spinach and other dark-green leafy vegetables, deep orange fruits (apricots, cantaloupe) and vegetables (squash, carrots, sweet potatoes, pumpkin). The richest natural sources of vitamin A are fish liver oils, particularly halibut and shark.

Vitamin A activity is present in milk as retinol, retinyl esters and as carotenes (Refer table 1). The concentration of vitamin A and carotenoids in milk is strongly influenced by the carotenoid content of the feed. Milk from animals fed on pasture contains higher levels of carotenes than that from animals fed on concentrate feeds. There is also a large seasonal variation in vitamin A concentration; summer milk contains an average of 62 mcg retinol and 31 mcg carotene per 100 g while the values for winter milk are 41 and 11 mcg per 100g of milk. Vitamin A is relatively stable to most dairy processing operations (Ref Tables 2 & 4). In general, vitamin A activity is reduced by oxidation and exposure to light.

Vitamin D (cholecalciferol and Ergocalicferol) - Unlike other vitamins, cholecalciferol (vitamin D_3) can be formed from a steroid precursor, 7-dehydrocholesterol, by the skin when exposed to sunlight; with sufficient exposure to the sun, no preformed vitamin D is required from the diet. The contribution of Vitamin D in biological functions and its deficiency results in to rickets, in which bone is inadequately mineralized, resulting in growth retardation and skeletal abnormalities. Vitamin D2 (ergocalciferol) is formed by the photo conversion of ergosterol, a sterol present in certain fungi and yeasts. Ergocalciferol was widely used for many years as a therapeutic agent.

Whole cows' milk contains only about 4IU vitamin D per 100 g and 1 litre of milk per day will supply only 10-20% of the RDA. Therefore, milk is often fortified with vitamin D.

Vitamin D originates from three sources:

1. Sunlight exposure: vitamin D_3 is synthesized directly when human skin is exposed to sunlight. This is the main source of vitamin D for most people. Commercial tanning beds and solaria that emit 2–6 % UVB radiation are also effective in mimicking sunlight in the synthesis of vitamin

D_3 in the skin, though as discussed later, they are associated with increased risk of early-onset melanoma and their prudent use is recommended by health authorities.

2. 'Natural' food sources in the diet: small quantities of vitamin D_3 occur in foods of animal origin with the richest sources being fish oils, flesh of fatty fish, eggs, and liver. Small quantities of vitamin D_2 occur in some fungi especially if they are exposed to the sun.

3. Supplements and fortified foods: vitamins D_2 and D_3 are available from supplements and fortified foods (most commonly milk, margarine, fruit juices, bread, and breakfast cereals).

As with other fat-soluble vitamins, the concentration of vitamin D in dairy products (Ref. Table 1) is increased pro rata by concentration of the fat (e.g. in the production of butter or cheese). Vitamin D is relatively stable (Ref Table 2 & 4) during storage and to most dairy processing operations. Studies on the degradation of vitamin D in fortified milk have shown that the vitamin may be degraded by exposure to light.

Vitamin E (Tocopherols and related compounds): Eight compounds have vitamin E activity, four of which are derivatives of tocopherol and four of tocotrienol; all are derivatives of 6-chromanol. Vitamin E activity can be expressed as tocopherol equivalents (TE), where 1 TE is equivalent to the vitamin E activity of 1 mg α-D-tocopherol. The biological activity of β- and γ-tocopherols and α-tocotrienol is 50, 10 and 33% of the activity of α-D-tocopherol, respectively. Vitamin E deficiency is normally associated with diseases of fat malabsorption and is rare in humans. Deficiency is characterized by erythrocyte haemolysis and prolonged deficiency can cause neuromuscular dysfunction. Extremely high doses of the vitamin may interfere with the blood clotting process. The major food sources of vitamin E are polyunsaturated vegetable oils and products derived therefrom (e.g. maragrine, salad dressings), green and leafy vegetables, wheat germ, whole-grain cereal products, liver, egg yolk, nuts and seeds.

The concentration of vitamin E in cows' milk is quite low (0.09mg per 100g). Most dairy products contain low levels of vitamin E (Ref table 1) and thus are not important sources of this nutrient. Like other fat-soluble vitamins, the concentration of vitamin E in dairy products is increased pro rata with fat content. Vitamin E is relatively stable below 100°C but is destroyed at higher temperatures (e.g. deep-fat frying) (Ref Table 2 &4). The vitamin may also be lost through oxidation during processing. Oxidative losses are increased by exposure to light, heat or alkaline pH, and are promoted by the presence of pro-oxidants, lipoxygenase or catalytic trace elements (e.g. Fe3+, Cu2+). Exogenous vitamin E in milk powders supplemented with this nutrient appears to be stable for long storage periods if the powders are held at or below room temperature.

The potential of feed supplemented with vitamin E to increase the oxidative stability of milk has been investigated, as has the potential use of exogenous tocopherols added directly to the milk fat.

Vitamin K (Phylloquinone and related compounds) the structure of vitamin K is characterized by methylnaphthoquinone rings with a side chain at position 3. It exists naturally in two forms: phylloquinone (vitamin K_1) occurs only in plants, while menaquinones (vitamin K_2)

are a family of compounds with a side chain consisting of between 1 and 14 isoprene units. Menaquinones are synthesized only by bacteria (which inhabit the human gastrointestinal tract and thus provide some of the vitamin K required by the body).

The physiological role of vitamin K is in blood clotting and is essential for the synthesis of at least four of the proteins (including prothrombin) involved in this process. Vitamin K also plays a role in the synthesis of a protein (osteocalcin) in bone. Vitamin K deficiency is rare but can result from impaired absorption of fat. Vitamin K levels in the body are also reduced if the intestinal flora is killed (e.g. by antibiotics).

Vitamin K toxicity is rare but can be caused by excessive intake of vitamin K supplements. Symptoms include erythrocyte haemolysis, jaundice, and brain damage and reduced effectiveness of anticoagulants. Whole cows' milk contains 0.4-1.8 mcg vitamin K per 100g while human milk contains about 0.2mcg per 100g. Human colostrum contains higher concentrations of vitamin K, which are necessary since bacteria capable of synthesizing vitamin K take time to become established in the intestine of the neonate. The unit operations in dairy processing are unlikely to have an effect on the stability of this nutrient.

2. Water Soluble vitamins

a. B-Group Vitamins

i. Thiamin (vitamin B_1) - Thiamin acts as a co-enzyme in the form of thiamin pyrophosphate (TPP) which is an essential co-factor for many enzyme-catalysed reactions in carbohydrate metabolism. TPP-dependent pyruvate dehydrogenase catalyses the conversion of pyruvate to acetyl CoA in mitochondria. The acetyl CoA produced in this reaction enters the Krebs cycle and also serves as a substrate for the synthesis of lipids and acetylcholine.

The characteristic disease caused by prolonged thiamin deficiency is beriberi, the symptoms of which include oedema, enlarged heart, abnormal heart rhythms, heart failure, wasting, weakness, muscular problems, mental confusion and paralysis.

Thiamin is widespread in many nutritious foods but pig meat, liver, whole-grain cereals, legumes and nuts are particularly rich sources. Milk contains, on average, 0.03 mg thiamin per 100 g. Most (50-70%) of the thiamin in bovine milk is in the free form; lesser amounts are phosphorylated (18-45%) or protein-bound (7- 17%). Most of the thiamin in bovine milk is produced by micro-organisms in the rumen and, therefore, feed, breed of the cow or season have relatively little effect on its concentration in milk. Thiamin levels in milk products are generally 0.02-0.05 mg per 100g (Ref Table 1). As a result of the growth of the Penicillium mould, the rind of Brie and Camembert cheese is relatively rich in thiamin (0.5 and 0.4 mg per 100 g, respectively). Thiamin is relatively unstable and is easily cleaved by a nucleophilic displacement reaction at its methylene carbon. Thiamin is thus more stable under slightly acid conditions. It is relatively stable during dairy processing (Ref Table 2&4).

ii. Riboflavin (vitamin B2) consists of an isoalloxazine ring linked to an alcohol derived from ribose. The ribose side chain of riboflavin can be modified by the formation of a phosphoester (forming flavin mononucleotide, FMN). FMN can be joined to adenine monophosphate to form flavin adenine dinucleotide (FAD). FMN and FAD act as co-enzymes by accepting or donating two hydrogen atoms and thus are involved in redox reactions. Flavoprotein enzymes are involved in many metabolic pathways. Riboflavin is a yellow-green fluorescent compound and, in addition to its role as a vitamin, it is responsible for the colour of milk serum.

Symptoms of riboflavin deficiency include cheilosis (cracks and redness at the corners of the mouth), glossitis (painful, smooth tongue), inflamed eyelids, and sensitivity of the eyes to light, reddening of the cornea and skin rash. Milk is a good source of riboflavin; whole milk contains about 0.17mg per l00g. Most (65-95%) of the riboflavin in milk is present in the free form; the remainder is present as FMN or FAD. Milk also contains small amounts (about 11% of total flavins) of a related compound, 10-(2'-hydroxyethyl) flavin, which acts as an antivitamin.

Riboflavin is stable in the presence of oxygen, heat and at acid pH. However, it is labile to thermal decomposition under alkaline conditions. The concentration of riboflavin in milk is unaffected by pasteurization and little loss is reported for UHT-treated milks. Loss of riboflavin in milk packaged in materials that do not protect against light can be caused by either sunlight or by lights in retail outlets. Packaging in paperboard containers is the most efficient method for minimizing this loss, although glass containing a suitable pigment has also been used. Riboflavin is more stable in high-fat than in low-fat or skim milk, presumably as a result of the presence of antioxidants (e.g. vitamin E) in the milk fat which protect riboflavin against photo-oxidation.

iii. Niacin (Vitamin B_3) is a generic term which refers to two related chemical compounds, nicotinic acid and its amide, nicotinamide, both are derivatives of pyridine. Niacin is obtained from food or can be synthesized from tryptophan (60 mg of dietary tryptophan has the same metabolic effect as 1 mg niacin). Niacin forms part of two important co-enzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are co-factors for many enzymes that participate in various metabolic pathways and function in electron transport.

The classical niacin deficiency disease is pellagra, which is characterized by symptoms including diarrhoea, dermatitis, dementia and eventually death. Large doses of niacin can cause the dilation of capillaries, resulting in a painful tingling sensation.

The richest dietary sources of niacin are meat, poultry, fish and whole-grain cereals. Milk contains about 0.1 mg niacin per l00g and thus is not a rich source of the preformed vitamin. Tryptophan contributes roughly 0.7 mg NE per 100 g milk. In milk, niacin exists primarily as nicotinamide. Niacin is relatively stable to most food-processing operations. It is stable to exposure to air and resistant to autoclaving (and is therefore stable to pasteurization and UHT treatments). Like other water-soluble vitamins, niacin can be lost by leaching.

iv. Biotin (Vitamin B_7) consists of an imidazole ring fused to a tetrahydrothiophene ring with a valeric acid side chain. Biotin acts as a co-enzyme for carboxylases involved in the synthesis and catabolism of fatty acids and for branched-chain amino acids and gluconeogenesis.

Biotin deficiency is rare, symptoms of biotin deficiency include scaly dermatitis, hair loss, and loss of appetite, nausea, hallucinations and depression.

Milk contains about 1.9 mcg biotin per 100 g, apparently in the free form (Ref Table 1). The concentration of biotin in cheese ranges from 1.4 (Gouda) to 7.6 (Camembert) mcg per l00 g. Skim-milkpowder contains high levels of biotin (20mcg per 100g) owing to the concentration of the aqueous phase of milk during its manufacture. Biotin is stable during food processing and storage and is unaffected by pasteurization.

v. Panthothenic acid (Vitamin B_5) is a dimethyl derivative of butyric acid linked to β-alanine. Pantothenate is part of the structure of co-enzyme A (CoA), and as such is vital as a co-factor for numerous enzyme-catalysed reactions in lipid and carbohydrate metabolism.

Pantothenate deficiency is rare, occurring only in cases of severe malnutrition; characteristic symptoms include vomiting, intestinal distress, insomnia, fatigue and occasional diarrhoea.

Pantothenate is widespread in foods; meat, fish, poulty, whole-grain cereals and legumes are particularly good sources. Milk contains, on average, 0.35 mg panthothenate per 100 g. Pantothenate exists partly free and partly bound in milk and its concentration is influenced by breed, feed and season. Pantothenate is stable at neutral pH but is easily hydrolysed by acid or alkali at high temperatures. Pantothenate is reported to be stable to pasteurization.

vi. Pyridoxine and related compounds (vitamin B_6)- occurs naturally in three related forms: pyridoxine (the alcohol form), pyridoxal (aldehyde) and pyridoxamine (amine). All are structurally related to pyridine. The active co-enzyme form of this vitamin is pyridoxal phosphate (PLP), which is a co-factor for transaminases which catalyse the transfer of amino groups. PLP is also important for amino acid decarboxylases and functions in the metabolism of glycogen and the synthesis of sphingolipids in the nervous system. In addition, PLP is involved in the formation of niacin from tryptophan and in the initial synthesis of haem.

Deficiency of vitamin B_6, is characterized by weakness, irritability and insomnia and later by convulsions and impairment of growth, motor functions and immune response. High doses of vitamin B_6 often associated with excessive intake of supplements, are toxic and can cause bloating, depression, fatigue, irritability, headaches and nerve damage.

Important sources of B_6 include green, leafy vegetables, meat, fish and poultry, shellfish, legumes, fruits and whole grains. Whole milk contains, on average, 0.06mg per 100g, mainly in the form of pyridoxal (80%); the balance is mainly pyridoxamine (20%), with trace amounts of pyridoxamine phosphate. In general, dairy products are not major sources of B_6 in the diet. All forms of B_6 are sensitive to UV light and may be decomposed to biologically inactive compounds. Vitamin B_6 may also be decomposed by heat. The aldehyde group of pyridoxal and the amine group of pyridoxamine show some reactivity under conditions that may be encountered during milk processing. Losses during pasteurization and UHT treatments are relatively small, although losses of up to 50% can occur in UHT milk during its shelf-life.

vii. Folate (Vitamin B_9) consists of a substituted pteridine ring linked through a methylene bridge to p-aminobenzoic acid and glutamic acid. Up to seven glutamic acid residues can be attached by y-carboxyl linkages, producing polyglutamyl folate which is the major dietary and intracellular form of the vitamin. Folate is a co-factor in the enzyme-catalysed transfer of single carbon atoms in many metabolic pathways, including the biosynthesis of purines and pyramidines (essential for

DNA and RNA) and interconversions of amino acids. Folate interacts with vitamin B_{12} in the enzyme-catalysed synthesis of methionine and in the activation of 5-methyl-H4, folate to H4 folate. H4 Folate is involved in a complex and inter-linked series of metabolic reactions.

Folate deficiency impairs cell division and protein synthesis; symptoms include megaloblastic anaemia, digestive system problems (heartburn, diarrhoea, and constipation), suppression of the immune system, glossitis and problems with the nervous system (depression, fainting, fatigue, mental confusion). Higher intakes of folate have been suggested for women of child-bearing age to prevent the development of neural tube defects in the developing fetus.

Rich dietary sources of folate include leafy green vegetables, legumes, seeds and liver. Milk contains about 6mcg folate per 100g (Ref Table 1). The dominant form of folate in milk is 5-methyl-H4 folate. Folate in milk is mainly bound to folate-binding proteins and about 40% occurs as conjugated polyglutamate forms. The concentration of folate in yogurt is about 18 mcg per 100 g, principally in the form of formyl folate. The higher level of folate in yogurt is due to biosynthesis, particularly by *Streptococcus salivarius subsp. thermophilus*, and perhaps to some added ingredients.

Folate is a relatively unstable nutrient; processing and storage conditions that promote oxidation are of particular concern since some of the forms of folate found in foods are easily oxidized. The rate of the oxidative degradation of folate in foods depends on the derivative present and the food itself, particularly its pH, buffering capacity and concentration of catalytic trace elements and antioxidants. Folate is sensitive to light and may be subject to photodecomposition. Heat treatment influences folate levels in milk. Pasteurization and the storage of pasteurized milks have relatively little effect on the stability of folate but UHT treatments can cause substantial losses. The heat stability of folate-binding proteins in milk should also be considered in the context of folate in dairy foods.

viii. Cobalamin and its derivatives (vitamin B_{12}) consists of a porphyrin-like ring structure, with an atom of Co chelated at its centre, linked to a nucleotide base, ribose and phosphoric acid. A number of different groups can be attached to the free ligand site on the cobalt. Cyanocobalamin has -CN at this position and is the commercial and therapeutic form of the vitamin, although the principal dietary forms of B_{12} are 5'-deoxyadenosylcobalamin (with 5'-deoxyadenosine at the R position), methylcobalamin (-CH,) and hydroxocobalamin (-OH). Vitamin B_{12} acts as a co-factor for methionine synthetase and methylmalonyl CoA mutase. The former enzyme catalyses the transfer of the methyl group of 5-methyl-H4 folate to cobalamin and thence to homocysteine, forming methionine.

Vitamin B_{12} deficiency normally results from indequate absorption rather than inadequate dietary intake. Pernicious anaemia is caused by vitamin B_{12} deficiency; symptoms include anaemia, glossitis, fatigue and degeneration of the peripheral nervous system and hypersensitivity of the skin.

Unlike other vitamins, B_{12} is obtained exclusively from animal food sources, such as meat, fish, poultry, eggs, shellfish, milk, cheese and eggs. Vitamin B_{12} in these foods is protein-bound and released by the action of HCl and pepsin in the stomach. Bovine milk contains, on average, 0.4 mcg per 100 g (Ref Table 1). The predominant form is hydroxycobalamin and more than 95% of this nutrient is protein bound. The concentration of B_{12} in milk is influenced by the Co intake of the cow. Vitamin B_{12} is stable to pasteurization and storage of pasteurized milks (<10% loss). UHT heat treatment, and in particular storage of UHT milk, causes greater losses. Storage temperature has a major influence on the stability of B_{12} in UHT milk. Losses during storage at 7°C are minimal for up to 6 months but at room temperature (the normal storage conditions for UHT milk), losses can be significant after only a few weeks. Oxygen levels in UHT milk do not appear to influence the stability of B_{12}.

b. Ascorbic acid (Vitamin C)

Vitamin C is an essential nutrient for man as he lacks the capacity to synthesise it. Ascorbic acid is a strong reducing agent. It involved in collagen synthesis, bone and teeth calcification and many other reactions in the body as a reducing agent.

Vitamin C deficiency causes scurvy characterised by weakness, bleeding gums and defective bone growth. It also helps in the absorption of dietary iron by keeping in the reduced form. Toxic effects of vitamin C have been reported and include nausea, abdominal cramps, and diarrhoea, urinary tract problems and kidney stones.

The richest sources of ascorbic acid are fruits and vegetables; milk is a poor source. Milk contains about 1 mg ascorbate per l00g, although reported values range from about 0.85 to 2.75 mg per 100 g. These differences reflect the fact that ascorbate levels can be reduced markedly during the handling and storage of milk. Ascorbate is readily oxidized at the pH of milk. The rate of oxidation is influenced by factors including temperature, light, and the concentration of oxygen and the presence of catalytic trace elements. Ascorbic acid is of great importance in establishing and maintaining redox equilibria in milk, the protection of folate and in the prevention of oxidized flavour development in milk. The photochemical degradation of riboflavin catalyses the oxidation of ascorbate.

At least 75% of the vitamin C in milk survives pasteurization, and losses during storage of pasteurized milk are usually minimal. However, considerable losses of vitamin C have been reported in milk packaged in transparent containers. The extent of losses during UHT treatment depends on the amount of oxygen present during heat treatment and subsequent storage, and on storage temperature.

Stability of Vitamins during processing and storage

Factors affecting the stability of Vitamins

The deterioration of vitamins can take place naturally during the processing and preparation of foods, their ingredients, particularly those subjected to heat treatment and Storage. The factors that affect the degradation of vitamins are the same whether the vitamins are naturally occurring in the food or are added to the food from external sources. Sensitivity of the vitamins to various factors is listed in Table No 3.

Stability of vitamins in Milk during processing

Besides being an excellent food, milk is an ideal medium for the growth of micro-organisms, including pathogens, which may be present in raw milk, either as a result of infections in the milking animal or pathogens introduced during subsequent handling.

To ensure a safe product of good keeping quality it is necessary to control microbial contamination. This is accomplished by heat treatment or by the conversion of liquid milk into dry powder or other products (such as indigenous dairy products, cheese, yoghurt etc.) having built in protection against spoilage.

During processing certain heat labile nutrients undergo changes and hence the nutritive value of milk may be impaired. Pasteurization and sterilization/UHT of liquid milk, before consumption, are common methods of heat processing. Effect of heat treatment and storage on various vitamins present in milk are listed in Tables 2 & 4.

Table 1. Vitamins in milk and milk products													
Products	Retinol (µg)	Carotene (µg)	D (µg)	E (mg)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	B12 (µg)	Folate (µg)	B5 (mg)	Biotin (µg)	Vit C (mg)
Cow Milk Whole	150 [a,b]		4[a]	0.09	0.04	0.17	0.1	0.06	0.4	6	0.35	1.9	1
Buffalo milk Whole	240 [a],b		7 [a]	-	0.04	0.14	0.1	-		-	-	-	1
Toned Milk	115 [a,b]		3 [a]	-	0.04	0.15	0.1	-		-	-	-	1
Cow milk skimmed	Tr,[b]		0	Tr	0.04	0.18	0.1	0.06	0.4	6	0.32	2.0	1
Buffalo milk Skimmed	Tr,[b]		0	-	0.04	0.18	0.1	-		-	-	-	1
Butter	3300 [a,b]		92 [a]	-	Tr	0.01	0.1	-	0.05	-	-	-	0
SMP	40 [a,b]		Tr	-	0.35	1.96	1.1	-	36	-	-	-	7
Ghee	3800 [a,b]		99 [a]	-	0	0	0	-	0	-	-	-	0
Cheese (Surti)	830 [a,b]		20 [a]	-	0.01	0.2	0.1	-	4	-	-	-	0.6
Skimmed Pasteurized Milk	1	Tr	Tr		0.04	0.18	0.1		0.4				1
Sterilised whole milk in containers	52	21	0.03	0.09	0.03	0.14	0.1	0.04	0.1	Tr	0.28	1.8	Tr
Evaporated Whole Milk	105	100	0.09	0.19	0.07	0.42	0.2	0.07	0.1	11	0.75	4.0	1
Cheddar Cheese	325	225	0.26	0.53	0.03	0.40	0.1	0.10	1.1	33	0.36	3.0	Tr
Edam	175	150	(0.19)	0.48	0.03	0.35	0.1	0.09	2.1	40	0.38	1.8	Tr
Gouda	245	145	(0.24)	0.53	0.03	0.30	0.1	0.08	1.7	43	0.32	1.4	Tr
Processed Cheese Plain	270	95	0.21	0.55	0.03	0.28	0.1	0.08	0.9	18	0.31	2.3	Tr
UHT, Drinking Yoghurt	Tr	Tr	Tr	Tr	0.03	0.16	0.1	0.05	0.2	12	0.19	0.9	0
Low Fat yoghurt plain	8	5	0.01	0.01	0.05	0.25	0.1	0.09	0.2	17	0.45	2.9	1
Whole Milk yoghurt Plain	28	21	0.04	0.05	0.06	0.27	0.2	0.10	0.2	18	0.50	2.6	1
Ice cream	115	195	0.12	0.21	0.04	0.25	0.1	0.08	0.4	7	0.44	2.5	1

() Estimated Values, a- Values in IU, b- Vitamin A, Tr-Traces

Table 2. Stability of Vitamins during Processing

Vitamins	Pasteurization	UHT	Sterilization (In bottles)	Pasteurization & Evaporation	Drying
Vitamins A and carotene content	No Loss	Negligible loss in > 100°C Losses of vitamin A can occur in UHT milk during its long shelf-life at ambient temperatures.		20% and carotene is not affected	56-65% Carotene 30% loss when Pasteurised, Homogenised and Spray dried
Vitamin B Complex	There is no loss of Riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin by heat treatment. B_1, B_{12}: 10-20% B_3,B_5, B_7: Stable during processing B9: <5%	Thiamine (B_1) & B_{12} 10-20% Folic Acid: 10% B_6:Negligible losses B6: 27%	B_{12}: 90% Thiamine: 35% Folic Acid: 50% B6: 20%	B1: 30-50% B_{12}: 90% (in bottle)	--
Fortified milk (Vitamin D):	1.15% Loss in pasteurization Boiling 1.45%	--	1.92%	--	--
Vitamin D	Negligible/No losses of vitamin D_2 of these heat treatments upon milk and stable in most dairy operations				
Vitamin C	10% HTST 20%: Holder/Batch	10%	50%	15%-60%	Roller Dried:30 Spray Dried:20
	Vitamin C present in fresh milk as relatively heat-stable ascorbic acid, is oxidized by dissolved oxygen to Dehydroascorbic acid which is then readily destroyed by subsequent heat-treatment and storage.				
Vitamin E	Vitamin E content of milk is not influenced by pasteurization or evaporation but a loss of 9 % may occur after drying and reconstitution.				
Vitamin K	No effect				

Table 3. Sensitivity of vitamins

Vitamins	Light	Oxidizing agents	Reducing agents	Heat	Humidity	acids	Alkalis
Vitamin A	+++	+++	+	++	+	++	+
Vitamin D	+++	+++	+	++	+	++	++
Vitamin E	++	++	+	++	+	+	++
Vitamin K	+++	++	+	+	+	+	+++
Vitamin C	+	+++	+	++	++	++	+++
Thiamin	++	+	+	+++	++	+	+++
Riboflavin	+++	+	++	+	+	+	+++
Niacin	+	+	++	+	+	+	+
Vitamin B_6	++	+	+	+	+	++	++
Vitamin B_{12}	++	+	+++	+	++	+++	+++
Pantothenic Acid	+	+	+	++	++	+++	+++
Folic Acid	++	+++	+++	+	+	++	++
Biotin	+	+	+	+	+	++	++

+ Hardly or not sensitive, ++ Sensitive, +++ Highly sensitive

Table 4 Stability of Vitamins during storage of Milk	
Vitamins A & carotene content	• Vitamin A is stable in pasteurized milk at refrigeration temperatures provided the milk is protected from light, but substantial losses can occur in milk packaged in translucent bottles. (See Table 5 for retention in different packaging materials). • Losses of vitamin A can occur in UHT milk during its long shelf-life at ambient temperatures • Added vitamin A is less stable to light than the indigenous vitamin. • The increased surface area of dried milk products accelerates the loss of vitamin A. 30% loss during 5 Months storage of dried milk at 4-15°C. • Vitamin A stable for up to 6months in sterilised milk at 4-20°C in dark, 50% loss after 6 weeks storage at 38°C.
Vitamin B Complex	• Thiamine(B_1): the storage of pasteurized milk- 10%, UHT Milk stored for 1-2 years: 20-40% • Pyridoxine (B_6): 35-50% in UHT milk during its shelf life. • Cynocobalamine (B_{12}): Losses is significant in UHT milk stored few weeks in ambient temperature and no loss at 7 for 6 months. • Riboflavin (B_2): Liquid milk exposed to light can lose between 20 and 80% of its riboflavin content in two hours, with the rate and extent of loss being dependent upon the light intensity, the temperature and the surface area of the container exposed.
Vitamin D	• Extended exposure to light and oxygen are needed to cause significant losses of vitamin D. • Vitamin D_2 is stable in milk during heat treatments (pasteurization, boiling and sterilization). • Vitamin D_2 was stable during storage at refrigerated temperature (4-7°C for 7days) in glass and plastic bottles, whereas in polyethylene pouches the loss was significantly higher.
Vitamin C	• Losses of ascorbic acid from pasteurized milk, with and without added ferrous lactate, were 35–40 %. • Decrease in vitamin C content after 3 days storage at room temperature represent 35% of the initial value, after 1 month in a 3-layered packaging material 99%, in a 6-layered packaging 51%, and after 4 months in the 6-layered packaging material 75% of the vitamin C degraded in Sterilized and UHT treated fortified milks.

Table 5. Retention (%) of Vitamins in Liquid Milk Stored in Different Containers				
Vitamin	**Containers**	**3 hr Daylight**	**72 hr Dark**	**72hr fluorescent**
A	Opaque Carton	100	100	100
	Translucent Plastic	90	100	84
B_1	Opaque Carton	100	100	100
	Translucent Plastic	96	96	100
B_2	Opaque Carton	100	100	100
	Translucent Plastic	71	100	91
C	Opaque Carton	77	79	67
	Translucent Plastic	4	80	4
Folic Acid	Opaque Carton	91	91	100
	Translucent Plastic	87	87	89

Ref: Haisman, D.R. *et al.* 1992. The effect of light on the flavor and nutritional content of milk. Food Technology. 46(2):16-19.

3. Minerals in Milk

The mineral fraction, which is a small fraction of milk (about 8–9 g/L), contains cations (calcium, magnesium, sodium and potassium) and anions (inorganic phosphate, citrate and chloride). In milk, these ions play an important role in the structure and stability. Minerals present in the milk mentioned in the table. The values are varied depending on the various factors like breed, feed etc. Thus, the calcium and phosphate contents are higher in milks rich in proteins.

Milk makes a contribution to human needs for virtually all the minerals and trace elements known to be essential for health (See Table 6). Cow's milk and milk products, such as cheese and yogurt, are very good sources of dietary calcium, Phosphorous (30-45%) Magnesium (16-21%) in western countries. These are often present in a form which is well absorbed and utilized by the body (high bioavailability), e.g., calcium and zinc.

Although the contribution to zinc requirements made by milk is relatively low. Mean zinc concentration in milk is 3.9 mg/1, but large variations in the zinc content of milk (2.0–6.0 mg/1) have been reported. There is a large decrease (50%) in the concentration of zinc in cow's colostrum during the first 3 days of lactation, with little change thereafter. Milk is not a good source of Iron and concentration decreases by 35–50% and copper by 50% during the first 3 days of lactation and remains relatively constant thereafter.

Fortification of Milk and Milk Products

Milk and milk products are a suitable vehicle for fortification

Fortification of foods with vitamins and minerals can be an effective way to combat micronutrient deficiencies. Identification of suitable vehicle for fortification is key steps in designing and consideration of program. Milk and milk products considered to be a best vehicle for micronutrient fortification (See table 7) because of its acceptance by the large group in all income group of the population.

Generally milk is best carrier because vitamin A and D are fat soluble and milk is a good source of calcium consistent with bone health messages. This has been successfully fortified with vitamins A and D for many years.

In some countries (See Table 8) the addition of vitamin D to margarine has been mandated but usually this is for reasons of achieving nutrient equivalence with butter and not as an explicit fortification intervention to compensate for a reduction in the primary source of vitamin D.

Table 6 Macro and Trace elements in Milk and Milk products									
Products	Cow Milk (Per litre)	Pasteurized skimmed	Dried Skimmed	Evaporat ed (Whole)	Conden sed (Whole)	Butter (Whole Milk)	Yoghurt	Ice Cream	Cheddar
Macro Mineral (Mg/100g)									
Sodium	430	43	550	180	140	606a	80	60	723
Potassium	1550	155	1590	360	360	27	280	174	75
Chloride	890	89	1070	250	230	994	170	110	1040
Calcium	1180	118	1280	290	290	18a	200	100	739
Phosphorus	930	93	970	260	240	23	170	91	505
Magnesium	110	11	130	29	29	2	19	12	29
Trace elements (100g)									
Iron (mg)	0.3	0.03	0.27	0.26	0.23	Trace	0.1	Trace	0.3
Copper (mg)	0.09	Trace	Trace	0.02	Trace	0.01	Trace	Trace	0.03
Zinc (mg)	4.0	0.4	4.0	0.9	1.0	0.1	0.7	0.3	4.1
Manganese (mg)	30 mcg)	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Selenium (µg)	10	1.0	11	3.0	3.0	Trace	2.0	2.0	4.0
Iodine (µg)	100-700	31	150	11	74	38	63	32	-

a Unsalted butter contains 9mg /100 g sodium and 19mg/100 g chloride.
Ref: Compiled from Encyclopedia of Dairy Science 2nd Edition

Table 7. Milk can be fortified with following micronutrients				
Nutrients	**Products**	**Liquid Milk**	**Milk Powder**	**Milk With cereal**
Vitamins	**β-Carotene**	+	+	+
	A	+	+	+
	D	+	+	+
	E	+	+	+
	B_1	+	+	+
	B_2	+	+	+
	B_6	+	+	+
	C	+	+	+
	Niacin	+	+	+
	Folic acid	+	+	+
	B_{12}	+	+	+
Minerals	**Fe**	O**	+	+
	Ca	+	+	+
	Zn	+	-	+

+- Possible; o- trials needed; - Not available
**: Milk fortification with iron occurred in Argentina.
Ref: Food fortification: A tool for fighting hidden hunger by Alberto Nilson and Jaime Piza.

Table 8. Mandatory fortification of food with nutrients in different countries		
Nutrient fortificant	Food Fortified	Country/Region
Vitamin A	Sugar	Guatemala, Honduras, Costa Rica, El Salvador, Nicaragua, Panama, Zambia, Brazil
	Dried skimmed milk for complementary food programmes	Brazil
	Skimmed milk	Guatemala
	Sterilized, pasteurized low-fat milk	Mexico
	Milk	Honduras, Mexico
	Dried milk powder	Venezuela
	Evaporated milk, condensed milk	Malaysia, Thailand, Mexico
	Filled milk	Philippines, Malaysia
	Margarine	Chile, Colombia, Denmark, Ecuador, El Salvador, Guatemala, Honduras, Peru, South Africa, India, Indonesia, Malaysia, Philippines, Turkey, Mexico
	Oil products (ghee)	Pakistan, West Africa, Brazil
	Noodles	South East Asia
	Wheat flour	Pakistan
	Monosodium glutamate	Indonesia and Philippines
Vitamin D	Dried skimmed milk for complementary food programmes	Brazil
	Skimmed milk	Guatemala
	Milk	United states, Honduras

Vitamin D	Sterilized low-fat milk, pasteurized low-fat milk, evaporated whole and low-fat milk	Mexico
	Dried milk powder	Venezuela
	Filled milk	Philippines
	Margarine	Chile, Colombia, Ecuador, Honduras, Peru, South Africa, Indonesia, Malaysia, Philippines, Turkey, Mexico
Thiamine	Wheat flour	Bolivia, Canada, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, USA, Venezuela, Indonesia
	Pasta	Chile, Guatemala
	Precooked maize flour	Venezuela
	Enriched flour	Nigeria
	Filled milk	Philippines
	Wheat flour	Australia
Riboflavin	Wheat flour	Bolivia, Canada, Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, USA, Venezuela, Indonesia
	Pasta	Chile, Guatemala
	Precooked maize flour	Venezuela
	Enriched flour	Nigeria
	Enriched maize meal	South Africa
Niacin	Wheat flour	Bolivia, Canada, Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, USA, Venezuela
	Pasta	Chile, Guatemala
	Precooked maize flour	Venezuela
	Enriched flour	Nigeria
	Enriched maize meal	South Africa
Folic acid	Wheat flour	Bolivia, Canada, Chile, Colombia, Costa Rica, Dominican Republic, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, Venezuela, USA, Canada, 20 Latin American Countries, Australia
	Precooked maize flour	Venezuela
Iron	Wheat flour	Bolivia, Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, USA, Venezuela, Peru, Indonesia
	Pasta	Chile, Guatemala
	Precooked maize flour	Venezuela
	Enriched flour	Nigeria
	Biscuits	South Africa
	Salt	India
	Sugar	Brazil
Calcium	Wheat flour	Guatemala, USA
	Enriched flour	Nigeria
Zinc	Wheat flour	Indonesia
	Sugar	Brazil
Iodine	Salt	Switzerland, Philippines, United States , Australia, India
	Wheat flour, Bread	Australia
	Biscuits	South Africa

Fortification of Processed Liquid Milk and Milk Powder

Fortification of skimmed milk with vitamins A and/or D is mandatory in several countries. In the USA, some dairies voluntarily fortify milk with vitamins C and E and calcium, in addition to vitamins A and D, and dried milk and flavored milk powders are often fortified with vitamins A and D, calcium, and iron. Importantly, milk and milk product fortification can be modified to meet the nutritional requirements of specific target groups like children or the elderly. Although vitamin D is a fat-soluble vitamin, it can be added to both "fat-rich" products, such as whole milk or cheese, and to "fat-poor" foods, such as skim milk, fat free yogurt, orange juice, etc. FSSAI operationalized the food fortification regulations in India and permitted the fortification of Vitamin A and Vitamin D in Standardized, Toned, Double toned or skimmed milk in phase I and also proposed Fortification of micronutrients Ca, Zn, Mg and Omega 3 in milk by 2019-20.

Table 9 Recommended values Milk and Milk Products Fortification in other countries

Country	Products	Vitmin A (IU)	Vitmin D (IU)
Argentina	Fluid & dry milk(whole & skim)	2,500/L	400/L
Brazil	Dry skim milk for complementary food programmes	15,000 - 25,000/kg	2000 - 2400/kg
Guatemala	Skim milk	2,000 -3,000/L	400 -600/L
Honduras	Milk	2,000/L	400/L
Malaysia	Evaporated/unsweetened Condensed milk	6,700/kg	-
Malaysia	Sweetened condensed Milk	6,700/kg	-
Malaysia	Filled evaporated/filled condensed milk	6,700/kg	-
Mexico	Sterilized low-fat Milk	4,000/L	400/L
Mexico	Pasteurized low-fat milk	4,000/L	400/L
Mexico	Evaporated whole & low-fat milk	4,000/L	400/L
Philippines	Filled evaporated/filled condensed milk	4,866/kg	(973/kg)
USA	Fortified nonfat dry milk (reconstituted)	2,115/L	425/L

USA	Evaporated milk	(4,225/L)	845/L
USA	Evaporated skim milk	4,225/L	845/L
Venezuela	Dry milk powder	4,000/L	400/L
India	Processed Milk	770/L	550/L

Chile and Argentina introduced Iron fortification of milk for many years. A number of dairies in Ireland fortified milk with vitamin D and calcium following the report of a high prevalence of hypovitaminosis D in the elderly. Most of the vitamins and minerals are added to Infant formulae to meet the nutritional and regulatory requirements.

Dairy products have been the most popular delivery vehicles for a number of functional and healthy ingredients, from vitamin and mineral fortification to addition of bioactives to promote the health benefits. Since dairy products are normal part of our diet, it is easy to understand that vitamins and minerals have been incorporated in these products.

Fortification of Cheese and Other fermented Milk products

Cheese and cheese products are permitted fortified foods in the USA. In USA Cheese and cheese products are allowed to fortify 81-89 IU/100g, Acidified milk & Cultured milk 42 IU/100g, Yoghurt 89 IU/100g with Vitamin D. In Canada, the law mandates fortification of milk (180 IU/250 mL), milk alternatives and margarine (530 IU/100 gm) and few yogurts are fortified with vitamin D. Stability of vitamin D3 during cheese processing and aging of cheddar cheese and low fat cheese was 91% and 55%, respectively Vitamin D3 exhibits >90% stability in fortified foods, including high-temperature short-time-processed 2% milk, UHT (ultra-high temperature processing; heating milk for 1–2 s, at >135°C)—processed

2% fat chocolate milk and low fat strawberry yogurt and orange juice after 30 days of storage at 4 °C. Cheese can also be enriched with Folic acid, Lycopene, magnesium, polyphenols, probiotics and omega 3 and 6 fatty acids.

Microencapsulation is a suitable technology used to deliver these nutrients to avoid losses during processing and interaction with food matrices. In Germany, enrichment of cheese with iodine through the use of iodised salt has been approved. In the high acidic and Low pH product like yoghurt vitamin A is not suitable for fortification. Iron-fortified yogurt has a relatively high iron bioavailability however, oxidized and metallic flavor observed with salts are due to the catalytic role of iron and the presence of iron, respectively. Chocolate milk was fortified by iron and had acceptable flavor properties. The tricalcium citrate can be used as calcium source in yogurts and other dairy products at concentrations of more than 1 g/L calcium. Fortifying yogurt or dairy products with fiber is of increasing interest to create functional foods with health benefits and improve their functionality.

Fortification criteria's

1. Vitamins are sensitive to heat, light, humidity and oxidizing and reducing agents. Overages must be consider based on the processing, packaging and storage conditions (Ref Table 10).
2. Suitable fortificant shall be selected based on the bioavailability of the active compound and food matrices.
3. Vitamin – Vitamin interactions: Four of the 13 vitamins have been identified as having interactions with each other with deleterious effects. These are ascorbic acid (vitamin C), thiamin (vitamin B1), riboflavin (vitamin B2) and vitamin B12.

4. Nutrients – Matrix Reactions: The availability/ phase conversion of nutrient takes place when micronutrient reacts with the food matrix resulting in non-availability of nutrient.
5. Safety Factors: Fortification shall be done as per specified Guidelines and validated data for its toxicity at higher concentrations. There should be sufficient insurance against excessive intake of the fortificant. Unlike water soluble vitamins, fat soluble vitamins exhibited toxicity at higher concentrations.

Table 10. Recommended Overages[a] (%) for Selected Nutrients and Milk Forms

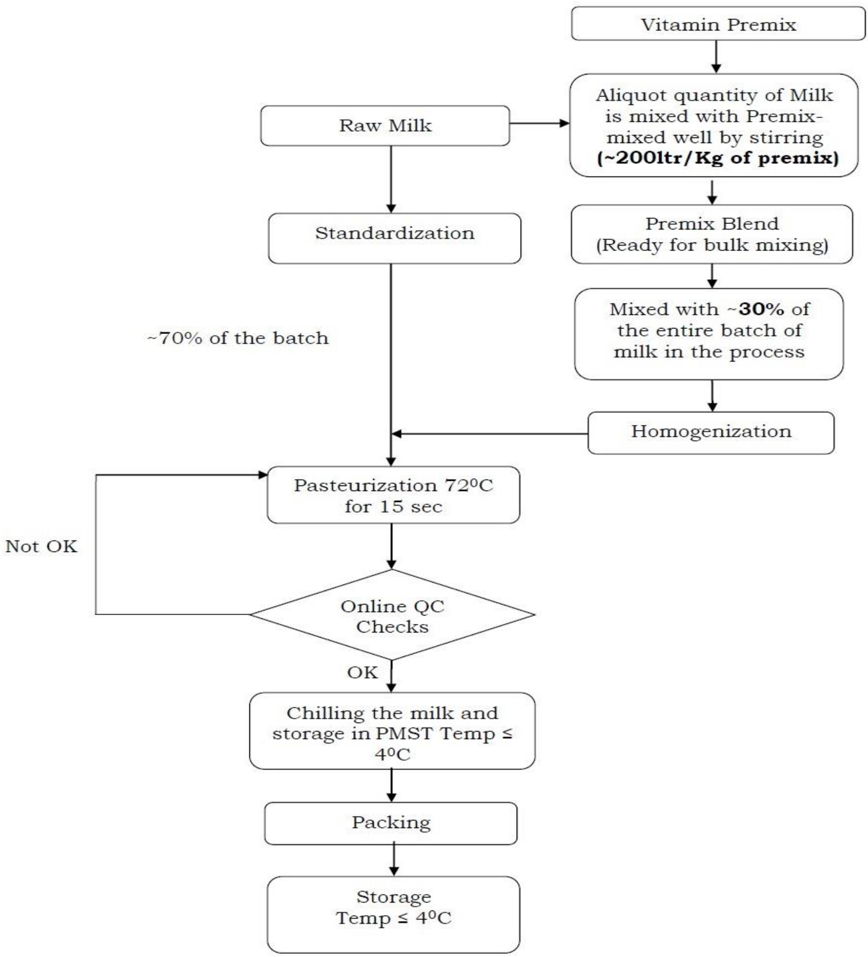
Nutrient	Pasteurized	UHT	Dry milk	Milk desserts
Vitamin A	20	30	40	20
Vitamin D	20	30	40	20
Vitamin E	10	30	20	10
Vitamin B1	25	50	20	25
Vitamin B2	15	40	20	15
Niacin	15	20	20	15
Vitamin B6	30	30	20-30	30
Vitamin B12	15	30	40	20
Folate	20	40	40	20
Vitamin C	30	100	50	30
Iron	5	5	5	5
Calcium	5	5	5	5

[a] Based only on losses during processing

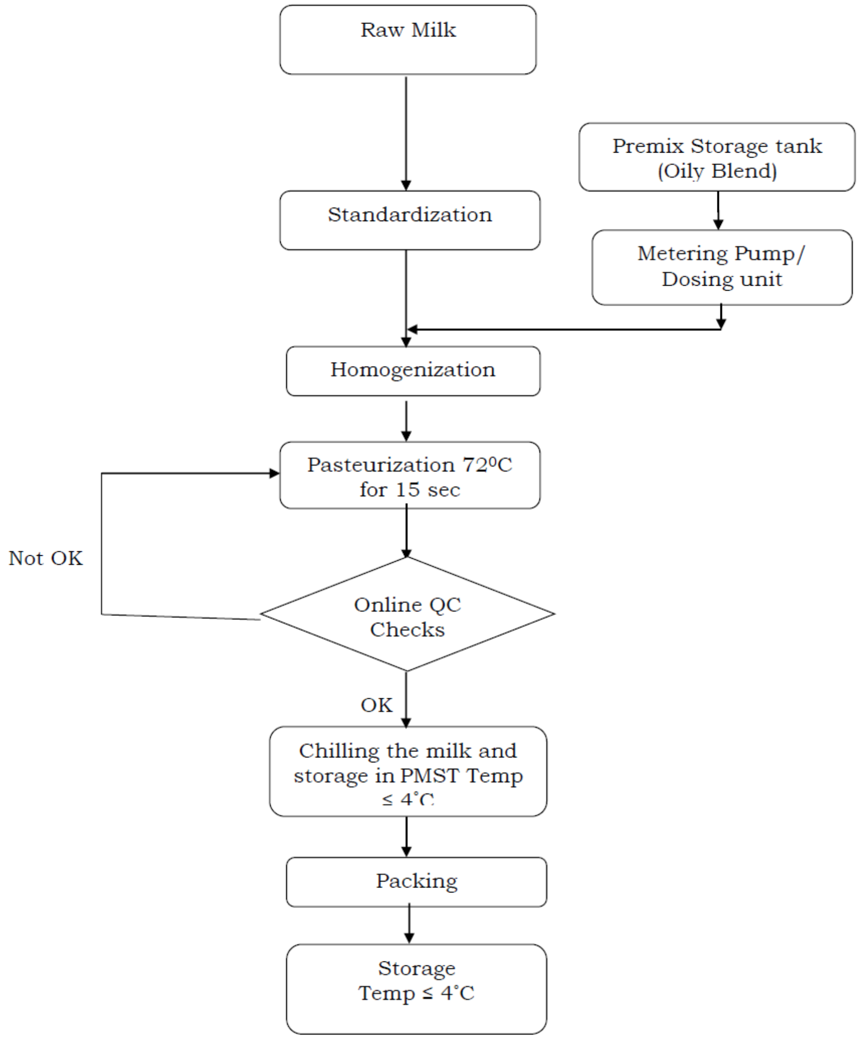
Technical considerations for Liquid Milk fortifications

Vitamin fortification can be accomplished by the addition of vitamins at various steps in the processing system, preferably after separation, including the pasteurizing vat, to the HTST constant level tank, or on a continuous basis into the pipeline after standardization and prior to pasteurization in accordance with the manufacturer's recommendations. This process covers order of processing and GMP & GHP as per process requirements for production of fortified milk and its storage.

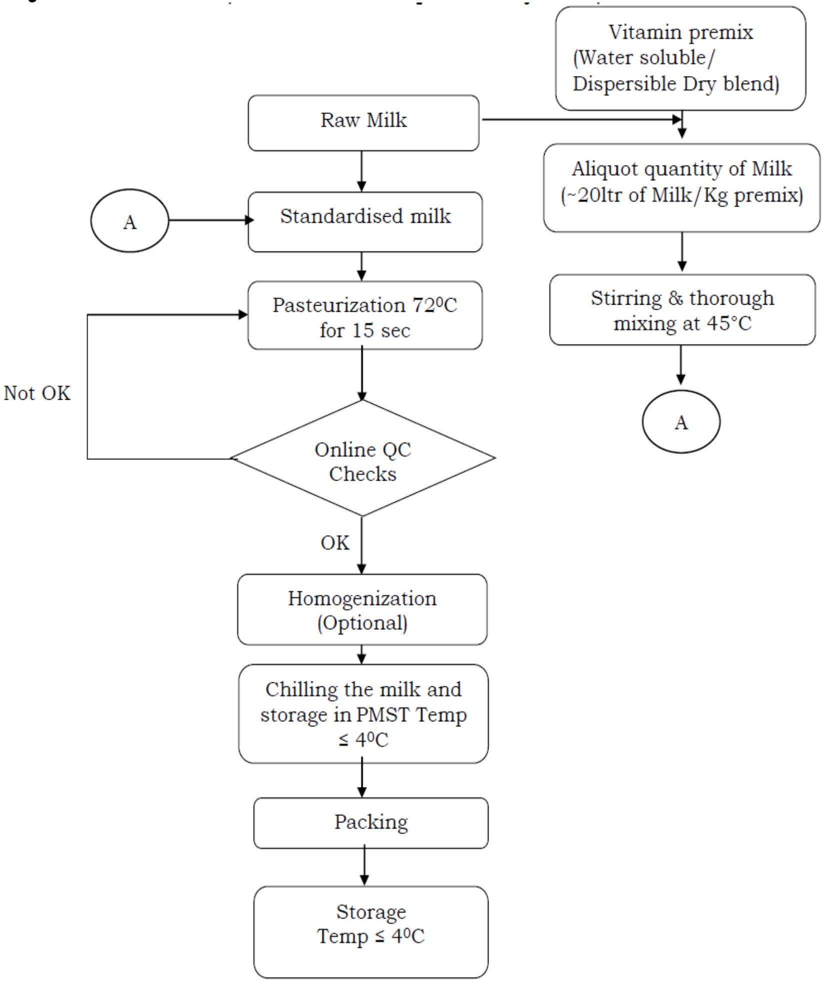
Fortification of Milk (Batch Process)



Fortification of Milk (Continuous Process)



Fortification of Milk with (Water soluble/ Dispersible Dry blend



Method of Fortification

1. Receiving fortificants and premix

1.1 Collect the required quantities of Fortificants from the store after these have been found fulfilling the QA compliance for specifications and other general requirements.

1.2 Store fortificants as per supplier's direction/ Product labelling requirements to get maximum shelf life.

2. Preparation of Premix and Processing

2.1. Batch Process

2.1.1. Take aliquot quantity of milk (~200ltr/Kg of fortificant)/ as per suppliers recommendation.

2.1.2. Care must be taken for accurate measurement of vitamins for addition and weigh required quantity, avoid add back of concentrate.

2.1.3. Mix the entire quantity by stirring, this Milk-fortificant premix is ready for bulk fortification.

2.1.4. Mix the above Milk-fortificant premix to 30% of the total batch of fortified milk to be processed.

2.1.5. Homogenize the above quantity in case of oily fortificants. Homogenization is optional for aqueous fortificants.

2.1.6. Add this homogenized premix to the total milk (rest of the 70%) up on standardization. 2.1.7. Pasteurize the entire quantity of milk by heating min 72°C/ 15 sec and immediate chilling of milk to 4°C.

2.2. Continuous Process (Oily Blend)

2.2.1. In the continuous process premix shall be stored in closed containers at suitable temperature condition.

2.2.2. The metering device/ dosing unit shall be installed after standardization step to pump the exact quantity of fortificant by adjusting its flow rate based on the level of fortificant in the final product.

2.2.3. Pump must be installed so as to be activated only when the unit is in forward flow (the pump shall not be operational during the Flow Diversion).

2.2.4. Use a check valve on the injection line to prevent milk from being pushed back into the line. This depends on the pump displacement.

2.2.5. Check the meter calibration regularly, including both the pump and the tubing, by determining delivery rate accuracy. Use only properly calibrated tubing for peristaltic pump systems and replace the tubing regularly.

2.2.6. Storage vessels used for supplying vitamin concentrate to metering pumps should be emptied on a regular basis.

2.2.7. A regular systematic cleaning and sanitizing schedule must be maintained for these vessels, pumps and tubing.

2.2.8. Homogenization of milk by applying required pressure is essentially required for uniform mixing of premix.

2.2.9. Pasteurization of milk of milk by heating min 72°C/ 15 sec and immediate chilling of milk to 4°C.

2.3.Continuous Process (Water Soluble/ Dispersible dry blend)

2.3.1. Take aliquot quantity of milk (~20ltr/Kg of fortificant)/ as per suppliers recommendation.

2.3.2. Mix the above blend properly at 45°C or as per supplier recommendation by stirring and ensure complete solubility of the dry vitamin blend.

2.3.3. Add this blend to the milk which needs to the standardized milk.

2.3.4. Pasteurize milk by heating min 72°C/ 15 sec and immediately chill the milk to 4°C.

2.3.5. Homogenization is optional in case of aqueous based mix.

2.4. Storage of milk

2.4.1. After clearance from the QA for compliance to its chemical and biochemical requirements, pasteurized milk shall be stored in dedicated pasteurized silos / storage tanks.

2.4.2. A precise quality control plan must be outlined to determine the level of fortificant(s) in the fortified milk.

2.4.3. Analyze finished products at regular intervals. Results should be reported in International Units for vitamins.

General Requirements for Premix storage and Handling

1. Vitamins are sensitive to heat, light, humidity and oxidizing and reducing agents.
2. Customize the quantity of concentrated vitamins/ premix based on the batch size, it is recommended to use entire quantity of premix up on removing from the container.
3. Assay of premix/vitamin concentrates shall be ensured periodically for bulk containers.
4. The amount of vitamin concentrates used must be recorded and cross-referenced with the amount of product fortified to ensure that the actual amount of concentrate used closely matches what is required for the total product made.
5. The stability of vitamins needs to be monitored as per supplier's directions during storage.
6. The premix shall be stored in amber coloured / opaque bottles in cool and dry place, avoid exposure to direct sunlight.

7. In case of products fortified, vitamin overages may be added appropriately to compensate for the loss during prolonged storage.

Fortified Milk testing for Vitamin A & D

Quantifying vitamin A & D in fortified milk is expensive and needs special analytical skills. The test procedures are lengthy, require sophisticated equipment including analytical grade (AR) reagents and Skilled and Trained personnel. The method based on HPLC can be used by external, well equipped / sophisticated analytical laboratory. However there are some colorimetric methods which can be performed at Dairy QC/QA laboratory (detailed procedure mentioned below). Nowadays Kit/ ELISA methods are also available for analysis of Vitamin A and D at field level.

Colorimetric methods of analysis- Vitamin A

1. Neeld and Pearson method (1963)

Principle:

The sample is saponified with ethanolic potassium hydroxide solution and vitamin A is extracted into petroleum ether. The unsaponified fraction which is obtained after boiling contains vitamin A. This is extracted thrice with petroleum ether. The pooled petroleum ether is washed with aqueous KOH and then with water to remove excess alkali. The ether extract is dried and the residue obtained is dissolved in chloroform and triflouroacetic acid (TFA) and optical density is measured at 620 nm.

Reagents: 60% Potassium hydroxide solution, Ethanol, Petroleum ether (boiling range 40°C to 60°C), 0.5 N potassium hydroxide, Chloroform, Anhydrous sodium sulphate, All-*trans*-retinol, Pyrogallol, Triflouroacetic acid

(TFA), TFA reagent: Mix 1 part TFA and 2 parts chloroform (v/v).

Laboratory Equipment's:

1. Spectrophotometer –Range 620nm
2. Rotary evaporator or low temperature vacuum evaporator

Preparation of Standards:

Prepare stock solution of vitamin A (Retynyl palmitate) (20 µg/ml) in ethanol. From this stock solution, make solutions of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µg/ml in chloroform (required IU/µg to be prepared based on the label claim).

Vitamin conversion

1IU Retinyl palmitate = 0.55 µg (mcg)

1IU Retinyl acetate = 0.344 µg (mcg)

Extraction of samples

- Take 10-20 ml milk in a 50 ml stoppered test tube.
- Add 5 ml of absolute ethanol containing 0.1% (w/v) ascorbic acid or 1% pyrogallol (w/v) and 2ml of 60% KOH.
- Agitate the tubes carefully and place in a water bath at 80°C for 20 min.
- After saponification, cool the tubes with running water and place in an ice water bath.
- Add 10 ml petroleum ether (40-60°C) and shake for 15 minutes.
- Transfer the upper ether layer in another tube. Repeat the extraction thrice and collect the ether portion.
- Transfer the combined ether extract to a separating funnel, wash with 10 ml of 0.5 N KOH and subsequently with distilled water (2-3 times) to remove excess alkali.

- Pass the ether extract through phase separator filter paper to remove water, if any.
- Evaporate the ether extract under nitrogen in a water bath maintained at 37°C.
  (Note: Perform all the extractions under subdued incandescent light using amber colored glassware.)
- Dissolve the residue in known volume of chloroform.
- Take 2 ml of sample/standard solution in a test tube and add 2 ml of TFA reagent. Blue color is developed which is not very stable. Immediately measure the optical density at 620 nm in a spectrophotometer.
- Plot the standard curve and find out the concentration of vitamin A in the sample.

2. Carr-Price method (1926)

Principle:
Estimation of vitamin A is based on the reaction of preformed vitamin A with antimony trichloride (Carr-Price reaction). When a solution of antimony trichloride in chloroform is added to a dilute solution of a vitamin A containing sample, a blue colour appears which soon reaches a maximum intensity and then rapidly fades or changes to reddish brown or other colours, depending on the nature of sample. Under carefully controlled conditions, the blue colour persists long enough to make accurate readings possible. A comparison of the blue colour of the unknown, with colour formed by a standard solution of vitamin A, is used to determine vitamin A in unknown sample.

Reagents: Chloroform, 95% ethyl alcohol, antimony trichloride - 25% solution in dry chloroform (discard if solution is turbid), KOH solution - 50% in distilled water, vitamin A standard - 0.1 g of vitamin A acetate, anhydrous sodium sulphate, diethyl ether, 0.5 N KOH in distilled water.

Procedure:

In case of dairy products containing fat, saponification and extraction is necessary, for vitamin A estimation.

- Weigh 0.lg of standard vitamin A acetate and 1g of milk fat/milk powder. Transfer these separately to two saponification flasks.
- To each, add 40 ml ethyl alcohol and 7 ml of 50% KOH. Reflux in boiling water bath for 30 minutes.
- Cool and add 30 ml of distilled water. Mix thoroughly and transfer into a separating funnel.
- Extract thrice, with 50 ml portions of ether and discard aqueous phase.
- Combine the ether extracts (these contain vitamin A) in another separating flask; wash with 100 ml water followed by 50 ml of 0.5 N KOH.
- Again wash with 100 ml portions of water till the washing give no color with phenolphthalein.
- Remove moisture from the ether extract by adding 5-10g of anhydrous sodium sulphate and allowing it to settle. Thereafter, decant the ether extract carefully into another flask. Rinse the first flask with ether to remove any traces and add this wash to ether extract.
- Evaporate the extract to dryness. Dissolve the residue obtained in 100 ml of chloroform.
- Dilute 1ml of this solution to 10 ml with chloroform to get final concentration of 0.1 mg/ml for standard. For sample, dilution may be carried out depending upon the concentration.
- To 1ml of the sample/standard chloroform solution, add 9 ml of antimony trichloride solution.

- Read the blue colour obtained at 620 nm within 4 seconds. This can be done by adding antimony trichloride just when taking colorimetric reading.
- From the readings of standard, equate the values mathematically to determine concentration of vitamin A in the sample.

HPLC Methods

Vitamin A- AOAC 2011.07 & AOAC 2012.10.

Vitamin D- AOAC 995.05, AOAC 2011.11 & AOAC 2002.05, 20th Edn., 2016.

The above methods of estimating Vitamin A and D in milk can be applicable at analytical laboratory, since needs skilled manpower and sophisticated analytical instruments.

Frequency of Testing

Premix/ Fortificant

- Vitamin premix for fortification –Ensure that premix supplied manufactured of single lot/Batch.
- Fix the premix batch size with the supplier and get the Certificate of Analysis (COA) for each lot of consignment supplied.
- Each lot of premix shall be sent to external laboratory for analysis.
- Assay of premix/vitamin concentrates shall be ensured quarterly for bulk containers have longer storage.

Fortified Milk

- The final fortified milk shall be tested for Vitamin A and D as per sampling plan mentioned in the Quality Manual/SOP.
- During initiation of the project random sample may be sent to external laboratory for quantification of vitamin A and D for validation of operations and assuring the label claim.

References

1. Vitamins in milk and dairy products, Chapter 6, Dairy Chemistry and Biochemistry by P.F.Fox and P.L.H. McSWEENEY published by Blackie Academic and Professional (1998).
2. R.K Sharma, Effect of heat treatment on the nutritive value of milk. Indian Dairy man 32, 8, 1980. PP 619-621.
3. Chemistry of processing, Chapter by Charles V. Morr and Ronald L. Richter in Fundamentals of dairy chemistry, Third edition by Nobel P Wong and Robert Jennes (1988).
4. Ravinder Kaushik , Bhawana Sachdeva , Sumit Arora, Vitamin D2 stability in milk during processing, packaging and storage, LWT - Food Science and Technology 56 (2014) 421-426.
5. Nutritional evaluation of Food Processing, Third edition, edited by Endel Karmas and Robert S. Harris Published by Van Nostrand Reinhold Company, New York (1988).
6. Andrea steskova, Monika Morochovicova and Emilia Leskova, Vitamin C degradation during storage of fortified foods. Journal of Food and Nutrition Research, Vol. 45, No. 2, (2006), pp. 55-61.
7. Dietary Importance, Chapter by J Buttriss in Encyclopedia of Food science and Nutrion, Academic Press, (2003).
8. Milk in Human Health and Nutrition, Chapter by S Patton in Encyclopedia of Dairy Science, Second edition, Academic Press, (2011).

9. Food Fortification and Supplementation – Technological, Safety and Regulatory aspects, Edited by Peter Berry Ottaway, Published by Woodhead Publishing Limited, (2008).
10. Alberto Nilson and Jaime Piza, Food fortification: A tool for fighting hidden hunger, Food and Nutrition Bulletin, vo. 19, no. 1, (1998), The United Nations University.
11. Staple food fortification to improve public health- brochure by DSM.
12. Fortification Basics-Milk- brochure by DSM.
13. Fortification Basics-Choosing a vehicle- brochure by DSM.
14. Szajnar K., Znamirowska A., Kalicka D., Zaguła G. Fortification of yoghurts with calcium compounds. J. Elem., 22(3): 869-879 (2016).
15. H. Hashemi Gahruie et al. Scientific and technical aspects of yogurt fortification: A review Food Science and Human Wellness 4 (2015) 1–8.
16. Richa Pritwani, Pulkit Mathur, Strategies to Combat Micronutrient Deficiencies: A Review International Journal of Health Sciences & Research Vol.5; Issue: 2; (2015).
17. Mona S Calvo, Susan J Whiting, and Curtis N Barton, Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr (2004); 80 (suppl):1710S– 6S.
18. Proceedings of the national summit for food fortification-FSSAI, October (2016).
19. Technical Specifications for Fortified UHT milk for Palestine, Version-V14.0 Date of issue: 28 April (2014).

20. Monique Lacroix et al., Cheese Fortification, Chapter 6 in Handbook of Food Fortification and Health From Concepts to Public Health Applications, Volume 2, Humana Press, (2013).
21. D. M. Graham, Alteration of Nutritive Value Resulting from Processing and Fortification of milk and Milk Produds, Journal of Dairy Science Vol. 57, no. 6 pp 738-745 (1974).
22. National Dairy Development Board, Standard Operating Procedures: Liquid Milk Fortification, Document No: NDDB/FC/01 and 1A Version 01 (2017).
23. National Dairy Development Board, Standard Operating Procedures: Fortified Milk Testing, Document No: NDDB/FC/01B Version 01 (2017).
24. Handbook of food analysis Part XI, Dairy Products-BIS (1981).
25. Determination of Fat Soluble Vitamins in Milk, Chapter 13 in Handbook of Milk analysis, Published by National Dairy Research Institute. pp 84-96 (2008).

Fortification of Milk and Milk Products

I find this bulletin:

Useful ☐ Informative ☐

Only entertaining ☐ Boring ☐

I think the format of this bulletin needs/does not need change.

I would like information in any subsequent issue on --------

Please send your letters to:

Dr. DK Sharma
General Manager QA & PPD

National Dairy Development Board
Post Box No. 40
Anand 388001
Gujarat.

Fax. No. +91(2692) 260-157
Email: dksharma@nddb.coop /
FSQ-milkcoops@nddb.coop