CC – 12, Unit- 6, (Part- 1) Food Chemistry

Water soluble Vitamins – structure, function and stability

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Vitamins are a diverse group of organic compounds that are nutritionally essential micro-nutrients. They are present in small amounts in foods. They are generally susceptible to change in structure and stability when the environmental conditions are not favorable. Losses of vitamins occur during processing, handling, packaging, exposure to heat, pH, presence or absence of oxygen and cooking. In our system, they act as co-enzymes and pre-cursors of many vital reactions, antioxidants against free-radicals and are very essential for overall health. When the body is deficient in one or more vitamins they lead to diseases. To know more about the importance of water soluble vitamins, the session is divided into five major sub-divisions namely:

1) Water soluble vitamins, functions and their forms

2) Structure of water soluble vitamins

3) Stability of water soluble vitamins

4) Bioavailability of water soluble vitamins

5) Other vitamin-like compounds

1) Water soluble vitamins, functions and their forms: Among the vitamins; ascorbic acid, thiamin, riboflavin, niacin, vitamin $B_{6,}$ folic acid, biotin, pantothenic acid and vitamin B_{12} are classified as water soluble vitamins. The sources of vitamins in general are from plants, animals, micro-organisms and fortified food products. The common feature of these vitamins is their solubility in water. Most of the water soluble vitamins cannot be stored in the body and hence their regular intake is of utmost importance.

1.a *Thiamin*: Different forms of thiamin are free thiamin, thiamine monophosphate and thiamine pyrophosphate or thiamine diphosphate, thiamine triphosphate, adenosine thiamine triphosphate and adenosine thiamine diphosphate. Thiamin pyrophosphate is the active form. Their activation requires phosphorylation by kinases into the diphosphate and triphosphate esters. Thiamin pyrophosphate is essential for the conversion of pyruvate to acetyl CoA to generate energy. It is a co-enzyme for transketolase in the oxidation of glucose by the hexose monophosphate shunt. Most of the circulating thiamin is bound to proteins such as albumin and erythrocytes. Their absorption is by active transport or passive diffusion.

1.b *Riboflavin:* It is essential in tissue respiration, growth and is a transporter of hydrogen ions. Different forms of riboflavin in the system are free riboflavin, flavin adenine dinucleotide or FAD and flavin adenine mononucleotide or FMN. The co-enzyme forms of riboflavin that is

FAD and FMN are the active forms which involve the action of flavokinase. These forms are essential in the initial oxidation of fatty acids and several intermediates of glucose metabolism. Their activation requires hydrolytic cleavage of free riboflavin from its flavoprotein complexes by various phosphatases. As thiamin, riboflavin is also bound to plasma proteins such as albumin. Its absorption is by a carrier mediated process which requires adenosine triphosphate (ATP).

1.c *Niacin:* It is essential in glycolysis, fat synthesis and tissue respiration. The different forms of niacin are nicotinic acid and nicotinamide. It is very essential for energy production and metabolism of cells. It is part of the pyridine nucleotide coenzymes nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) which facilitate transport of hydrogen. These molecules are co-substrates for more than 200 enzymes involved in metabolism of carbohydrates, amino acids and fatty acids. It can be synthesized from tryptophan. Most of the foods have NADH and NADPH which undergo digestion to release the absorbable forms nicotinamide and nicotinic acid. These forms are absorbed in the intestine by carrier mediated facilitated diffusion. Niacin in the plasma is not bound to any molecule and is taken up by tissues through passive diffusion. Few tissues have transport system for nicotinic acid. In the tissue, it is re-converted to NADH and NADPH.

1.d *Pantothenic acid:* It is essential in the intermediary metabolism of carbohydrate, fat and protein. It is an integral part of co-enzyme A (CoA) which is involved in energy production from the macronutrients and acyl carrier protein (ACP) which is used in synthesis reactions. CoA is important in energy metabolism for pyruvate to enter the tricarboxylic acid cycle (TCA cycle) as acetyl-CoA, and for α-ketoglutarate to be transformed to succinyl-CoA in the cycle The CoA and ACP forms are found in foods and their absorption requires hydrolysis to phosphopantetheine and later the conversion to pantothenic acid. It is absorbed by passive diffusion and active transport in the jejunum. It is transported in its free acidic form in the plasma and taken up by diffusion into erythrocytes. Later the peripheral tissues take up pantothenic acid by a sodium dependent active transport and in few tissues by facilitated diffusion.

1.e *Pyridoxine or Vitamin B_6:* It comprises of three forms; the alcohol derivative pyridoxine, the aldehyde derivative pyridoxal and the amine pyridoxamine. Pyridoxal phosphate is active form. It is formed from pyridoxal by phosphorylation catalyzed by pyridoxal kinase, however the absorption into the jejunum and ileum is in their de-phosphorylated form. Pyridoxal phosphate are bound to protein and act as prosthetic group or co enzyme of enzymes which are involved in transamination, decarboxylation, transsulfuration, desulfuration and non-oxidative deamination reactions, primarily in metabolism of proteins. Pyridoxal phosphate is coenzyme for enzymes that are involved in the synthesis of heme, neurotransmitters, catecholamines and coenzyme A synthesis. It is essential in the biosynthesis of niacin from tryptophan and unsaturated fatty acids from essential fatty acids.

1.f *Folates:* Dietary folates are absorbed as monoglutamate methylated forms which are 5-methyltetrahydrofolic acid and 5-formyltetrahydrofolic acid. Absorption occurs at the jejunum by active transport or by passive diffusion when ingested in large amounts. Dietary folates exist as polyglutamyl form with several glutamate residues whereas folic acid, the synthetic form is a oxidized monoglutamate form. Polyglutamates are hydrolyzed by conjugases to corresponding monoglutamates for intestinal absorption. Once they are absorbed, they are converted into 5-methyltetrahydrofolate which is a major circulating form of folates in the body. The different forms of tetrahydrofolic acid act as enzyme co-substrates in synthesis reactions in the metabolism of amino acids such as methionine, formation and maturation of red and white blood cells; and for biosynthesis of RNA and DNA by single carbon atom donors or acceptors. It is vital during early fetal development.

1.g *Vitamin B_{12}:* Vitamin B_{12} functions in two metabolically active forms adenosylcobalamin and methylcobalamin, however other forms are cyanocobalamin and hydroxycobalamin. They play a major role in metabolism of propionate, amino acids and single carbons. They are synthesized by the microflora of the gut but cannot be absorbed. A deficiency causes impaired cell division through arrested synthesis of DNA therefore they are essential for the biosynthesis or nucleic acids and nucleoproteins. In the food system, it is usually bound to proteins and is released by the action of pepsin. It combines with cobalophilins in the stomach and moves to small intestine. The cobalophilins detach from cobalamin by hydrolysis. Cobalamin later gets bound to intrinsic factor which is crucial since majority of B_{12} is absorbed by this active transport.

1.h *Biotin:* Biotin in foods is largely bound to proteins and are released by proteolytic digestion to yield free biotin, biocytin or biotiny peptide. By the action of biotinidase, biotin is released from biocytin and biotiny peptide. Free biotin is absorbed in the proximal small intestine primarily by carrier-mediated diffusion and transported into the plasma. Biotin is mainly a carboxyl carrier and acts by addition or removal of CO_2 and is involved in the gluconeogenesis and fatty acid synthesis. It removes the NH_2 group from amino acids.

1.i *Ascorbic acid:* Ascorbic acid or vitamin C functions in oxidation-reduction reaction. Humans receive ascorbic acid from their diet which is absorbed by active transport and passive diffusion. Two forms of vitamin C exist in the system, dehydroascorbic acid which is the oxidized form and the reduced form ascorbate or ascorbic acid, the former is better absorbed. It is involved in the synthesis and production of collagen, neurotransmitters serotonin and norepinephrine, interferons conferring immunologic potential and carnitine. It is an efficient antioxidant and prevents oxidative damage. It converts ferric to ferrous state of iron thus facilitating iron absorption overall and from the non-heme sources. It is also important in wound healing.

2) Structure of water soluble vitamins:

2.a *Thiamin:* It is named as the thio-vitamine or sulphur containing vitamin. It is a colorless organosulphur compound with the chemical formula $C_{12}H_{17}N_4OS$. Its structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge. The thiazole is substituted with methyl and hydroxyethyl side chains as displayed on the screen (Figure 1).

2.b *Riboflavin:* The name is derived from ribose which is a sugar and flavus which means yellow. The ring moiety flavin imparts yellow color. Riboflavin appears as yellow orange crystals. The molecular formula is $C_{17}H_{20}N_4O_6$. Riboflavin has 3 benzene rings and 6 tetrahedron bond shapes as displayed on the screen (Figure 2).

2.c *Niacin:* Niacin is a colorless compound which is a derivative of pyridine with a carboxylic acid at C-3 position. Nicotinamide has a carboxamide or $CONH_2$ instead of carboxylic acid. The molecular formula of niacin is $C_6 H_5NO_2$ (Figure 3).

2.d *Pantothenic acid:* It is the amide between pantoic acid and β-alanine. The molecular formula of pantothenic acid is $C_9H_{17}NO_5$ (Figure 4).

2.e *Pyridoxine:* By replacing the $-CH_2OH$ group on position 4 of the pyridoxine molecule with $-CH_2NH_2$ and -CHO respectively, two related compounds, pyridoxamine and pyridoxal can be formed which are also vitamin active. The molecular formula of pyridoxine is $C_8H_{11}NO_3$ (Figure 5). .

2.f *Biotin:* Biotin in nature appears as white crystalline needles. It is composed of an ureido (tetrahydroimidizalone) ring fused to a tetrahydrothiophene ring with a valeric acid side chain. Tetrahydrothiophene is a five membered ring containing four carbon atoms and one sulphur atom. The molecular formula is $C_{10}H_{16}N_2O_3S$ (Figure 6).

2. g *Vitamin B_{12}:* The molecular formula of Vitamin B_{12} is $C_{63}H_{88}CoN_{14}O_{14}P$. The structure of B_{12} is based on a corrin ring. The ring consists of 4 pyrrole subunits, joined on opposite sides by a $C\text{-}CH_3$ methylene link, on one side by a C-H methylene link, and with the two of the pyrroles joined directly. It is thus like a porphyrin, but with one of the bridging methylene groups removed. The nitrogen of each pyrrole is coordinated to the central cobalt atom. The central metal ion is cobalt. Four of the six coordination sites are provided by the corrin ring, and a fifth by a dimethylbenzimidazole group. The sixth coordination site, the center of reactivity, is variable, being a cyano group (-CN), a hydroxyl group (-OH), a methyl group ($-CH_3$) or a 5'-deoxyadenosyl group (here the C5' atom of the deoxyribose forms the covalent bond with Co), respectively, to yield the four B_{12} forms; cyanocobalamin, hydroxocobalamin, methylcobalamin and adenosylcobalamin respectively. The structure of B_{12} is the most complex of all vitamins (Figure 7).

2.h *Folate:* The molecular formula of folate is $C_{19}H_{19}N_7O_6$. Folic acid consists of pteridine nucleus, p-aminobenzoic acid and glutamate. In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one-carbon forms of tetrahydrofolate (e.g., 10-formyl, 5,10-methylene, and 5-methyl). The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and *p*-aminobenzoylglutamate, which have no biologic activity (Figure 8).

2.i Vitamin C: L-ascorbic acid is a carbohydrate like compound whose acidic and reducing properties are contributed by the 2,3-enediol moiety. It is highly polar and readily soluble in aqueous solution. Ascorbic acid contains two optically active isomeric forms, C-4 optical isomer D-ascorbic acid and C-5 optical isomer L-isoascorbic acid which is also known as erythorbic acid. The molecular formula is $C_6H_8O_6$ (Figure 9).

3) Stability of water soluble vitamins:

Physical methods: Processing such as milling and refining has shown decrease in water soluble vitamins such as pantothenic acid, riboflavin, biotin, niacin, thiamin, folic acid and vitamin B_6 which are concentrated in the germ and bran of the grain. Polishing of grains significantly reduce B-vitamins. They are easily washed out during food storage and processing. Refrigeration can minimize the loss of water soluble vitamins. Preliminary treatments such as trimming and washing of fruits and vegetables cause loss of vitamins present in the peel, skin or the discarded inedible part of the food. Cuts, damage, washing and cooking will leach water soluble vitamins.

Temperature: Thermal processes such as blanching which is an essential step during processing of fruits and vegetables to inactivate enzymes and to reduce microbial load on the surface has shown larger losses of water soluble vitamins. Canning which involves blanching as the preliminary step has shown loss of folate, pantothenic acid, thiamin, vitamin C, riboflavin and niacin. Thiamin is heat sensitive in neutral and alkaline foods and is unstable in air. High temperature short time retains vitamins than low temperature long time. Microwave heating has the potential for a greater retention of heat-labile vitamins compared with other more conventional methods because the heating time is shorter. Retention of vitamin C, B_1, B_2 and B_6 is higher in microwave heating than in conventional heating method. Freezing does not cause loss of vitamins.

Moisture: Water activity (a_w) greatly influences the stability of vitamins. Higher the water activity greater is the degradation rate. The process employed, temperature and exposure time decides the extent of loss of water soluble vitamins in dehydrated foods. Direct contact of the food material to the heated surface causes higher loss of water soluble vitamins than indirect methods such as spray drying.

Oxygen: Vitamins sensitive to oxidation such as ascorbic acid, folate and thiamin are lost during drying of foods.

Chemicals: Chemicals such as chlorine, sulphites, nitrites interact with vitamins by oxidation and reduction to inactivate water soluble vitamins. Sulfite ions directly react with thiamin causing inactivation. Sulfites also react with carbonyl groups and convert active vitamin B_6 aldehydes to their inactive sulfonated derivatives. Ascorbic acid prevents formation of N-nitrosamine in nitrite treated meat. The stability of ascorbic acid and thiamin is higher at lower pH and higher pH decreases in stability of ascorbic acid, thiamin, pantothenic acid and folates.

Light: Vitamin C, riboflavin, pyridoxine, folic acid and vitamin B_{12} become unstable when exposed to light.

Irradiation: Irradiation causes loss of thiamin whereas riboflavin and vitamin C shows better stability.

The overview of stability of water soluble vitamins in different media is given in Table 1:

Table 1: Stability of water soluble vitamins in different media

Water soluble Vitamins	**Neutral**	**Acid**	**Alkali**	**Oxygen**	**Light**	**Heat**	**Maximum cooking loss (%)**
Vitamin C	U	S	U	U	U	U	100
Thiamin	U	S	U	U	S	U	80
Riboflavin	S	S	U	S	U	U	75
Niacin	S	S	S	S	S	S	75
Pyridoxine	S	S	S	S	U	U	40
Folic acid	U	U	U	U	U	U	100
Vitamin B_{12}	S	S	S	U	U	S	10
Pantothenic acid	S	U	U	S	S	U	50
Biotin	S	S	S	S	S	U	60

U- unstable, S – stable. Ref: Harris RS (1971), General discussion on the stability of nutrients, in nutritional evaluation of food processing, AVI Publishing Co., Westport.

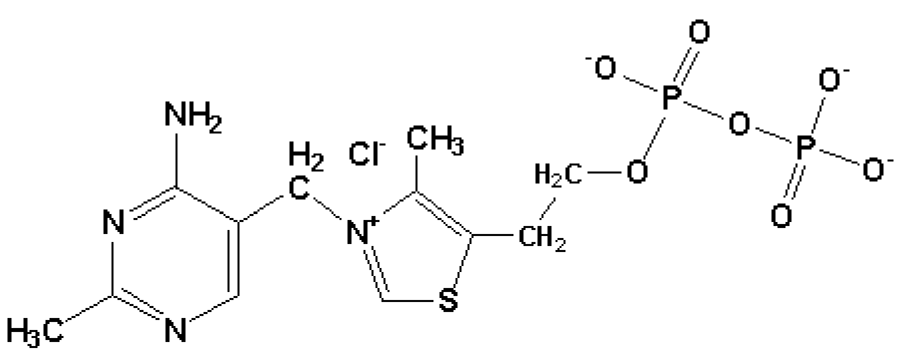
4) Bioavailability of water soluble vitamins: Bioavailability refers to the degree to which an ingested nutrient undergoes intestinal absorption and metabolic function or utilization within the body. Bioavailability depends on the concentration of the vitamin present during consumption, composition of the diet, intestinal transit time, efficiency of the intestine to absorb nutrients, enzyme concentration, rate of conversion of vitamins to its active form and interaction of vitamins with other food components such as proteins, carbohydrates, fat etc. Various studies have been conducted to improve the absorption of vitamins. In a study conducted by Vinson J. A *et al*, a significant improvement in the plasma levels of Vitamin E and C was observed when these vitamins were consumed with *Aloe vera* preparations. Fortified foods and supplements are effective than high folate foods when it comes to bioavailability of folates. Therefore, added folic acid is better absorbed than natural folic acid. Diet high in fruits and vegetables possess better bioavailability of folates. Few antithiamine factors such as thiaminase I and II, and polyphenols make thiamin unavailable for absorption. Dietary fibre improves the absorption of riboflavin. Chronic alcoholism decreases the bioavailability of most of the B-vitamins.

5) Other vitamin like compounds: Compounds such as choline, carnitine, myo-inositol, ubiquinone and bioflavonoids are classified under vitamin like compounds as the name suggests they have characteristics of vitamins and are essential. Few of them such as carnitine can be biosynthesized and few such as choline have to be provided in the diet. Choline is a structural component in the membrane phospholipids as phosphatidylcholine and in the neurotransmitter acetylcholine. Carnitine helps in energy production by the transport of long chain fatty acids into the mitochondria for oxidation in a process called carnitine transport shuttle. Myo-inositol in the form of phosphatidylinositol serves as an anchor for membrane proteins by covalent bonding. It is essential for the biosynthesis of eicosanoids. They are important sources of intracellular signals and act as secondary cell messengers in response to hormonal stimuli. Ubiquinones are essential for the reversible redox reactions in the mitochondrial electron transport chain. Bioflavonoids help in reducing the risk of cardiovascular diseases and cancers.

Conclusion:

Vitamins are essential components of the diet. Stability of vitamins depends on various factors such as temperature, processing methods, light and radiation. The bioavailability of vitamins depends on the concentration of the vitamin present during consumption, composition of the diet, intestinal transit time, efficiency of the intestine to absorb nutrients, enzyme concentration, rate of conversion of vitamins to its active form and interaction of vitamins with other food components. Bioavailability can be affected by alcoholism or a non-functional gut due to the presence of a disease. Like vitamins there are other compounds which are conditionally essential for the body.

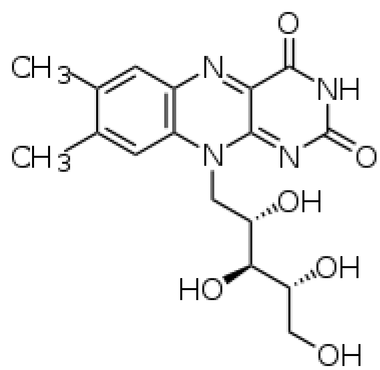
Figure 1: Thiamine



Thiamine pyrophosphate

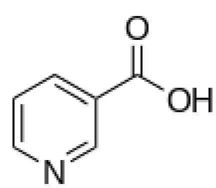
http://scienceunraveled.com/2012/06/20/why-do-we-need-thiamine-or-better-known-as-vitamin-b1/

Figure 2: Riboflavin



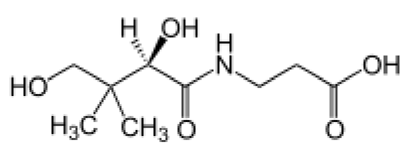
https://en.wikipedia.org/wiki/Riboflavin

Figure 3: Niacin



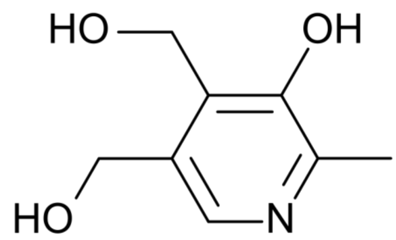
https://en.wikipedia.org/wiki/Niacin

Figure 4: Pantothenic_acid



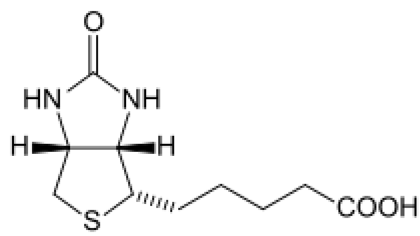
https://en.wikipedia.org/wiki/Pantothenic_acid

Figure 5: Pyridoxine



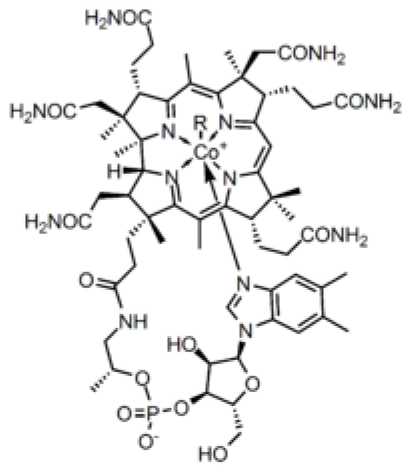
https://en.wikipedia.org/wiki/Pyridoxine

Figure 6: Biotin



https://en.wikipedia.org/wiki/Biotin

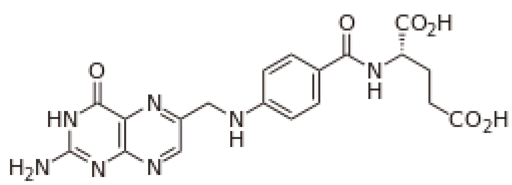
Figure 7: Vitamin B12



R = 5'-deoxyadenosyl, Me, OH, CN

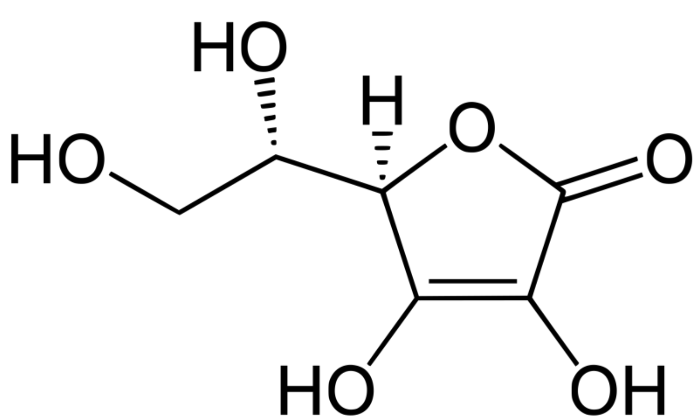
https://en.wikipedia.org/wiki/Vitamin_B12

Figure 8: Folic acid



https://en.wikipedia.org/wiki/Folic_acid

Figure 9: Vitamin C



https://en.wikipedia.org/wiki/Vitamin_C