

Paper No. 01

Paper Title: Food Chemistry

Module-26: Water-soluble vitamins – Properties, Stability & modes of degradation

The water-soluble vitamin group contains eight vitamins collectively known as the B-complex vitamins plus vitamin C (ascorbic acid).

Table 26.1. Vitamins and some commonly used synonyms

<i>Water-soluble</i> vitamins	Synonyms
vitamin B1	thiamin
vitamin B2	riboflavin
vitamin B6	pyridoxal, pyridoxine, pyridoxamine
vitamin B12	cobalamins, cyanocobalamin, hydroxocobalamin
niacin	nicotinic acid (vitamin PP)
niacinamide	nicotinamide (vitamin PP)
pantothenic acid	—
folic acid	folacin (vitamin M)
biotin	vitamin H
vitamin C	ascorbic acid

Thiamin (vitamin B1)

Thiamin is widely distributed in living tissues. In most animal products it occurs in a phosphorylated form, and in plant products it is predominantly in the nonphosphorylated form. Commercially it is available as either thiamin hydrochloride or thiamin mononitrate. Both these salts have specific areas of application and their use depends on the product matrix to which they are added. A considerable amount of research has been carried out on the heat stability of thiamin and its salts, particularly in the context of cooking losses. Early work on thiamin losses during bread-making showed an initial cleavage of the thiamin to pyrimidine and thiazole.⁷ The destruction of thiamin by heat is more rapid in alkaline media. Vitamin B1 losses in milk, which has an average fresh content of 0.04 mg thiamin per 100 g, are normally less than 10% for pasteurised milk, between 5 and 15% for UHT milk and between 30 and 40% for sterilised milk. Between 30 and 50% of the vitamin B1 activity can be lost during the production of evaporated milk.

Losses of thiamin during the commercial baking of white bread are between 15 and 20%. Part of this loss is due to the yeast fermentation, which can convert thiamin to cocarboxylase, which is less stable than thiamin. Thiamin is very sensitive to sulphites and bisulphites as it is cleaved by sulphite. This reaction is rapid at high pH, and is the cause of large losses of the vitamin in vegetables blanched with sulphite, and in meat products such as comminuted meats where sulphites and bisulphites are used as preservatives. Where the pH is low, such as in citrus fruit juices, the bisulphite occurs mainly as the unionised acid, and thiamin losses in such systems are not significantly different from those in products not containing bisulphite.

Studies on the rate of sulphite-induced cleavage of thiamin during the preparation and storage of minced meat showed that losses of thiamin were linear with sulphur dioxide concentrates up to 0.1%. The storage temperature did not have a significant effect on the losses. It has also been reported that thiamin is cleaved by aromatic aldehydes. Thiamin is decomposed by both oxidising

and reducing agents. If it is allowed to stand in alkaline solution in air it is oxidised to the disulphide and small amounts of thiothiazolone.

A range of food ingredients has been shown to have an effect on the stability of thiamin. In general, proteins are protective of the vitamin, particularly food proteins such as egg albumin and casein. When heated with glucose, either as a dry mixture or in solution, a browning analogous to a Maillard reaction can occur. This reaction is similar to the reaction between sugars and amino acids and may be important in the loss of thiamin during heat processing. Work has shown that fructose, invertase, mannitol and inositol can actually retard the rate of destruction of thiamin.

Thiamin is unstable in alkaline solutions and becomes increasingly unstable as the pH increases. The stability of the vitamin in low pH solutions such as fortified fruit drinks is very good. In common with that of some other vitamins, the stability of thiamin is adversely affected by the presence of copper ions. This effect can be reduced by the addition of metal-chelating compounds such as calcium disodium ethylenediamine tetra-acetate (EDTA). The heavy metals only appear to influence thiamin stability when they are capable of forming complex anions with constituents of the medium.

The enzymes, thiaminases, which are present in small concentrations in a number of animal and vegetable food sources, can degrade thiamin. These enzymes are most commonly found in a range of seafoods such as shrimps, clams and raw fish, but are also found in some varieties of beans, mustard seed and rice polishings. Two types of thiaminases are known and these are designated thiaminase I and thiaminase II. The former catalyses the decomposition of the thiamine by a base-exchange reaction, involving a nucleophilic displacement of the methylene group of the pyrimidine moiety. Thiaminase II catalyses a simple hydrolysis of thiamin.

A problem associated with the addition of vitamin B1 to food products is the unpleasant flavour and odour of the thiamin salts. The breakdown of thiamin, particularly during heating, may give rise to off-flavours, and the compounds derived from the degradation of the vitamins are believed to contribute to the 'cooked' flavours in a number of foods. However, both thiamin hydrochloride and mononitrate are relatively stable to atmospheric oxygen in the absence of light and moisture, and both are normally considered to be very stable when used in dry products with light and moisture-proof packaging.

Stability of Water soluble vitamins

Vitamin	Neutral	Acid	Alkaline	Air/Oxygen	Light	Heat	Max. cooking loss
Ascorbic acid	U	S	U	U	U	U	100
Biotin	S	S	S	S	S	U	60
Choline	S	S	S	U	S	S	5
Vitamin B12	S	S	S	U	U	S	10
Folate	U	U	U	U	U	U	100
Niacin	S	S	S	S	S	S	75
Pantothenic acid	S	U	U	S	S	S	50
Vitamin B6	S	S	S	S	U	U	40
Riboflavin	S	S	U	S	U	U	75
Thiamin	U	S	U	U	S	U	80

Note: S – Stable (no important destruction); U – Unstable (Significant destruction). Caution: These conclusions are oversimplifications and may not accurately represent stability under all circumstances.

Riboflavin (vitamin B2)

Riboflavin is the most widely distributed of all the vitamins and is found in all plant and animal cells, although there are relatively few rich food sources. It is present naturally in foods in two bound forms, riboflavin mononucleotide and flavin adenine dinucleotide. Plants and many bacteria can synthesise riboflavin and it is also found in dietary amounts in dairy products. Riboflavin is available commercially as a crystalline powder that is only sparingly soluble in water. As a consequence, the sodium salt of riboflavin-5 ϕ -phosphate, which is more soluble in water, is used for liquid preparations.

The most important factor influencing the stability of this vitamin is light, with the greatest effect being caused by light in the 420 to 560nm range. Fluorescent light is less harmful than direct sunlight, but products in transparent packaging can be affected by strip lighting in retail outlets. Riboflavin and riboflavin phosphate are both stable to heat and atmospheric oxygen, particularly in an acid medium. In this respect, riboflavin is regarded as being one of the more stable vitamins. It is degraded by reducing agents and becomes increasingly unstable with increasing pH. While riboflavin is stable to the heat processing of milk, one of the main causes of loss in milk and milk products is from exposure to light. 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. Although vitamin B2 is sensitive to light, particularly in a liquid medium such as milk, it remains stable in white bread wrapped in transparent packaging and kept in a lit retail area.

Niacin

The term 'niacin' is generic for both nicotinic acid and nicotinamide (niacinamide) in foods. Both forms have equal vitamin activity, both are present in a variety of foods and both forms are available as commercial isolates. Niacin occurs naturally in the meat and liver of hoofed animals and also in some plants. In maize and some other cereals it is found in the form of niacytin, which is bound to polysaccharides and peptides in the outer layers of the cereal grains and is unavailable to man unless treated with a mild alkali. Both forms of niacin are normally very stable in foods because they are not affected by atmospheric oxygen, heat and light in either aqueous or solid systems.

Pantothenic acid

In nature, pantothenic acid is widely distributed in plants and animals, but is rarely found in the free state as it forms part of the coenzyme A molecule. It is found in yeast and egg yolk, and in muscle tissue, liver, kidney and heart of animals. It is also found in a number of vegetables, cereals and nuts. Pantothenic acid is optically active and only its dextro-rotary forms have vitamin activity. Losses of pantothenic acid during the preparation and cooking of foods are normally not very large. Milk generally loses less than 10% during processing, and meat losses during cooking are not excessive when compared to those of the other B vitamins.

Free pantothenic acid is an unstable and very hygroscopic oil. Commercial preparations are normally provided as calcium or sodium salts. The alcohol form, panthenol, is available as a stable liquid but is not widely used in foods. The three commercial forms, calcium and sodium d-pantothenate and dpanthenol, are moderately stable to atmospheric oxygen and light when protected from moisture. All three compounds are hygroscopic, especially sodium pantothenate. Aqueous solutions of both the salts and the alcohol form are thermolabile and will undergo hydrolytic cleavage, particularly at high or low pH. The compounds are unstable in both acid and alkaline solutions and maximum stability is in the pH range of 6 to 7. Aqueous solutions of d-panthenol are more stable than the salts, particularly in the pH range 3 to 5.

Folic acid/folates

Folic acid (pteroylglutamic acid) does not occur in nature but can be produced commercially. The naturally occurring forms are a number of derivatives collectively known as folates or folacin, which contain one or more linked molecules of glutamic acid. Polyglutamates predominate in fresh food, but on storage these can slowly break down to monoglutamates and oxidise to less biologically available folates. The folic acid synthesised for food fortification contains only one glutamic group.

Most of the stability studies have been carried out with the commercially available folic acid, which has been found to be moderately stable to heat and atmospheric oxygen. In solution it is stable at around pH 7 but becomes increasingly unstable in acid or alkali media, particularly at pH less than 5. Folic acid is decomposed by oxidising and reducing agents. Sunlight, and particularly ultraviolet radiation, has a serious effect on the stability of folic acid. Cleavage by light is more rapid in the presence of riboflavin, but this reaction can be retarded by the addition of the antioxidant BHA to solutions containing folic acid and riboflavin.

The stability of the folates in foods during processing and storage is variable. Folic acid loss during the pasteurisation of milk is normally less than 5%. Losses in the region of 20% can occur during UHT treatment and about 30% loss is found after sterilisation. UHT milk stored for three months can lose over 50% of its folic acid. The extra heat treatment involved in boiling pasteurised milk can decrease the folic acid content by 20%. Losses of around 10% are found in boiled eggs, while other forms of cooking (fried, poached, scrambled) give between 30 and 35% loss. Total folic acid losses from vegetables as a result of heating and cooking processes can be very high.

A study carried out on the stability of folic acid in spinach during processing and storage showed major differences between water blanching and steam blanching, with a folate retention of 58% with steam blanching and only 17% with water. Frozen spinach was found to retain 72% folate after 3 months storage.

Vitamin B6 (pyridoxine)

Vitamin B6 activity is shown by three compounds, pyridoxol, pyridoxal and pyridoxamine and these are often considered together as pyridoxine. Vitamin B6 is found in red meat, liver, cod roe and liver, milk and green vegetables. The commercial form normally used for food fortification is the salt, pyridoxine hydrochloride.

Pyridoxine is normally stable to atmospheric oxygen and heat. Decomposition is catalysed by metal ions. Pyridoxine is sensitive to light, particularly in neutral and alkaline solutions. One of the main causes of loss of this vitamin in milk is sunlight with a 21% loss being reported after 8 hours exposure. Pyridoxine is stable in milk during pasteurisation but about 20% can be lost during sterilisation. Losses during UHT processing are around 27%,¹³ but UHT milk stored for 3 months can lose 35% of this vitamin. Average losses as a result of roasting or grilling of meat are 20%, with higher losses (30–60%) in stewed and boiled meat. Cooking or canning of vegetables results in losses of 20–40%.

Vitamin B12

The most important compound with vitamin B12 activity is cyanocobalamin. This has a complicated chemical structure and occurs only in animal tissue and as a metabolite of certain microorganisms. The other compounds showing this vitamin activity differ only slightly from the cyanocobalamin structure. The central ring structure of the molecule is a 'corrin' ring with a central cobalt atom. In its natural form, vitamin B12 is probably bound to peptides or protein.

Vitamin B12 is commercially available as crystalline cyanocobalamin, which is a dark red powder. As human requirements of vitamin B12 are very low (about 1–2mg a day), it is often supplied as a

standardised dilution on a carrier. Cyanocobalamin is decomposed by both oxidising and reducing agents. In neutral and weakly acid solutions it is relatively stable to both atmospheric oxygen and heat. It is only slightly stable in alkaline solutions and strong acids. It is sensitive to light and ultraviolet radiation, and controlled studies on the effect of light on cyanocobalamin in neutral aqueous solutions showed that sunlight at a brightness of 8.6×10^4 lux caused a 10% loss for every 30 minutes of exposure, but exposure to levels of brightness 3.2×10^3 lux had little effect. Vitamin B12 is normally stable during pasteurisation of milk but up to 20% can be lost during sterilisation, and losses of 20–35% can occur during spray drying of milk. The stability of vitamin B12 is significantly influenced by the presence of other vitamins.

Biotin

The chemical structure of biotin is such that eight different isomers are possible and of these only the dextro-rotatory or d-biotin possesses vitamin activity. D-biotin is widely distributed, but in small concentrations, in animal and plant tissues. It can occur both in the free state (milk, fruit and some vegetables) and in a form bound to protein (animal tissues and yeast). It is commercially available as a white crystalline powder.

Biotin is generally regarded as having a good stability, being fairly stable in air, heat and daylight. It can, however, gradually be decomposed by ultraviolet radiation. Biotin in aqueous solutions is relatively stable if the solutions are either weakly acid or weakly alkaline. In strong acid or alkaline solutions the biological activity can be destroyed by heating.

Avidin, a protein complex, which is found in raw egg white, can react with biotin and bind it in such a way that the biotin is inactivated. Avidin is denatured by heat and biotin inactivation does not occur with cooked eggs.

Vitamin C

Although a number of compounds possess vitamin C activity, the most important is l-ascorbic acid. Vitamin C is widely distributed in nature and can occur at relatively high levels in some fruits and vegetables and is also found in animal organs such as liver and kidney. Small amounts can be found in milk and other meats. Ascorbic acid is the enolic form of 3-keto-1-gulofuranolactone. The endiol groups at C-2 and C-3 are sensitive to oxidation and can easily convert into a diketo group. The resultant compound, dehydro-l-ascorbic acid, also has vitamin C activity. The d-isomers do not have vitamin activity.

The l-ascorbic acid in foods is easily oxidised to the dehydro-l-ascorbic acid. In fresh foods the reduced form normally predominates but processing, storage and cooking increase the proportions of the dehydro form. Commercially, vitamin C is available as l-ascorbic acid and its calcium, sodium and magnesium salts, the ascorbates. It is also available as ascorbyl palmitate and can be used in this form as an antioxidant in processed foods. Ascorbic acid and the ascorbates are relatively stable in dry air but are unstable in the presence of moisture. Ascorbic acid is readily oxidised in aqueous solutions, first forming dehydro-l-ascorbic acid which is then further and rapidly oxidised. Conversion to dehydroascorbic acid is reversible but the products of the latter stages of oxidation are irreversible.

Ascorbic acid is widely used in soft drinks and to restore manufacturing losses in fruit juices, particularly citrus juices. Research has shown that its stability in these products varies widely according to the composition and oxygen content of the solution. It is very unstable in apple juice but stability in blackcurrant juice is good, possibly as a result of the protective effects of phenolic substances with antioxidant properties.

The effect of dissolved oxygen is very significant. As 11.2 mg of ascorbic acid is oxidised by 1.0 mg of oxygen, 75–100 mg of ascorbic acid can be destroyed by one litre of juice. Vacuum treatment stages are normally added to the process to deaerate the solution to reduce the problem. It is also important to avoid significant head-spaces in containers of liquids with added ascorbic acid as 3.3mg of ascorbic acid can be destroyed by the oxygen in 1cm³ of air.¹⁴ Different production and filling processes can have a significant effect on the retention of vitamin C in drinks. For example, the ascorbic acid loss in a drink packed in a 0.7 litre glass bottle with a partial deaeration of the water and vacuum deaeration of the drink immediately before filling was 16% of the same product filled without any deaeration.

Traces of heavy metal ions act as catalysts to the degradation of ascorbic acid. Studies on the stability of pharmaceutical solutions of ascorbic acid showed that the order of the effectiveness of the metallic ions was $\text{Cu}^{+2} > \text{Fe}^{+2} > \text{Zn}^{+2}$. A Cu^{+2} -ascorbate complex has been identified as being intermediate in the oxidation of the ascorbic acid in the presence of Cu^{+2} ions. Other work on model systems has shown that copper ion levels as low as 0.85 ppm was sufficient to catalyse oxidation, and that the reaction rate was approximately proportional to the square root of the copper concentration.

Work with sequestrants has shown that ethylenediamine tetra-acetate (EDTA) has a significant effect on the reduction of ascorbic acid oxidation, with the optimal level of EDTA required to inhibit the oxidation of vitamin C in blackcurrant juice being a mole ratio of EDTA to $[\text{Cu} + \text{Fe}]$ of approximately 2.3. Unfortunately, EDTA is not a permitted sequestrant for fruit juices in many countries. The amino acid cysteine has also been found to inhibit ascorbic acid oxidation effectively.

Copper and iron ions play such a significant part in metal catalysed oxidation of ascorbic acid that the selection of process equipment can have a marked effect on the stability of vitamin C in food and drink products. Contact of product with bronze, brass, cold rolled steel or black iron surfaces or equipment should be avoided and only stainless steel, aluminium or plastic should be used. The rate of ascorbic acid degradation in aqueous solutions is pH-dependent with the maximum rate at about pH 4. Vitamin C losses can occur during the frozen storage of foods, and work has shown that oxidation of ascorbic acid is faster in ice than in the liquid water. Frozen orange concentrates can lose about 10% of their vitamin C content during twelve months' storage at -23°C (-10°F). Light, either in the form of sunlight or white fluorescent light, can have an effect on the stability of vitamin C in milk, with the extent of the losses being dependent on the translucency and permeability of the container and the length and conditions of exposure. Bottled orange drinks exposed to light have been found to lose up to 35% vitamin C in three months.

The destruction of vitamin C during processing or cooking of foods can be quite considerable, with losses during pasteurisation being around 25%, sterilization about 60% and up to 100% in UHT milk stored for three months. Milk boiled from pasteurised can show losses of between 30 and 70%. Large losses of vitamin C are also found after cooking or hot storage of vegetables and fruits. The commercial dehydration of potatoes can cause losses of between 35 and 45%. Destruction of vitamin C during the processing of vegetables depends on the physical processing used and the surface area of product exposed to oxygen. Slicing and dicing of vegetables will increase the rate of vitamin loss. Blanching of cabbage can produce losses of up to 20% of the vitamin C, whilst subsequent dehydration can account for a further 30%.

Vitamin–vitamin interactions

One of the least expected and less understood aspects of maintaining the stability of vitamins in foods is the detrimental interaction between vitamins. This can lead to the more rapid degradation of one or more of the vitamins in a food or beverage. These interactions should be taken into

consideration when vitamins are used to restore or fortify products presented in the liquid (aqueous) phase such as soft drinks or fruit juices. Most of the work in the area of vitamin–vitamin interactions has been carried out by the pharmaceutical industry in relation to the development of liquid multivitamin preparations. Four of the thirteen 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. The principal interactions are given in Table 26.2. Other interactions have been identified that can be advantageous, particularly in increasing the solubility of the less soluble vitamins in aqueous solutions. For example, niacinamide has been shown to act as a solubiliser for riboflavin and folic acid.

Table 26.2. Principal vitamin–vitamin interactions

Activator	Increased instability
Ascorbic acid	Folic acid
Ascorbic acid	Vitamin B12
Thiamin	Folic acid
Thiamin	Vitamin B12
Riboflavin	Thiamin
Riboflavin	Folic acid
Riboflavin	Ascorbic acid

Note: Vitamin loss during processing, Vitamin & food product shelf – life & Protection of vitamins in foods May be referred from previous chapter.

Addition of Nutrients to Foods

Throughout the early 20th century, nutrient deficiency represented a major public health problem globally. Pellagra was endemic in many countries, while deficiencies of riboflavin, niacin, iron, and calcium were widespread too in developing countries. The development of legally defined standards of identity under the 1938 Food, Drug, and Cosmetic Act provided for the direct addition of several nutrients to foods, especially certain dairy and cereal-grain products. The nearly complete eradication of overt vitamin-deficiency disease provides evidence of the exceptional effectiveness of fortification programs and the general improvement in the nutritional quality of the U.S. food supply.

Definitions of terms associated with the addition of nutrients of foods include:

1. **Restoration:** Addition to restore the original concentration of key nutrients.
2. **Fortification:** Addition of nutrients in amounts significant enough to render the food a good to superior source of the added nutrients. This may include addition of nutrients not normally associated with the food or addition to levels above that present in the unprocessed food.
3. **Enrichment:** Addition of specific amounts of selected nutrients in accordance with a standard of identity as defined by the U.S. Food and Drug Administration (FDA).
4. **Nutrification:** This is a generic term intended to encompass any addition of nutrients to food.

The addition of vitamins and other nutrients to food, while clearly beneficial in current practice, also carries with it the potential for abuse and, thus, risk to consumers. For these reasons, important guidelines have been developed that convey a reasonable and prudent approach. The guidelines state that the nutrient added to a food should be:

1. Stable under customary conditions of storage, distribution, and use.
2. Physiologically available from the food.
3. Present at a level where there is assurance that there will not be excessive intake.

4. Suitable for its intended purpose and in compliance with provisions (i.e., regulations) governing safety.

