Stability of vitamins during food processing and storage

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Abstract: The effects of processing and storage of food on the amounts of vitamins that are present in dietary sources are discussed. The factors that affect the stability of vitamins are reviewed. These factors vary from vitamin to vitamin; the most important being heat, moisture, oxygen, pH and light. The problems that arise for the food technologist from the nutritional labelling of food products are discussed. When more than one vitamin is the subject of a quantitative label claim for a food, it is very unlikely that the vitamins will deteriorate at the same rate. If the amounts of these vitamins are included in nutritional labelling, the shelf life of the food is determined by the life of the most unstable component.

Key words: vitamins, food processing, food storage, nutritional labelling, food technology.

19.1 Introduction

Vitamins, by their definition, are essential to health and have to be obtained from the diet on a regular basis as, with the exception of vitamin D, they cannot be produced by the body. In terms of medicine and nutrition, our knowledge of vitamins is relatively recent. Although Lind discovered an association between lime juice and scurvy in 1753, it was over 170 years later that vitamin C was eventually isolated. The understanding of vitamin B_{12} only goes back to the 1950s and new roles for folates are still being discovered. Man’s supply of vitamins is obtained from a varied diet of vegetables, cereals, fruits and meats and the quantities of vitamins that are present in the dietary sources can be affected significantly by the processing and storage of the food.
19.2 Vitamins

The vitamins are a heterogeneous group of substances that fall into the classification of being vital nutrients that have to be obtained from the diet. Although a number of substances were termed vitamins between the 1930s and 1950s, nutritional science now recognises only 13 substances, or groups of substances, as being true vitamins. The 13 substances are divided into two categories, the fat-soluble vitamins and the water-soluble vitamins. These are listed in Table 19.1.

Even within the two sub-categories, the vitamins have almost no common attributes in terms of chemistry, function or daily requirements. In terms of requirements some, such as vitamins C, E and niacin, are needed in tens of milligrams a day whilst others, such as vitamins D and B₁₂, are only required in single microgram amounts. It can be seen from these examples that there is no relationship between the form of delivery (i.e. fat or water soluble) and the daily requirements.

The heterogeneity also applies to the chemical structure and the functions of the vitamins. Chemically, there are no similarities between the substances. Some are single substances, such as biotin, whilst others, such as vitamin E, are groups of compounds all exhibiting vitamin activity.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Synonyms</th>
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<td><strong>Fat-soluble</strong></td>
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<td>Retinol</td>
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<tr>
<td>Pro-vitamin A</td>
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<td>Vitamin K₁</td>
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<td>Niacin</td>
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<td>Folate</td>
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<td>Biotin</td>
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<td>Vitamin C</td>
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19.3 Factors affecting vitamin stability

One of the very few attributes that the vitamins have in common is that none is completely stable in food. The stability of the individual vitamins varies from the relatively stable, such as in the case of niacin, to the relatively unstable, such as vitamin B<sub>12</sub>. The factors that affect the stability vary from vitamin to vitamin and the principal ones are summarised in Table 19.2. The most important of these factors are heat, moisture, oxygen, pH and light.

The deterioration of vitamins can take place naturally during the storage of vegetables and fruits and losses can occur during the processing and preparation of foods and their ingredients, particularly those subjected to heat treatment. 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 synthetic sources. However, the form in which a synthetic source is used (e.g. a salt or ester) may enhance its stability. For example, the vitamin E (tocopherol) esters are more stable than the tocopherol form.

With the increased use of nutritional labelling of food products, vitamin levels in foods have become the subject of label claims that can be easily checked by the enforcement authorities. This poses a number of problems for the food technologist. When more than one vitamin is the subject of a quantitative label claim for a food, it is very unlikely that the vitamins will deteriorate at the same rate. If the amounts of these vitamins are included in nutritional labelling, the shelf life of the food is determined by the life of the most unstable component.

In order to comply with the legal requirements of maintaining the label claim throughout the declared life of a food product, the technologist needs to obtain a reasonably accurate estimation of the stability of each of the vitamins in the product. This has to be evaluated in the context of the food system (e.g. solid, liquid), the packaging and probable storage conditions and is achieved by conducting well-designed stability tests.

It is interesting to note that most of the scientific literature on the stability of vitamins in foods and supplements relates to work carried out in the

<table>
<thead>
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<th>Table 19.2 Factors affecting the stability of vitamins</th>
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<tr>
<td>1. Temperature</td>
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<td>2. Moisture</td>
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<td>4. Light</td>
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<td>5. pH</td>
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<td>6. Oxidising and reducing agents</td>
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<td>7. Presence of metallic ions (e.g. copper, iron)</td>
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<td>8. Other components of food such as sulphur dioxide</td>
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<td>9. Presence of other vitamins</td>
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<td>10. Combinations of the above</td>
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</table>
19.4 Classification of vitamins

19.4.1 Fat-soluble vitamins

Vitamin A

Nutritionally, the human body can obtain its vitamin A requirements from two sources: from animal sources as forms of retinol, and from plant sources from β-carotene and related carotenoids. Both sources provide a supply of vitamin A, but by different metabolic pathways. In terms of stability the two sources are different from each other.

Retinol

Vitamin A is one of the more labile vitamins with retinol being less stable than the retinyl esters. The presence of double bonds in its structure makes it subject to isomerisation, particularly in an aqueous medium at acid pH. The isomer with the highest biological activity is the all-trans vitamin A. The predominant cis isomer is 13-cis or neovitamin A, which only has a biological activity of 75\% of the all-trans isomer; and 6-cis- and 2, 6-di-cis-isomers, which may also form during isomerisation, have less than 25\% of the biological activity of the all-trans form of vitamin A. Natural vitamin A sources usually comprise about one third neovitamin A whereas most synthetic sources generally contain considerably less. For aqueous products where isomerisation is known to occur, mixtures of vitamin A palmitate isomers at the equilibrium ratio have been produced commercially. Vitamin A is relatively stable in alkaline solutions.

Vitamin A is sensitive to atmospheric oxygen with the alcohol form being less stable than the esters. The decomposition is catalysed by the presence of trace minerals. As a consequence of its sensitivity to oxygen, vitamin A is normally available commercially as a preparation that includes an antioxidant and often a protective coating. Although butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are permitted in a number of countries for use as antioxidants in vitamin A preparations, the recent trend has been towards the use of tocopherols (vitamin E).

Both retinol and its esters are inactivated by the ultraviolet component of light. In general, vitamin A is relatively stable during food processing involving heating, with the palmitate ester more stable to heat than retinol. It is normally regarded as stable during milk processing, and food composition tables give only small differences between the retinol contents of fresh whole milk, sterilised and ultra high temperature (UHT) treated milk.
However, prolonged holding of milk or butter at high temperatures in the presence of air can be shown to result in a significant decrease in the vitamin A activity.

β-carotene as provitamin A
There are a number of carotenoids with provitamin A activity. A provitamin is a compound that can be converted in the body to a vitamin. These compounds are generally found as naturally occurring plant pigments that give the characteristic yellow, orange and red colours to a wide range of fruits and vegetables. Some can also be found in the liver, kidney, spleen and milk. The provitamin A with the greatest nutritional and commercial importance is β-carotene.

The stability of the carotenoids is similar to that of vitamin A in that they are sensitive to oxygen, light and acid media. It has been reported that treatment with sulphur dioxide reduces carotenoid destruction in vegetables during dehydration and storage. A study with model systems showed that the stability of β-carotene was greatly enhanced by sulphur dioxide added either as a sulphite solution to cellulose powder before β-carotene absorption or as a headspace gas in containers of β-carotene. Although the β-carotene stability was improved by increasing the nitrogen levels in the containers, the stability was even greater when the nitrogen was replaced by sulphur dioxide. Comparative values for the induction period were 19 h for β-carotene samples stored in oxygen only, 120 h in nitrogen and 252 h in sulphur dioxide.6

Investigations into the effect of sulphur dioxide treatment on the β-carotene stability in dehydrated vegetables have given varying results and it has been postulated that the effects of the drying and storage conditions on the stability of the sulphur dioxide has a consequential effect on the stability of the β-carotene in dehydrated products.7

Studies on the heat stability of both α-carotene and β-carotene showed that the β-carotene was about 1.9 times more susceptible than α-carotene to heat damage during normal cooking and blanching processes.8

There is some evidence of a protective effect from ascorbic acid on β-carotene and other pro-vitamin A carotenoids both in liquid and powder form.9,10 It would appear that, in these circumstances, the ascorbic acid is acting as an antioxidant protecting the carotenoids from rapid oxidation. Products containing β-carotene should be protected from light and headspace air should be kept to the minimum.

Vitamin E
A number of naturally occurring substances exhibit vitamin E activity, including the α, β, γ and δ tocopherols and alpha tocotrienols. Dietary sources of vitamin E are found in a number of vegetables and cereals, with some vegetable oils such as wheatgerm, sunflower seed, safflower seed and maize oils being particularly good sources. Both synthetic and naturally
sourced forms of vitamin E are available commercially. Although natural sources of tocopherols, which have the highest biological activity, are in the ‘D’ form, synthetic versions can only be produced in the ‘DL’ form. Both the ‘D’ and ‘DL’ forms are also commercially available as esters.

There is a considerable difference in the stability of the tocopherol forms of vitamin E and the tocopherol esters. Although vitamin E is generally regarded as being one of the more stable vitamins, the unesterified tocopherol is less stable owing to the free phenolic hydroxyl group.

Vitamin E is unusual in that it exhibits reduced stability at temperatures below freezing. The explanation given for this is that the peroxides formed during fat oxidation are degraded at higher temperatures but are stable at temperatures below 0 °C and, as a consequence, can react with the vitamin E.11 It has also been shown that α-tocopherol may function as a pro-oxidant in the presence of metal ions such as iron.

α-Tocopherol is readily oxidised by air, is stable to heat in the absence of air, but is degraded if heated in the presence of air; thus, it is readily oxidised during the processing and storage of foods. One of the most important naturally occurring sources of tocopherols are the vegetable oils, particularly wheat germ and cottonseed oils. While deep-frying of the oils may result in a loss of vitamin E of around 10%, it has been found that the storage of fried foods, even at temperatures as low as −12 °C, can result in very significant losses. DL-α-Tocopheryl acetate is relatively stable in air but is hydrolysed by moisture in the presence of alkalis or strong acids to free tocopherols.

**Vitamin D**

Present in nature in several forms, dietary vitamin D occurs predominantly in animal products with very little being obtained from plant sources. Vitamin D₃, or cholecalciferol, is derived in animals, including man, from ultraviolet irradiation of 7-dehydrocholesterol which is found in the skin. Human requirements are obtained both from endogenous production in the skin and from dietary sources.

Vitamin D₂ (ergocalciferol) is produced by the ultraviolet irradiation of ergosterol, which is widely distributed in plants and fungi. Both vitamins D₂ and D₃ are manufactured for commercial use. Both vitamins D₂ and D₃ are sensitive to light and can be destroyed relatively rapidly if exposed to light. They are also adversely affected by acids. Preparations of vitamin D in edible oils are more stable than the crystalline forms, and the vitamin is normally provided for commercial usage as an oil preparation or stabilised powder containing an antioxidant (usually tocopherol). The preparations are usually provided in lightproof containers with inert gas flushing.

The presence of double bonds in the structure of both forms of vitamin D can make them susceptible to isomerisation under certain conditions. Studies have shown that the isomerisation rates of ergocalciferol and cholecalciferol are almost equal. Isomerisation in solutions of cholecalciferol
resulted in an equilibrium being formed between ergocalciferol and precalciferol, with the ratios of the isomers being temperature dependent. The isomerisation of ergocalciferol has been studied in powders prepared with calcium sulphate, calcium phosphate, talc and magnesium trisilicate. It was found that the isomerisation was catalysed by the surface acid of these additives.\textsuperscript{12}

Crystalline vitamin D\textsubscript{2} is sensitive to atmospheric oxygen and will show signs of decomposition after a few days’ storage in the presence of air at ambient temperatures. Crystalline cholecalciferol (vitamin D\textsubscript{3}) is also destroyed by atmospheric oxygen but is relatively more stable than vitamin D\textsubscript{2}, possibly because it has one fewer double bond. The vitamin D\textsubscript{3} naturally occurring in foods such as milk and fish appears to be relatively stable to heat processing.

**Vitamin K**

Vitamin K occurs in a number of forms. Vitamin K\textsubscript{1} (phytomenadione or phylloquinone) is found in green plants and vegetables, potatoes and fruits, whereas vitamin K\textsubscript{2} (menaquinone) can be found in animal and microbial materials. The presence of double bonds in both vitamins K\textsubscript{1} and K\textsubscript{2} makes them liable to isomerisation. Vitamin K\textsubscript{1} has only one double bond in the side chain in the 3-position, whereas in K\textsubscript{2} double bonds recur regularly in the side chain. Vitamin K\textsubscript{1} exists in the form of both \textit{trans} and \textit{cis} isomers. The \textit{trans} isomer is the naturally occurring form and is the one that is biologically active. The \textit{cis} form has no significant biological activity.

The various forms of vitamin K are relatively stable to heat and are retained after most cooking processes. The vitamin is destroyed by sunlight and is decomposed by alkalis. Vitamin K\textsubscript{1} is only slowly decomposed by atmospheric oxygen. Vitamin K is rarely added to food products and the most common commercially available form is K\textsubscript{1} (phytomenadione), which is insoluble in water. A water-soluble K\textsubscript{3} is available as menadione sodium bisulphite.

### 19.4.2 Water-soluble vitamins

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

**Thiamin (vitamin B\textsubscript{1})**

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 non-phosphorylated form. Commercially, it is mainly 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. 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 caused by 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. Large losses of thiamin can also be found in fortified fruit juices and drinks and liquid food supplements where sulphites are present as a preservative either for the product or for a major ingredient. Where the pH is low, such as in citrus fruit juices, the bisulphite occurs mainly as the un-ionised 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 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 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 thiamin 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.²⁰ 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.

**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′-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 560 nm range. Fluorescent light is less harmful than direct sunlight, but products in transparent packaging can be affected by strip lighting in retail outlets.²¹,²²

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. Although 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 2 h, 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 the same 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 as they are stable to atmospheric oxygen, heat and light in both aqueous and 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-rotatory 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 with 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, d-panthenol, is available as a stable liquid but is not widely used in foods. The three commercial forms, calcium and sodium d-pantothenate and d-panthenol, are moderately stable to atmospheric oxygen and light when protected from moisture. All three compounds are hygroscopic, with sodium pantothenate being the worst. 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 and 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 has only one glutamic group.

For many years, folic acid has been the only source of this vitamin for food fortification and supplementation. An isolated folate, 5-methyltetrahydrofolic acid, became available in 1999. By 2005, following official safety reviews and approvals, it was considered suitable for use in foods and supplements.23

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. This reaction can be retarded by the addition of the antioxidant BHA to solutions containing folic acid and riboflavin.24

The stability of the folates in foods during processing and storage is variable. Folate 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 folate. The extra heat treatment involved in boiling pasteurised milk can decrease the folate content by 20%. Losses of around 10% are found in boiled eggs, whereas other forms of cooking (fried, poached, scrambled) give between 30 and 35% loss. Total folate losses from vegetables as a result of heating and cooking processes can be very high.

A study carried out on the stability of folate 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 three months storage.25,26 Stability studies carried out on 5-methyltetrahydrofolic acid showed that its degradation in all the model systems could be described by first-order reaction kinetics.27 The thermostability of the folate was enhanced at a pH of 7. The study also investigated the pressure stability of the folate in fruit and vegetable juices subjected to high-pressure processing and at different temperature/pressure combinations. It was found that it was relatively pressure stable at temperatures lower than 40 °C and that both the temperature and pressure stabilities were enhanced in the presence of ascorbic acid.

Vitamin B₆ (pyridoxine)

Vitamin B₆ activity is shown by three compounds, pyridoxol, pyridoxal and pyridoxamine. 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 eight hours’ exposure.\(^{17}\)

Pyridoxine is stable in milk during pasteurisation but about 20% can be lost during sterilisation. Losses during UHT processing are around 27%,\(^{28}\) but UHT milk stored for three months can lose 35% of this vitamin. Average losses as a result of roasting or grilling of meat are 20%, with higher losses (30 to 60%) in stewed and boiled meat.\(^{29}\) Cooking or canning of vegetables results in losses of 20 to 40%.

**Vitamin B\(_{12}\)**

The most important compound with vitamin B\(_{12}\) activity is cyanocobalamin. This has a complicated chemical structure and occurs only in animal tissue and as a metabolite of certain micro-organisms. 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 B\(_{12}\) is probably bound to peptides or protein.

Vitamin B\(_{12}\) is commercially available as crystalline cyanocobalamin, which is a dark red powder. As human requirements of vitamin B\(_{12}\) are very low (about 1–5 \(\mu\)g 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 8000 foot candles caused a 10% loss for each thirty minutes of exposure, but exposure to levels of brightness below 300 foot candles had little effect.\(^{11}\)

Vitamin B\(_{12}\) is normally stable during pasteurisation of milk but up to 20% can be lost during sterilisation, and losses of 20 and 35% can occur during spray drying of milk. The stability of vitamin B\(_{12}\) 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 \(\text{d}\)-biotin, possesses vitamin activity. \(\text{d}\)-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. In aqueous solutions, biotin 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 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, but the D-isomers do not have vitamin activity.

In foods, L-ascorbic acid 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 to 100 mg of ascorbic acid can be destroyed by 1 l 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 headspaces in containers of liquids with added ascorbic acid as 3.3 mg of ascorbic acid can be destroyed by the oxygen in 1 cm$^3$ of air. 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 l glass bottle with a partial deaeration
Chemical deterioration and physical instability of food and beverages

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 \( \text{Cu}^{2+} \)-ascorbate complex has been identified as being intermediate in the oxidation of the ascorbic acid in the presence of \( \text{Cu}^{2+} \) ions.\(^{11}\) Other work on model systems has shown that copper ion concentrations as low as 0.85 ppm were sufficient to catalyse oxidation, and that the reaction rate was approximately proportional to the square root of the copper concentration.

Studies on sequestrants have shown that ethylenediamine tetra-acetate (EDTA) has a significant effect on the reduction of ascorbic acid oxidation, with the optimal concentration 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.\(^{31,32}\) Unfortunately, EDTA is not a permitted sequestrant for fruit juices in many countries. The amino acid cysteine has also been found effectively to inhibit ascorbic acid oxidation.

\( \text{Cu} \) and \( \text{Fe} \) ions play such a significant part in metal-catalysed oxidation of ascorbic acid that the selection of the process equipment can have a marked effect on the stability of vitamin C in food and drink products. Contact of the 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.\(^{33}\)

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.\(^{17}\)

The destruction of vitamin C during processing or cooking of foods can be quite considerable, with losses during pasteurisation being around 25%, during sterilisation about 60%, and up to 100% in UHT milk stored for three months. Milk boiled after pasteurisation 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 20% of the vitamin C, whilst subsequent dehydration can account for a further 30%. 34

19.5 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 13 vitamins have been identified as having interactions with each other with deleterious effects. 35 These are ascorbic acid (vitamin C), thiamin (vitamin B1), riboflavin (vitamin B2) and vitamin B12. The principal interactions are given in Table 19.3. 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>
<thead>
<tr>
<th>Activator</th>
<th>Increased instability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Vitamin B12</td>
</tr>
<tr>
<td>Thiamin</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Thiamin</td>
<td>Vitamin B12</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Thiamin</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Ascorbic acid</td>
</tr>
</tbody>
</table>

(Adapted from Berry Ottaway, 1993).

19.6 Effect of irradiation on vitamin stability in foods

The use of ionising radiation (irradiation) as a sterilisation technique for foods has been accepted in a number of countries, including the European Union. In many countries, the foods and ingredients that are allowed to be irradiated are restricted by law and the process is normally only used for foods at risk of high levels of microbiological contamination.

It has been shown that vitamin levels in a food can be affected by irradiation and the losses can, in general, be related to the dose. At low doses
Chemical deterioration and physical instability of food and beverages (e.g. up to 1 kGy), the losses for most vitamins are not significant. At higher doses (3–10 kGy) it has been shown that vitamin losses can occur in foods that are exposed to air during the irradiation and subsequent storage. At the highest permitted radiation doses, care has to be taken to protect the food by using packaging that excludes the air and by carrying out the irradiation process at a low temperature.

There is evidence that the fat-soluble vitamins A, E and K and the water-soluble thiamin are the most sensitive to irradiation, whereas niacin, riboflavin and vitamin D are relatively stable. There is conflicting evidence for vitamins with some foods showing significant losses and others almost none. If it is intended that nutrition claims are to be made for irradiated foods, it is essential that studies are carried out on the content and stability of the vitamins after the treatment with the ionising radiation.

19.7 Vitamin loss during processing

As already discussed, all vitamins exhibit a degree of instability, the rate of which is affected by a number of factors. Naturally occurring vitamins in foods are susceptible to many of these factors during the harvesting, processing and storage of the food and its ingredients. It is particularly important that the effects of processing are taken into consideration when assessing vitamin stability in foods, as the food may have been subjected to a number of adverse factors during processing.

The most common factor during processing is the application of heat, which in some cases, such as canning, can be for a relatively long time. Most of the work on the stability of vitamins in fruits and vegetables during blanching and canning was carried out during the 1940s and 1950s. Although there have since been refinements both in processing and analytical techniques, many of the conclusions drawn from this research are still valid.

19.7.1 Blanching

A high temperature and, thus, a short water-blanch time gives a better vitamin retention than a low temperature and, thus, long blanch time. Overall, steam blanching is superior to water blanching. The addition of sulphite to the blanching water has been shown to significantly affect thiamin levels in fruits and vegetables. β-Carotene was found to be the best survivor during blanching. Riboflavin had retentions in the range 80 to 95%; vitamin C was in the range 70 to 90% under optimum conditions, and niacin 75 to 90%.

19.7.2 Heat processing

Studies on the heat processing of fruits and vegetables in both tin and glass containers showed significant losses of both vitamin C and thiamin. In some
cases, the vitamin C concentrations determined immediately after the heat processing were between 15 and 45% of the fresh product and these concentrations were further reduced during storage.

Thiamin reduced by about 50% during heat processing and further declined to between 15 and 40% of the original level after 12 months’ storage. Riboflavin losses were between 12 and 15% during processing but concentrations of about 50% of the original were observed after 12 months. Niacin was more stable with initial losses of 15 to 25% but with much less than riboflavin being lost during storage. β-Carotene was found to be relatively stable.

In milk, the fat soluble vitamins A and D are relatively stable to the heat treatments used for the processing of milk, as are the water-soluble vitamins riboflavin, niacin, pantothenic acid and biotin. Vitamin C, thiamin, vitamin B₆, vitamin B₁₂ and folic acid are all affected by the heat processing of milk, the more severe the process, the greater the loss is. With the exception of vitamin C, vitamin losses are generally less than 10% after pasteurisation of milk and between 10 and 20% after ultra high temperature (UHT) treatment. Average losses following sterilisation of milk are reported as 20% for thiamin, vitamin B₆ and vitamin B₁₂, and 30% for folic acid.

Studies have shown that the stability of vitamin C during the processing of milk is also affected by the oxygen content of the milk. Average losses for vitamin C were 25% after pasteurisation, 30% after UHT and 60% after sterilisation. However, vitamin C appears to be particularly well retained in condensed full cream milk.¹⁰

Vitamin B stability during the heat processing and cooking of meats varies widely. Cooking conditions can have a marked effect on stability and the retention of thiamin in beef and pork is related to roasting temperatures. If the vitamin content of the drippings is taken into consideration, it is generally found that riboflavin, niacin and vitamin B₁₂ are stable during the cooking of meat. Pantothenic acid losses in cooked meat are usually less than 10% although high losses of folate (both free and total) of over 50% have been found in pork, beef and chicken that had been boiled for 15 min.

Post-mortem ageing of beef can result in up to a 30% loss of niacin over seven days, although the remaining niacin is relatively stable on cooking. The baking of bread can induce losses of about 20% for thiamin, up to 17% for vitamin B₆ and up to one third of the natural folate content. Niacin and pantothenic acid are normally stable during baking.

### 19.7.3 Freezing

Although most of the vitamins are stable in frozen fruits and vegetables for periods of up to a year, losses of vitamin C have been found to occur at temperatures as low as −23°C.
### 19.7.4 Dehydration

Studies on the dehydration of blanched vegetables show that the dehydration process can result in additional losses. The dehydration of blanched cabbage (unsulphited) gave an additional 30% reduction in vitamin C content, 5 to 15% in the niacin content and about 15% of the thiamin.

### 19.8 Food product shelf life and its determination

As the tendency to include nutritional information on the labels of food products has increased, so have the liabilities of the manufacturers. For many, if not most, foods, the inclusion of nutrition information is optional but any statements made on the label come under the force of law. A company making an inaccurate voluntary nutritional declaration can be subject to prosecution.

Within a nutritional information statement, vitamins are the main category of declared nutrients where the quantities can significantly decrease during the shelf life of the food. The vitamin content of processed foods can decrease during storage and it has already been pointed out that losses of vitamin C can occur in frozen vegetables stored at \(-23\,^\circ\text{C}\) (4.2.9). If declarations of vitamin levels are required on the label, whether voluntary or statutory, the manufacturer needs to carry out suitable stability trials to determine the stability of each vitamin claimed on the label over the duration of the declared shelf life. The actual procedures used for the study will depend on the composition of the food, the processing and the form in which it is presented and stored. The type of packaging can have a significant effect on vitamin stability and the quality of the barriers to oxygen, moisture and light is very important. A requirement for label claims for vitamins can influence the selection of the form of packaging. The need to retain the vitamins often means that a compromise has to be achieved between the length of required shelf life and the barrier quality of the packaging.

Because of the wide variety of products, processes and packaging, it is not possible to give specific procedures for the determination of the shelf life of vitamins in a food. However, guidelines have been established for the determinations and predictions of shelf life. The determination of the vitamin levels at each stage of the shelf life study should be built into the protocol. As the degradation of most of the vitamins follows 'first order' or 'zero order' kinetics, it is possible for shelf life predictions to be made using a classical Arrhenius model on the assumptions that the model holds for all the reactions being studied; that the same reaction mechanism occurs throughout the temperature range of the study; that the energy of activation is between 10 and 20 \(\text{kcal mol}^{-1}\); and that the effects of moisture at ambient temperature are equivalent to maintaining the same relative humidity at higher temperatures.
19.8.1 Vitamin overages

No two vitamins will degrade at the same rate in a food at any one set of conditions. Where it is possible to add vitamins to a food, either to restore loss or to fortify the food, the food technologist has to determine the rates of deterioration of each vitamin and then increase the amount added to the product during manufacture to ensure that the label claim is met throughout the life of the product. The difference between the formulated and declared levels is known as the ‘overage’. The amount of overage will vary according to the inherent stability of the vitamin, the conditions under which the food is processed and packed, the packaging materials selected and the anticipated shelf life of the product.

Overages are normally expressed as a percentage of the declared value so that an input of 45 mg of vitamin C and a declared amount of 30 mg would give an overage of 50%. For food supplements where the added vitamins are the only significant source of these nutrients in the food, the overages are usually calculated as a percentage of the amount required in the product at the end of its shelf life.

When determining the overage for a vitamin in a product, consideration must also be given to the total amount of the vitamin in the product, particularly in the case of vitamins A and D where there may be safety concerns. As vitamins A and D are often the most unstable vitamins in a product, overages tend to be higher than those of the other vitamins. The consequences of large overages must be considered and, at all times, the amount of overage added must be the minimum necessary and well within any safety levels for the vitamin.

The shelf life of a product is often dictated by commercial pressures, which must take into account the time taken for the product to reach the consumer and the range of temperatures that it may be subjected to during the time between its manufacture and sale to the consumer. Once this information has been established, the vitamin overages to achieve the required shelf life have to be assessed. The only realistic estimations of the shelf life and required overages are those obtained by stability trials on the product carried out in the packaging to be used and at the anticipated storage conditions.

Methodology using the Arrhenius model has been developed which allows predictions to be made for both shelf life and overages.\(^{38,39}\) As already stated this is based on the assumption that the degradation of most of the vitamins follow ‘first order’ or ‘zero order’ kinetics. The precision of the technique has been found to be related to the number of storage temperatures that can be used and the number of samples that can be taken from each temperature. Typical storage temperatures used for stability studies are 0, 25, 35, 40 or 45, and 50 or 55°C. Ideally, at least three temperatures should be used and the selection of the higher temperatures depends on the composition of the product under test, as phase changes (e.g. solid to liquid) during storage should be avoided. The tests should
normally run for at least 24 weeks with samples from each temperature being removed at predetermined intervals and stored at 0 °C. All samples are stored at 0 °C until the final samples are taken and then they are all assayed at the same time. The data is analysed using the Arrhenius equations. The data obtained enables estimates to be made of overage amounts for each vitamin to meet a given shelf life; it assists in the comparison of different packaging materials and also helps to identify potential stability problems. Although it can be demonstrated that the technique has some limitations, work has shown that, if all the experimental controls are maintained, useful predictions of a product’s stability can be obtained. A comparison of the predicted and actual vitamin losses in a multivitamin tablet is given in Table 19.4.

### Table 19.4

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Predicted loss (%)</th>
<th>Actual loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>43.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>24.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Vitamin B_{12}</td>
<td>9.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Folic acid</td>
<td>12.0</td>
<td>10.5</td>
</tr>
</tbody>
</table>

19.9 Protection of vitamins in foods

For all products for which claims for vitamins are intended, it is essential that all stages of the processing, handling and storage of the product are evaluated to minimise the degradation of the vitamins. This can be accomplished by keeping residence times at high temperatures to a minimum and reducing or eliminating exposure to light and oxygen. For example, during the processing of fruit juices, fruit squashes and fruit drinks, the de-aeration of the solution can have a protective effect on the vitamin C levels in the product by reducing or eliminating the oxygen. Commercial sources of vitamins for addition to foods can be obtained in forms that have been encapsulated or coated to improve their stability. However, some of these preparations can only be used in low moisture foods. A good knowledge of vitamin stability is essential during the early stages of product development.
19.10 References