

Paper No. 01

Paper Title: Food Chemistry

Module-25: Fat Soluble Vitamins: Properties, Stability & Modes of degradation

Introduction

Vitamins, by their definition, are essential to health and have to be obtained from the diet on a regular basis because, 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 James Lind discovered an association between limejuice and scurvy in 1753, it was over 170 years later that vitamin C was eventually isolated. The understanding of vitamin B12 goes back only to the 1950s and new roles for folates were still being discovered in the late 1990s. 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.

The vitamins

Vitamins are a heterogeneous group of substances and are vital nutrients that must be obtained from the diet. Although a number of these 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 of which there are four (vitamins A, D, E and K) and the water-soluble vitamins of which there are nine (vitamins C, B1, B2, B6, B12, niacin, pantothenic acid and biotin). They are listed in [Table 25.1](#). 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 needed 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 25.1. Vitamins and some commonly used synonyms

<i>Fat-soluble</i> Vitamins	Synonyms
vitamin A	retinol
vitamin D2	ergocalciferol
vitamin D3	cholecalciferol
vitamin E	alpha, beta and gamma tocopherols and alpha tocotrienol
vitamin K1	phylloquinone, phytylmenadione
vitamin K2	farnocoumarin, menaquinone
vitamin K3	menadione

Factors affecting vitamin stability

One of the very few attributes that the vitamins have in common is that none is completely stable in foods. 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 B12. The factors that affect stability vary from vitamin to vitamin and the principal ones are summarised in [Table 25.2](#). 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.

Table 25.2. Factors affecting the stability of vitamins

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- Temperature
 - Moisture
 - Oxygen
 - Light
 - pH
 - Presence of metallic ions (e.g. copper, iron)
 - Oxidising and reducing agents
 - Presence of other vitamins
 - Other components of food (e.g. sulphur dioxide)
 - Combinations of the above
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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 itself.

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 food 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 (solid, liquid, etc.), the packaging and probable storage conditions and is achieved by conducting well-designed stability tests.

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 *b*-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.

Vitamin A is one of the more labile vitamins and retinol is 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. The

natural vitamin A sources usually contain about one-third neovitamin A while 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. While 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.¹ 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.

A provitamin is a compound that can be converted in the body to a vitamin and there are a number of carotenoids with provitamin A activity. Carotenoids 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 *b*-carotene. The stability of the carotenoids is similar to 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 *b*-carotene was greatly enhanced by sulphur dioxide added either as a sulphite solution to cellulose powder prior to *b*-carotene absorption or as a headspace gas in containers of *b*-carotene. While it was found that the *b*-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 hours for *b*-carotene samples stored in oxygen only, 120 hours in nitrogen and 252 hours in sulphur dioxide.

Investigations into the effect of sulphur dioxide treatment on the *b*-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 *b*-carotene in dehydrated products.³ Studies on the heat stability of both *a*-carotene and *b*-carotene showed that the *b*-carotene was about 1.9 times more susceptible than *a*-carotene to heat damage during normal cooking and blanching processes. Products containing *b*-carotene should be protected from light and headspace air 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 α 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. Whilst the natural sources of the tocopherols, which also have the highest biological activity, are in the *d* form, the 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. While vitamin E is regarded as being one of the more stable vitamins, the unesterified tocopherol is less stable due 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. 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. It is stable to heat in the absence of air but is degraded if heated in the presence of air and 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 cotton-seed 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 ultra-violet irradiation of 7-dehydrocholesterol found in the skin. Human requirements are obtained both from the 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 normally 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. Crystalline vitamin D₂ 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, D₃, is also destroyed by atmospheric oxygen but is relatively more stable than D₂, possibly due to the fact that it has one less double bond. The vitamin D₃ 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 K₁ (phytomenadione or phylloquinone) is found in green plants and vegetables, potatoes and fruits, while vitamin K₂ (menaquinone) can be found in animal and microbial materials. The presence of double bonds in both vitamins K₁ and K₂ makes them liable to isomerisation. Vitamin K₁ has only one double bond in the side chain in the 3-position whereas in K₂ double bonds recur regularly in the side chain. Vitamin K₁ exists in the

form of both *trans* and *cis* isomers. The *trans* isomer is the naturally occurring form and is the one that is biologically active. The *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 K1 is only slowly decomposed by atmospheric oxygen. Vitamin K is rarely added to food products and the most common commercially available form is K1 (phytomenadione), which is insoluble in water. A water-soluble K3 is available as menadione sodium bisulphite.

Stability of Fat soluble vitamins

Vitamin	Neutral	Acid	Alkaline	Air/Oxygen	Light	Heat	Max. cooking loss
Vitamin A	S	U	S	U	U	U	40
Carotenes	S	U	S	U	U	U	30
Vitamin D	S	S	U	U	U	U	40
Vitamin K	S	U	U	S	U	S	5
Tocopherols	S	S	S	U	U	U	55

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

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.

1. Blanching

In terms of blanching it was found that a high temperature–short time water blanch gave a better vitamin retention than a low temperature–long time blanch and that, overall, steam blanching was superior to water blanching. The addition of sulphite to the blanching water has been shown to affect significantly thiamin levels in fruits and vegetables. Beta 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%.

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 levels assayed immediately after the heat processing were between 15 and 45% of the fresh product and these values 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 levels 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.

Beta 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, with the more severe the process, the greater the loss. 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 sterilization 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 the 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.

B-vitamin 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 minutes. 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.

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 .

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.

5. 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 (e.g. up to 1 kilogray), the losses for most vitamins are not significant. At higher doses (3– 10kGy) 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.

Vitamins and food product shelf-life

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°C .

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.

Due to 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 follow '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 kcal/mole and that the effects of moisture at ambient temperature are equivalent to maintaining the same relative humidity at the higher temperatures.

Where it is possible to add vitamins to a food either to restore losses during processing or to fortify the food, it is common practice to add an amount above the label claims to compensate for losses during storage. This additional amount is called an overage and is normally quoted as a percentage of the claimed level. For example, if a label claim is made for 60 mg/serving of vitamin C and it is determined that a 10% overage is required to achieve a stored shelf life of one year, the input of vitamin C would be 110% of label claim, or 66mg/serving. The amount of overage added should be reasonable and well within any safety concerns for the vitamin.

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 deaeration 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.
