# 1
# Vitamins, Biopigments, Antioxidants and Related Compounds: A Historical, Physiological and (Bio)technological Perspective

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## 1.1
## Historical Aspects of the Search for Vitamins

In hindsight, the history of organic compounds that are now called *vitamins* can be traced back to the ancient Egyptians; they experienced that feeding animal liver to a person would help cure night blindness, an illness now known to be caused by vitamin A deficiency. About 400 BC, the Greek physician – and father of Western medicine – Hippocrates of Kos (460 to 370 BC) reported via his 'Corpus Hippocraticum' that eating liver could cure the same vision problem. Indeed, the value of eating certain foods to maintain health was thus recognised long before vitamins were ever identified (Bender, 2003).

In the thirteenth century, the Crusaders frequently suffered from scurvy, now known to be caused by a lack of vitamin C in their food (Carpenter, 2012). Scurvy was a particular deadly disease in which the tissue collagen is not properly formed, causing poor wound healing, bleeding of the gums, severe pain and, finally, death. It had also long since been a well-known disease, appearing towards mid-winter in Northern European countries. Much later, in the sixteenth century, the therapeutic effects of lemon juice against scurvy (then named scorbut) became gradually known during long sea and ocean discovery voyages. The disease name, scorbut, seems to be derived from the Old Nordic 'skyr-bjugr', meaning 'sour milk-abscess', believed to be caused by continuous use of sour milk or 'skyr' as main food on long sea journeys; the Medieval Latin term was *scorbutus*, later known as *Sceurbuyck* in French, *Scheurbuyck* in Dutch and *scorbuicke* in English and then as *scorbut*, but it is now known as *scurvy*. The chemical name of vitamin C, L-ascorbic acid, is actually derived from these old names (Davies, Austin and Partridge, 1991). Scurvy had caused the loss of most ship crew members on Vasco da Gama's journey rounding the Cape of Good Hope in 1499 and those of Ferdinand Magellan during his first circumnavigation of our globe during 1519–1522. The Scottish physician James Lind, a pioneer in naval hygiene, studied this disease in 1747 and described, in 1753, in his book 'A treatise of the scurvy', the beneficial effect of eating fresh vegetables and citrus fruits in preventing it. He recommended that the British Royal Navy use lemons and limes to avoid scurvy; this led to the nickname 'limeys' for

British sailors at that time. However, these findings were not widely practiced even by the Royal Navy's Arctic expeditions in the nineteenth century, where it was believed that scurvy could be prevented by practising good hygiene and exercise, rather than by a diet of fresh food. (Ant)Arctic expeditions thus continued to be plagued by scurvy and other deficiency diseases further into the twentieth century. The prevailing medical theory was that scurvy was caused by tainted canned foods!

For another nutritional deficiency disease (vitamin $B_3$ or niacin deficiency) already described for its dermatological effects in 1735 by Gaspar Casal in Spain, the Italian medical doctor Francesco Frapoli used the name pellagra (pelle = skin; agra = rough), referring to a rough skin appearance. Pellagra was common in people who obtained most of their food energy from maize, notably in the Americas, but also in Africa and China. Its emergence also depended on neglecting the once common practice of the 'nixtamalisation' process – a special method of milling the whole dried corn kernel – making niacin, bound as niacytin, nutritionally available in the kernel.

In the nineteenth century, in Japan, the Hikan child diseases (keratomalacia or necrosis of the cornea and xerophthalmia or eye dryness) were successfully treated by including cod liver oil, eel fat or chicken liver, as a source of vitamin A, in the diet. It was also found that cod liver oil and also direct sunlight had a curing effect on rickets (vitamin D deficiency), a disease already well described by the English physician Daniel Whistler in 1645 and based on earlier observations of his colleague Francis Glisson. During the late eighteenth and early nineteenth centuries, the use of food deprivation studies, especially with mice and rats, but also with humans, allowed scientists gradually to isolate and identify a number of vitamins. Lipids from fish oil were successfully used to cure rickets in rats, and the fat-soluble nutrient was named 'antirachitic A or vitamin A'; this first vitamin 'bioactivity' ever isolated, which cured rickets, is now named vitamin D.

In 1881, the Russian surgeon Nikolai Lunin, while studying the effects of scurvy at the University of Tartu (now Estonia), compared the effects of feeding mice with milk versus an artificial mixture of then known milk constituents (proteins, fats, carbohydrates and salts); the mice that received only the individual milk constituents died, while those fed milk developed normally. He concluded that 'a natural food such as milk must therefore contain small quantities of unknown substances essential for life'.

In the Far East, when hulled rice was replaced by dehulled or polished white rice as the staple food of the middle class, a sharp increase in the occurrence of beriberi, a Sinhalese term meaning 'serious weakness' (due to lack of vitamin $B_1$), was observed, and it became an endemic disease. In 1884, Takaki Kanehiro, a British trained medical doctor of the Imperial Japanese Navy, observed that beriberi was endemic among the low-ranking crew, just eating rice, but not among officers who also consumed a Western-style diet. He experimented with using crews of two battleships: one was fed only white rice, and the other received a diet of meat, fish, barley, rice and beans. The group that ate only white rice reported 161 crew members with beriberi and 25 deaths, while the other group had only 14

cases of beriberi and no deaths. This convinced the Japanese Navy that the white rice diet was the cause of beriberi.

In 1897, the Dutch physician Christiaan Eijkman, working in what is now Indonesia, further observed that poultry fed with polished rice developed polyneuritis, a disease similar to human beriberi. This disease could also be prevented and cured by feeding rice and the silver fleece of the rice kernel; his co-worker, Gerrit Grijns hypothesised that beriberi was caused by a 'protecting factor' (later known as *vitamin $B_1$*) that was obviously lacking in dehulled rice. In 1898, the English biochemist Frederick G. Hopkins postulated that some foods contain 'accessory factors', in addition to proteins, carbohydrates, fats and nucleic acids that are necessary for the healthy functioning of the human body. Later, Hopkins and Eijkman were awarded the Nobel Prize for Physiology/Medicine in 1929 for their research on vitamins. Around 1910, F.G. Hopkins in the United Kingdom and T.B. Osborne and L.B. Mendel in the United States initiated research on modern vitamins with animal models and substantiated a theory, stating that diseases, such as night blindness, scurvy, pellagra, rickets, beriberi, hypocobalaminemia and paraesthesia, were the result of a lack of certain essential food components in the diet. We know now that all these aforementioned diseases are the result of nutritional vitamin deficiencies, that is, vitamin A, vitamin C, vitamin $B_3$ or niacin, vitamin D, vitamin $B_1$ or thiamine, vitamin $B_{12}$ and vitamin $B_5$ deficiencies (Rosenfeld, 1997).

## 1.2 Vitamins: What's in a Name

The first vitamin complex was isolated in 1910 by the Japanese scientist Umetaro Suzuki, who succeeded in extracting a water-soluble complex of micronutrients from rice bran that prevented beriberi and named it 'aberic acid'. He published his discovery as an article in a Japanese scientific journal that, however, in a more accessible German translation failed to mention that it was a novel nutrient, thus gaining little attention! In 1912, the Polish biochemist Casimir Funk isolated the same beriberi-preventing complex of micronutrients from rice bran, displaying chemical properties of an amine; this led him in 1912 to coin the name 'vitamine' for this type of 'vital amine' compounds (Piro *et al.*, 2010). Funk also found in aqueous extracts of brewer's yeast a growth-promoting additive for the diet of young rats; it was called *vitamin B complex*. This vitamin B complex was, in the coming decades, to be resolved into its component vitamins: $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, $B_7$, $B_9$ and $B_{12}$. The name 'vitamine' soon became synonymous with Hopkins' 'accessory factors', and by the time it was shown that not all vitamins are amines, this word was already in general use. In 1920, Jack Cecil Drummond proposed that the final 'e' be dropped to de-emphasise the 'amine' reference, as more researchers began to realise that not all vitamins have an amine moiety.

In 1913, American nutritional biochemists Elmer V. McCollum and M. Davis demonstrated a lipo-soluble factor A in butter fat and egg yolk, and in 1915, a

**Table 1.1** Discovery years of vitamins and their original source.

| Discovery year | Vitamin | Used food source |
|---|---|---|
| 1910 | $B_1$ (Thiamine) | Rice bran, yeast |
| 1913 | A (Retinol) | Cod liver oil |
| 1920 | $D_3$ (Calciferol) | Cod liver oil |
| 1920 | $B_2$ (Riboflavin) | Meat, dairy, eggs |
| 1922 | E (Tocoferol) | Wheat germ oil, unrefined vegetable oils |
| 1926 | $B_{12}$ (Cobalamin) | Liver, animal products, eggs |
| 1928 | C (Ascorbic acid) | Citrus |
| 1928 | F (Essential fatty acids) | Plant oils |
| 1929 | $K_1$ (Phylloquinone) | Leaf vegetables |
| 1930 | F (Essential fatty acid) | Plant oils |
| 1931 | $B_5$ (Pantothenic acid) | Meat, whole grains |
| 1931 | $B_7$ (Biotin) | Meat, dairy products, eggs |
| 1934 | $B_6$ (Pyridoxine) | Meat, dairy products |
| 1936 | $B_3$ (Niacin) | Meat, grains |
| 1941 | $B_9$ (Folic acid) | Leafy vegetables |
| 1957 | $Q_{10}$ (Ubiquinone) | Beef heart tissue |

water-soluble factor B was found in wheat germ. It was Drummond who, in 1920, named the fat-soluble factor vitamin A; the water-soluble anti-beriberi factor was named vitamin B; the water-soluble anti-scorbut factor was first isolated in 1928 and named hexuronic acid, now vitamin C. In 1925, the fat-soluble anti-rickets factor was named vitamin D. After the 1920s, discovery and isolation of several other vitamins followed relatively quickly (see Table 1.1), and their structures, nutritional and chemical properties and chemical synthesis were studied in great detail in the following two decades.

In 1930, the Swiss chemist Paul Karrer elucidated the structure of beta-carotene, the main precursor of vitamin A and identified other carotenoids as pigments. Karrer and the British chemist Norman Haworth also made significant contributions to the chemistry of flavins, leading to the identification of riboflavin, for which they received the Nobel Prize in Chemistry in 1937. In 1931, the Hungarian physiologist Albert Szent-Györgyi and a fellow researcher, Joseph Svirbely, suspected that 'hexuronic acid' was actually vitamin C; they gave a sample to Charles Glen King, who proved its anti-scorbutic activity in his long-established guinea-pig scorbutic assay. In 1937, Szent-Györgyi received the Nobel Prize in Physiology/Medicine. In 1943, American biochemist Edward Albert Doisy and Danish biochemist Hendrik Dam were awarded the Nobel Prize in Physiology/Medicine for their discovery of vitamin K and for the elucidation of its chemical structure. In 1967, American George Wald became a Nobel laureate for his discovery that vitamin A participated directly in the physiological and chemical processes in the visual cycle.

Vitamin nomenclature was initially based on the use of letter symbols alphabetically arranged according to the time of discovery; soon it appeared that one-letter

named vitamins were multiple complexes, and this led to the addition of an index to the original letters ($B_1$, $B_2$, … ). Often, when the function of the vitamin became known, an appropriate letter symbol was chosen, that is, vitamin K, with K being the first letter of the German word 'Koagulation'; other names reflected deficiencies, that is, aneurin ($B_1$, now thiamine) for anti-polyneuritis vitamin; vitamin PP ($B_3$ or niacin) stood for 'pellagra-preventing' vitamin. Reasons that the list of vitamins skips certain letters of the alphabet are given as follows: certain compounds were discarded as false leads, were reclassified over time or were renamed because of being part of a complex. Letter names or trivial names are generally more in use than the IUPAC names. The division into fat-soluble and water-soluble vitamins as introduced about 100 years ago by McCollum and Davis is still universally in use today (Eggersdorfer *et al.*, 2012).

Another term that is often encountered in vitamin nomenclature is 'vitamer': by definition, a vitamer of a particular vitamin refers to any of a number of chemical compounds, generally having a similar molecular structure, each of which shows varying vitamin activity in a vitamin-deficient biological system (Table 1.2). As an example, vitamin A refers to at least six vitamer chemical structures, each displaying slightly differing properties: four of these are found naturally in plant foods and are carotenoids; the retinol and retinal forms occur in animal-based foods, and these are several times (up to six times) as effective in humans as the carotenoid forms; for example, the carotenoid forms of vitamin A cannot be absorbed by cats and ferrets and therefore display no vitamin A activity in them.

**Table 1.2** List of vitamins by generic descriptor, with some of their vitamers including active forms.

| Vitamin generic descriptor name | Vitamer chemical name(s) or chemical class of compounds |
|---|---|
| Vitamin A | Retinol, retinal and four carotenoids: the carotenes alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll, beta-cryptoxanthin |
| Vitamin $B_1$ | Thiamine, thiamine pyrophosphate (TPP) |
| Vitamin $B_{12}$ | Cyanocobalamin, hydroxycobalamin, methylcobalamin, adenosylcobalamin |
| Vitamin $B_2$ | Riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) |
| Vitamin $B_3$ | Niacin (nicotinic acid), niacinamide |
| Vitamin $B_5$ | Pantothenic acid, panthenol, pantetheine |
| Vitamin $B_6$ | Pyridoxine, pyridoxamine, pyridoxal, pyridoxal 5-phosphate |
| Vitamin $B_9$ | Folic acid, folinic acid, 5-methyltetrahydrofolate |
| Vitamin C | Ascorbic acid, Dehydroascorbic Acid, calcium ascorbate, sodium ascorbate, other salts of ascorbic acid |
| Vitamin D | Calcitriol, ergocalciferol ($D_2$), cholecalciferol ($D_3$) |
| Vitamin E | Tocopherols (alpha, beta, gamma and delta-tocopherol), tocotrienols (alpha-, beta-, gamma-, delta-tocotrienols) |
| Vitamin F | Linoleic acid and alpha-linolenic acid |
| Vitamin K | Phylloquinone ($K_1$), menaquinones ($K_2$), menadiones ($K_3$) |

## 1.3 Physiological Functions of Vitamins and Related Compounds

From a chemical point of view, vitamins are a very heterogeneous and diverse group of organic compounds, yet they can be considered as a particular single group of molecules. A vitamin is an organic chemical, an essential vital nutrient that an organism requires in limited amounts, one that the organism cannot synthesise on its own in sufficient quantities and that normally must be obtained through the diet. This implicates that the term 'vitamin' is conditional upon a particular organism and the food habits and situation. Vitamin C is a vitamin for humans, but not for most other animal organisms (except primates, guinea pigs, bats, some birds and fishes) nor for plants or microbes. Vitamin supplementation is important for the treatment of certain health conditions and for malnutrition as indicated earlier (Bender, 2003).

There are other essential nutrients such as dietary minerals and essential amino acids that are usually not considered as vitamins by convention. However, over the past decades, novel vitamin-like compounds have been discovered and studied in every detail and are now being considered as real vitamins; some are already commercialised. They include the essential fatty acids (EFAs), also called *vitamin F* or *polyunsaturated fatty acids* (PUFAs), the coenzyme ubiquinone (vitamin $Q_{10}$) and several quinoprotein factors such as pyrroloquinoline quinone (PQQ). Other molecules are still considered as growth factors and include inositol, glutathione, L-carnitine, carnosine, gamma-aminobutyric acid (GABA) and flavonoids. They perform diverse essential physiological functions or behave as antioxidants.

Most vitamins have to be provided via daily food/feed intake, but certain vitamins can be formed partially or indirectly within the body. Examples are:

- compounds – often called provitamins – with no apparent or low vitamin activity that can be converted into a vitamin within the body:
  - provitamin A or beta-carotene (in vegetables and fruits) converted into vitamin A
  - the amino acid tryptophan (in protein-rich food) converted into vitamin $B_3$ (niacin)
  - provitamin $B_5$ (panthenol) converted into vitamin $B_5$ (pantothenic acid)
  - provitamin $D_2$ or ergosterol (in yeasts, fungi, plants) converted into vitamin $D_2$ (ergocalciferol)
  - provitamin $D_3$ or 7-dehydrocholesterol (in our skin) converted into vitamin $D_3$ (cholecalciferol).
- other vitamins that are formed by the intestinal microbiota (Guarner and Malagelada, 2003; Le Blanc *et al.*, 2013), that is,
  - vitamin $K_2$ (menaquinone)
  - some B vitamins ($B_1$ or thiamin, $B_2$ or riboflavin, $B_7$ or biotin, $B_{12}$ or cobalamin)

It is also well known that most fermented foods and drinks are enriched in their vitamin content derived from the beneficial microorganisms involved in their production by fermentation (Farnworth, 2003).

Vitamins have a catalytic role in the body, in enabling optimal biosynthesis, conversion and degradation of macromolecules, such as nucleic acids, proteins, lipids and carbohydrates or their building blocks. The physiological/biochemical function of most water-soluble vitamins is now well known: they are part of coenzymes, involved in enzymatic group transfer and thus responsible for specific biochemical reactions to occur (Padh, 2009). A survey is summarised in Table 1.3; see also Table 1.5.

The physiological functions of fat-soluble vitamins and water-soluble vitamin C are more varied and complex. Some examples are given in Table 1.4; see also Table 1.5.

Much debate exists about the positive effects of high doses of water-soluble vitamins on human and animal physiology; on the other hand, several hypervitaminoses of fat-soluble vitamins are well known. Compounds that specifically counteract the functioning of vitamins are known as *antivitamins* or *vitamin antagonists*; their negative action can be based on degradation of the vitamins or on the complexation of the vitamins into a non-resorbable complex, that is, avidin (in raw egg white) with biotin. Dicoumarin excludes vitamin K from the prothrombin synthesis system, and amethopterin is an antagonist of folic acid. Antivitamins present in our daily food are usually destroyed during food processing and cooking.

**Table 1.3** Water-soluble vitamins and their corresponding coenzymes.

| Vitamin | Coenzyme | Group transfer |
|---|---|---|
| $B_1$ (Thiamine) | Thiamine pyrophosphate (TPP) | $C_2$-aldehyde, decarboxylation |
| $B_2$ (Riboflavin) | Flavin adenine mononucleotide (FMN) | Hydrogen |
| | Flavin adenine dinucleotide (FAD) | Hydrogen |
| $B_3$ (Niacin) | Nicotinamide adenine dinucleotide (NAD+) | Hydrogen |
| | Nicotinamide adenine dinucleotide phosphate (NADP+) | Hydrogen |
| $B_5$ (Pantothenic acid) | Coenzyme A | Acyl |
| $B_6$ (Pyridoxine) | Pyridoxalphosphate | Amino, decarboxylation |
| $B_7$ (Biotin) | Biocytin | Carboxyl |
| $B_9$ (Folic acid) | Tetrahydrofolic acid | Formyl |
| $B_{12}$ (Cyanocobalamin) | $B_{12}$ coenzyme | Carboxyl, H-X rearrangements |

**Table 1.4** Physiological functions of vitamin C and fat-soluble vitamins.

| Vitamin | Important physiological functions |
|---|---|
| Vitamin C | Cosubstrate of monooxygenases; role in redox reactions; hydroxylation of amino acids; hormone synthesis; iron absorption |
| Vitamin A | Active form (11-*cis*-retinol) is part of rhodopsin, the light-sensitive molecule in the eye; biosynthesis of proteoglycans; epithelial cell formation; immunostimulation |
| Vitamin $D_3$ | Active form (1,25-dihydroxycholecalciferol) regulates Ca and P metabolism; bone and teeth formation; prevention of osteoporosis |
| Vitamin E | Antioxidant towards unsaturated compounds; protects membrane integrity |
| Vitamin F | Long-chain polyunsaturated fatty acids form prostaglandins, thromboxanes and related compounds having physiological effect in the body such as being anti-inflammatory, preventing platelet aggregation |
| Vitamin $K_1$ | Formation of $\gamma$-carboxyglutamate residues in osteocalcin; bone formation |

## 1.4 Technical Functions of Vitamins and Related Compounds

In addition to their nutritional, physiological and medical importance, vitamins and related compounds have also found large-scale technical applications, for example, as antioxidants (D-isoascorbic acid as the $C_5$-epimer of vitamin C, glutathione, vitamin E), as acidulants (vitamin C) and as biopigments (carotenoids, riboflavin) in the food, feed, cosmetic, chemical, nutraceutical and pharmaceutical sectors. There is a special need for natural pigments of (micro)biological origin to replace synthetic pigments and colourants; certain carotenoids (beta-carotene, lycopene, astaxanthin) and *Monascus* pigments have already been used in this respect (Vandamme, 2002, 2011; Patakova, 2013). Details about technical applications of vitamins, pigments, antioxidants and other related molecules are discussed in the corresponding chapters in this volume.

## 1.5 Production and Application of Vitamins and Related Factors

The staple food of humans, including cereals, rice, potato, vegetables, fruits, fish, meat, milk and eggs, forms the basic source of vitamins and related growth factors. Adequate nutrition should thus supply this daily need of vitamins. This need, however, increases with an unbalanced diet, physical exercise, pregnancy, lactation, active growth, re convalescence, drug abuse, stress, air pollution and so on. Pathological situations, such as intestinal malresorption, stressed intestinal microbiota, liver/gall diseases, treatment with drugs, antibiotics or hormones and

**Table 1.5** Survey of the vitamins with main food sources, deficiency diseases, Recommended Dietary Allowance (RDA) and overdose diseases.

Vitamin A
Food sources: *retinol (in animal-derived food): liver, meat, butter, margarines, fatty fish, milk and derived products, cheese, egg yolk
*Provitamin A carotenoids (in plant-derived food): leafy vegetables, spinach, carrots, yellow and orange fruits
Deficiency diseases: night blindness, hyperkeratosis, keratomalacia, dry and scaly skin, brittle hair
Recommended dietary daily allowances (RDA): 350–750 μg (as retinol)
Overdose: >7500 μg retinol/day; fatigue, liver intoxication

Vitamin D
Food sources: *cholecalciferol ($D_3$): formed in our skin + UV in sunlight; also from animal-derived food: butter, margarines, fatty fish (herring, eel, salmon, mackerel), milk, cheese, egg yolk
*Ergocalciferol ($D_2$): yeast, wheat germ oil, cabbage, citrus fruits
Deficiency diseases: rickets, osteomalacia, osteoporosis
RDA: 10–15 μg
Overdose: >50 μg/day; hypercalcemia

Vitamin E
Food sources: plant oils rich in vitamin F, nuts, seeds, vegetables, fruits, bread, grains, cereals
Deficiency diseases: heamolytic anaemia, neurological disorders
RDA: 1–10 mg/day per gram
Overdose: >1 g/day

Vitamin F (EFAs)
Food sources : fish, especially oily fish (sardines, herring, salmon, etc.), egg yolks
Deficiency diseases: absence of long-chain PUFAs in neonatal children has adverse effects on brain and eye development. Therefore, they are now added to infant formula in over 70 countries
RDA: 1–2% of total daily calorie intake
Overdose: no known effects if consumed in humans at up to 7 g/day; also safe up to 30 g/kg body weight when fed to rats

Vitamin K
Food sources: *vitamin $K_1$ (phylloquinone): green leafy vegetables, fruits, milk, meat, egg yolk, cereals
*Vitamin $K_2$ (menaquinone): via gut microbiota
Deficiency diseases: impaired blood coagulation; haemorrhage
RDA: 10–35 μg/day
Overdose: not known

Vitamin $B_1$ (thiamine)
Food sources: bread, cereals, potatoes, vegetables, pork meat, milk products, eggs
Functions: synthesis of nucleic acids; essential in carbohydrate and energy metabolism; nerve impulse functioning
Deficiency diseases: beriberi; Wernicke–Korsakoff syndrome; depression; memory loss; neurological disorders; heart damage
RDA: 0.3–1 mg/day
Overdose: not known

(*continued overleaf*)

**Table 1.5** (*Continued*)

Vitamin $B_2$ (riboflavin)
Food sources: milk and other dairy products (to be stored in the dark), meat (liver), vegetables, fruits, bread, cereals
Functions: essential role in metabolism of carbohydrates, proteins, lipids; promotes conversion of tryptophan into niacin; conversion of vitamin $B_6$ and vitamin $B_9$ into active forms; mobilisation of iron
Deficiency diseases: glossitis; inflammation of skin, mucous membranes, seborrhoeic dermatitis, vision problems, secondary iron deficiency due to intestinal malabsorption, impairs $B_6$ and $B_9$ activation
RDA: 0.4–1.6 mg/day
Overdose: not known

Vitamin $B_3$ (niacin)
Food sources: meat (liver), fish, whole meal bread, vegetables, potatoes, yeast, nuts
Functions: essential role in energy metabolism; involved in numerous enzymatic reactions (synthesis of fatty acids and cholesterol); DNA repair and stress responses
Deficiency diseases: pellagra, via diet mainly based on maize
RDA: 8–18 mg/day; expressed as niacin equivalents, NE: 1 NE = 1 mg nicotinic acid or nicotinamide and = 60 mg food source tryptophan ($B_2$ is involved in this conversion)
Overdose: >500 mg nicotinic acid per day; liver and eye damage; blood vessel dilatation

Vitamin $B_5$ (pantothenic acid)
Food sources: meat, eggs, whole grain cereals, vegetables, pulses, fruits, milk products
Functions: role in carbohydrate and fatty acid metabolism; synthesis of cholesterol and fatty acids; formation of red blood cells, formation of sex and stress-related hormones
Deficiency diseases: burning feeling in extremities, depression, irritability, vomiting, stomach pains
RDA: 2–12 mg/day
Overdose: diarrhoea, increase the risk of bleeding

Vitamin $B_6$ (pyridoxine)
Food sources: meat (chicken, beef liver, pork and veal), eggs, bread, grain products, potatoes, pulses, vegetables, milk and products, cheese
Functions: important role in energy metabolism, in polyunsaturated fatty acids, phospholipids and amino acid metabolism; production of hormones, red blood cells and cells of the immune system; controls (along with vitamin $B_{12}$ and vitamin $B_9$) homocysteine levels in the blood; improves conversion of tryptophan into niacin and into serotonin
Deficiency diseases: anaemia, depression and nervous system disorders; impairment of the immune system; inflammation of skin and mucosa
RDA: 0.4–1.7 mg/day
Overdose: >50 mg/day; irreversible neuropathy of limbs

Vitamin $B_7$ (biotin)
Food sources: yeast, kidney, eggs, liver, milk and milk products, nuts, pindas
Functions: role in energy metabolism and formation of fatty acids; maintaining healthy skin and hair
Deficiency diseases: seldom; anaemia; depression; cracking in the corners of the mouth, swollen and painful tongue; dry eyes; loss of appetite; fatigue; insomnia
RDA : 10–100 µg/day; also formed by intestinal microbiota
Overdose: not known

**Table 1.5** (*Continued*)

| |
|---|
| Vitamin $B_9$ (folic acid)<br>Food sources: whole grain products, bread, cereals, green vegetables, fruits, milk and dairy products<br>Functions: red blood cell formation; involved in metabolism of histidine, glycine, methionine, DNA and RNA synthesis in the presence of $B_6$ or $B_{12}$; maintenance of cells; development of the brain and spinal marrow in foetus<br>Deficiency diseases: macrocytic anaemia; birth defects (spina bifida, harelip, cleft palate); growth retardation; increased homocysteine levels in the blood<br>RDA: 50–400 µg/day; prevents spina bifida (neural tube defects) in babies<br>Overdose : overdose can mask $B_{12}$ deficiency |
| Vitamin $B_{12}$ (cyanocobalamin)<br>Food sources: meat and other animal products (milk and dairy, cheese, eggs); not present in plant-derived food<br>Functions: formation of red blood cells; nerve system functioning; controls, together with vitamin $B_6$ and vitamin $B_9$, homocysteine levels in the blood; production of nucleic acids<br>Deficiencies: pernicious anaemia; neurological disorders; memory loss; deficiency risk also caused by stomach surgery (insufficient secretion of 'intrinsic factor', IF) or intestinal diseases (Crohn's disease); heart disease<br>RDA: 0.5–2.0 µg/day; uptake depends on level of IF, secreted by parietal stomach gland cells<br>Overdose: >200 µg/day |
| Vitamin C (ascorbic acid)<br>Food sources: many fruits (citrus, kiwi, raspberry, strawberry, guava, mango), vegetables (Brussels sprouts, cabbage, paprika, potatoes)<br>Functions: formation of collagen; metabolism of sugars, proteins and lipids; muscle and brain metabolism; bone formation; hormone synthesis; iron uptake from food; immune defence; antioxidant<br>Deficiencies: scurvy; fatigue; retarded wound healing; dry and splitting hair; inflammation of the gums; decreased ability to ward off infection<br>RDA: 35–110 mg/day<br>Overdose: extremely high doses (>2–5 g/day) increase the risk of kidney stones; diarrhoea and gastrointestinal disturbances |

enzyme deficiencies, can also lead towards vitamin shortages despite sufficient intake. Malnourishment in many underdeveloped countries but equally wrong food habits in developed countries also ask for direct nutritional and medical remediation, combined with daily diet adjustment. Vitamin-enriched and medicated feed are used worldwide to procure healthy livestock. Overdose of vitamins, especially fat-soluble ones, but also some water-soluble ones (high doses of *C*, $B_3$, $B_5$, $B_6$, $B_9$), can lead to hypervitaminoses and diseases. Table 1.5 presents a survey of vitamins with main food sources, deficiency diseases, recommended dietary daily allowance (RDA) and overdose diseases.

Concentrates or extracts derived from these vitamin-rich natural staple food products (of plant, animal or microbial origin), however, find relatively little use in the food, feed, pharmaceutical or cosmetic sector. Some of the reasons are:

- the level of vitamins in the natural plant/animal source is usually relatively low and fluctuates drastically (i.e. exceptions are PUFAs in plant oils and fish oils, vitamin D in fish oils).
- their organoleptic presentation and shelf life are often not optimal.
- vitamins are labile molecules during the process of harvest, preservation, storage or preparation of foodstuffs and are generally sensitive to pH, heat ($B_2$, $B_5$, $B_6$, $B_9$, C, E), light ($B_2$, $B_6$, $B_9$, $B_{12}$, C, D), oxygen ($B_9$, C, D, F); water-soluble vitamins are easily lost by aqueous extraction or other manipulations of these natural food-vitamin sources.

These drawbacks have led to the industrial manufacturing of most vitamins and related factors. Currently, several vitamins are produced chemically (A, $D_3$, E, K and $B_1$, $B_5$, $B_6$, $B_7$, $B_9$), although microbiological/biotechnological methods exist or are being developed, though not economically profitable as yet (Demain, 2000, 2007; Laudert and Hohmann, 2011). Others are produced (exclusively) by microbial fermentation with bacteria and/or fungi (C, $D_2$, $B_2$, $B_{12}$, EFAs). Some are produced by a combination of chemical steps and microbial/enzymatic steps ($B_3$, $B_5$, C) (Vandamme, 1989, 1992; Eggersdorfer *et al.*, 1996; De Baets, Vandedrinck and Vandamme, 2000; Shimizu, 2008; Laudert and Hohmann, 2011). Some are produced via microalgal culture in ponds or fermenter vessels (beta-carotene, PUFAs) (Cadoret, Garnier and Saint-Jean, 2012; Borowitzka, 2013).

The detailed biosynthetic pathways (and their metabolic regulation and controls) used by those microorganisms have been almost fully elucidated for most vitamins and similar compounds but only over the past two decades, mainly by studying model microbial strains and/or producer microorganisms, such as bacteria (*Escherichia coli, Serratia, Bacillus, Lactobacillus, Pseudomonas, Gluconobacter, Sinorhizobium, Agrobacterium, Propionibacterium, Rhodobacter, Arthrospira*), yeasts (*Saccharomyces*, *Candida, Xanthophyllomyces, Yarrowia*), fungi (*Blakeslea*, *Ashbya, Mortierella, Mucor, Monascus*), as well as green microalgae (*Dunaliella*, *Euglena, Haematococcus*), marine non-photosynthetic dinoflagellates (*Crypthecodinium*) and marine non-photosynthetic thraustochytrid–microalgae (*Schizochytrium*) (Laudert and Hohmann, 2011; Borowitzka, 2013; Ledesma-Amaro *et al.*, 2013; Bellou *et al.*, 2014). For some of the vitamins and related factors, microbial overproduction still remains a challenge. In the future, the advent of synthetic biology will allow for the complete construction of tailor-made microbial vitamin producer strains (Wang, Chen and Quinn, 2012).

This volume focuses especially on the biotechnological aspects of vitamins and related compounds – on biosynthesis and on their production processes. Apart from obtaining these vitamins and related compounds via a natural process – which is what microbial fermentation, biocatalysis and algal culture are all about, fermentation-based or enzymatic biocatalytic processes furthermore yield the desired enantiomeric compound, and they can be redirected via genetic and biotechnological modification of the involved bacteria, yeast and fungi or microalgae into high-yielding production systems. Especially, the advancement

of genetic engineering techniques and the introduction of metabolic engineering have recently allowed high-yielding microbial strains that are suitable for industrial production of vitamins and related compounds to be constructed, and this has led to their wider application.

A broad range of applications now exists for these vitamin preparations (and similarly for related factors) in the food, feed, cosmetic, technical and pharmaceutical sectors:

- *Revitamination*: restoring the original vitamin level of a foodstuff.
- *Standardisation*: addition of vitamins to compensate for natural fluctuations.
- *Vitamin enrichment*: further addition of vitamins to a level higher than the original one.
- *Vitamination*: addition of vitamins to products lacking them.
- *Technical additive*: beta-carotene as pigment, vitamins C and E as antioxidants, riboflavin as yellow pigment.
- *Medical applications*: to alleviate hypo- or even avitaminoses.

## 1.6 Outlook

Vitamins and related compounds belong to those few chemicals with a strong positive appeal to most people worldwide. How they were discovered and how they are produced are not very well known nor understood by most people, layman as well as even academics, as long as they are widely available!

This volume hopes to contribute this understanding, not only to scientists, microbiologists, biochemists, nutritionists and medical people, but also to process biochemists and industrial biotechnologists already involved in – or attracted to – the production enigmas and application potential of vitamins, biopigments, antioxidants and related molecules.

Even today, vitamins remain to be seen as fascinating yet still elusive molecules!

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