Vitamins

Definition

Vitamins are essential organic nutrients which, in contrast to other nutrients (carbohydrates, fats and proteins) do not provide any energy or growth boosters, but are necessary for smooth metabolism in the body. Vitamins appear in a large number of natural foods. They are micronutrients, i.e. nutrients of which only a small quantity (a few mg or µg) has to be taken up in order to fulfil its metabolic function. However, the uptake of this small amount is essential, as they are not synthesised by the organism itself or only to an insufficient extent. There is a Recommended Daily Allowance (RDA) for every vitamin. The actual quantity required per individual is defined by a number of different factors such as age, gender, environment, state of health and stress. An unbalanced diet or certain disorders can lead to a vitamin deficiency and possible deficiency disorders. The intake of food supplements or enriching of foods and industrial animal foods with vitamins can avoid a deficiency in humans and animals.

Classification and function

The chemical and physiological properties of vitamins are highly diverse. At present, there are 13 known vitamins, whereby each vitamin represents a group of structurally similar organic compounds with varying biological activity, so-called vitamers. Provitamins are vitamin precursors, i.e. naturally occurring components that are transformed into ‘true’ vitamins by normal bodily metabolism.

Vitamins are divided into two groups based on their solubility properties. The fat-soluble vitamins contain vitamins A, D, E and K and around 50 carotenoids. The group of water-soluble vitamins is made up of vitamin C (ascorbic acid) and all B vitamins, namely thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 of H), folic acid (B11 or B9) and cyanocobalamin (B12). The solubility properties also define the distribution of vitamins in separate food groups and have direct effects for the applied analytical methods. Fat-soluble vitamins mostly occur in high fat foods and can be stored in fatty tissue and the liver. The water-soluble vitamins B and C appear in all kinds of foods. The body is not able to store these vitamins very well (except for vitamin B12) and an excess leaves the body via urine and sweat.

Vitamins are regulators of synthesis and degradation processes and form the foundations of co-enzymes, hormones and other substances. They play a role in growth, the repair and proper functioning of the body and above all have a catalytic function. The majority of water-soluble vitamins are transformed in vivo into co-enzymes that play a crucial role in combination with metabolic enzymes in the catabolism of nutrients to produce energy for the organism. A few vitamins are produced in the body (by intestinal flora bacteria and in the skin under the influence of sunlight). Plants have the ability to synthesise vitamins themselves, with the exception of B12, and they consequently serve as the major source of these essential food components.

The majority of vitamins are highly unstable compounds and are quickly broken down because of their sensitivity to heating, oxidation, radiation, moisture, water activity, pH, corrosive enzymes and the catalytic effects of metals. To increase stability, derivatives are mostly used for the addition of vitamins to animal and human foods.
Vitamin analysis

In addition to bioassays for the establishment of the true nutritional value of a vitamin in a product, in vitro analysis techniques such as microbiological assays (MBAs) or physicochemical analysis methods such as HPLC are mainly used for the analysis of enriched animal and human foods. Physicochemical assays allow the quantification of the main constituents which are responsible for biological activity and provide a high level of precision. Currently, (ultra) high performance liquid chromatography (U)HPLC is the preferred method for the detection of vitamin content. HPLC can be used during sample preparation to clean up extracts or to separate and quantify components. MBAs were developed at the start of the 1940s and often still constitute the official methods for the definition of various B vitamins, but in this area, HPLC and LC-MS are also on the rise. The general analytical procedure for microbiological and physicochemical assays can be divided into a number of important steps: taking of samples, extraction, possible clean-up, measurement and calculation of the result.

Figure 1: General diagram of the analysis procedure
Extraction of vitamins

The analyses begin with an extraction of the vitamins from the matrix to make their measurement possible. The chosen method for extraction depends on the required result, the nature of the matrix, the present natural or synthetic form of the vitamins, interfering constituents, resistance of the vitamin to heat and extreme pH values and the selectivity and specificity of the analytical method used. For successful determination, it is essential that the vitamins are quantitatively extracted from the matrix in a form that can be accurately measured by the analytical technique used. An efficient extraction procedure homogenises and concentrates the sample, isolates the analyte from its bond with protein, eliminates known interfering substances to the extent possible and destroys endogenous enzyme activity. Extraction methods for vitamins from food matrices include acid or alkaline hydrolysis (saponification), enzymatic hydrolysis, direct solvent extraction and solid phase extraction.

Liquid chromatography, suitable or not?

HPLC can be used for the determination of added vitamins in enriched human and animal foods. The definition of naturally occurring vitamins via HPLC is more complex due to the different vitamins and as a result there is a need for more complex extraction procedures to take the bound forms into account. HPLC is not sufficiently sensitive for an accurate measurement of the extremely low vitamin B12 and vitamin B8 content in food. HPLC is also a popular technique for the determination of vitamin C, despite the low absorption of dehydroascorbic acid and its lack of electrochemical activity. However, HPLC does not guarantee that all biologically active forms of a vitamin will be taken into account that could be measured during a microbiological assay and, in this respect, the inherent specificity of HPLC can lead to an underestimation of the total vitamin activity.

The use of reversed phase HPLC columns that are suitable for both 100% organic and 100% aqueous mobile phases allows for a separation of strong polar analytes in aqueous mobile phases and hydrophobic analytes in organic mobile phases. As a result, they are suitable for the separation of both fat-soluble and water-soluble vitamins and enable the simultaneous determination of certain vitamin combinations. Recent methods are above all directed towards the fast and efficient simultaneous determination of as many vitamins as possible in as many matrices as possible. The finding of an appropriate extraction method, the elimination of matrix effects and the optimisation of HPLC conditions however, represent a substantial challenge for the simultaneous determination of vitamins. The most widely used detection methods are UV and fluorescence detection, of which FL detection shows a higher sensitivity. Photodiode array detection on the other hand, offers the possibility to identify analytes via UV absorption at various wavelengths and can test peak purity at the same time. A more recent development is the use of LC-MS as a sensitive and selective means for the determination of one or more vitamins in food.
The future of vitamins

Over the coming years, increasing emphasis will be placed on the accurate definition of types of vitamins and vitamin concentrations in foodstuffs (food supplements, infant foods, baby foods, etc.) in European legislation. The introduction of stricter regulations makes the development of well validated, internationally accepted analytical methods with high accuracy and precision even more necessary. However, it is not clear whether the current standardised methods can be used for the definition of certain vitamins in certain matrices. Unfortunately, the development of new methods for the analysis of vitamins is a very slow process, above all for water-soluble vitamins. A harmonisation and/or extension of the current standardised methods and matrices of the various official organisations would already be a first step to ensure compliance with the new regulations and to maintain an overview of the available methods.

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"You have a fear of Thiamine, Riboflavin, Niacin, etc.,...better known as a Vitamin B Complex."