Techniques of Vitamins Analysis

Introduction

- Accurate quantitative measurements for vitamins are required to ensure product quality according to product specification and regulatory compliance as well as to monitor recommended daily vitamin intake.
- The food supplements legislation stipulates that the amount of any individual substance with a nutritional or physiological effect must be indicated on the labelling of the food supplement.
- Nowadays, vitamin determinations in foods are routinely performed using microbiological assays or physicochemical methods of analysis.
- Microbiological assays, measure the combined response of the active substances and take into account utilization at the cellular level.
- Physicochemical assays permit the quantification of the principal substances that are responsible for the biological activity, and can achieve a high degree of precision.
- The great drawback of in vitro analytical techniques is that they do not account for the complexities of mammalian digestion and absorption and thus do not provide a reliable estimate of vitamin bioavailability.
- This is particularly the case for vitamins which are chemically bound with other constituents of the food matrix.

- Since the mid-1970s up to the present, the method of choice for determining the fat-soluble vitamins in foods has been high-performance liquid chromatography (HPLC).
- This is due to the technique's ability to separate the vitamins without the need for chemical derivatization, the nondestructive operation, and the detection selectivity.
- HPLC can be used in the preparative mode to purify sample extracts, as well as in the quantitative mode.
- Fat-soluble vitamins (FSVs) are soluble in non-polar solvents.
- FSVs include vitamin A (retinol, retinyl acetate, retinyl palmitate), vitamin D (D2: ergocalciferol, D3: cholecalciferol), vitamin E (α-, β-, γ-, δ-tocopherols and tocotrienols) and vitamin K (K1: phylloquinone, K2: menaquinone).
- In general, vitamins are highly unstable and degrade rapidly under several conditions such as heat, oxygen, light, moisture and certain pH.

- Water Soluble Vitamins (WSVs) are essential micronutrients and includes vitamin C and B complex vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin(vitamin B7), folic acid (vitamin B9) and cobalamins (vitamin B12).
- WSVs act mainly in the metabolism of carbohydrates, lipids and proteins, and involved in physiological roles such as maintenance of healthy muscle, skin, eyes, hair and liver.
- As the human body is unable to synthesize and store WSVs (except vitamin B12), they must be obtained through daily intake from food.
- Analytical method for analysis of WSVs in food samples is complex due to low and different concentration, interaction with other compounds such as protein, complexity of food matrices and poor stability of vitamin solutions.
- Microbiological assays, developed in the early 1940s, remain the official methods for determining vitamin B6, pantothenic acid (B5), folate (B9), and vitamin B12.

Microbiological Assays

- Microbiological assay is applicable only to the B vitamins.
- The rate of growth of a species of microorganism that requires a vitamin is measured in growth media that contain various known quantities of a foodstuff preparation containing unknown amounts of the vitamin.
- One must select a microorganism sensitive to the substance under assay—it should be cultivable with ease; the growth response should be easily measurable and the response should be specific; and it should be non-pathogenic.
- https://www.slideshare.net/ChowdaryPavani/vita-microbial-assay

Association of Official Analytical Chemists (AOAC) Methods

- Several methods have been used for determination of vitamin C in foods, including spectrophotometry, electrophoresis, titration, and high performance liquid chromatography (HPLC).
- The oxidation-reduction titration method using indophenol dye as indicator was stablished as the official method for vitamin C determination by the Association of Official Analytical Chemists (AOAC) for many years.
- In this method, ascorbic acid is oxidized to dehydroascorbic acid and the indophenol dye is reduced to a colorless compound, indicating the end point of the reaction .
- The iodometric titration for vitamin C determination was the official method for Public Health Laboratories in Brazil.
- The endpoint of this titration is determined by the first excess of iodine in the solution, that reacts with the starch indicator, forming a complex with an intense dark blue-violet color.
- This method is single, fast, and reliable, however, just as for the AOAC method, the end point of the titration cannot be easily detected when the food sample has intense color.
- The alternative to exclude color interference was to transfer the traditional iodometric titration to automatic potentiometric titrator, as described in this work.
- Thus, the end point of titration is indicated by platinum ring electrode, and no color interference occurs.
- In the last decades, HPLC and UHPLC methods were developed in substitution to titration method, showing high accuracy, but these equipment cost is high.

High-performance liquid chromatography

- Many separation systems have been developed for the determination of folate vitamin using HPLC including reversed-phase, ion pair, and anion exchange types.
- The developments in the liquid chromatographic analysis of folates have been comprehensively reviewed by several workers.
- The water-soluble nature of the folates, together with differences in ionic properties and hydrophobicity, make these compounds well suited for either ion exchange, or reversed-phase HPLC.
- Analysis of various naturally occurring folate polyglutamate derivatives can be accomplished on hydrolysed extracts (as the corresponding folate monoglutamates), or as intact poly-γ-glutamylfolates.
- Monoglutamyl folates are usually separated by reversed-phase HPLC, or by ion pair techniques.
- In reversed-phase separations, suppression or enhancement of the ionization of functional groups by pH can effectively be used to regulate retention on the column.
- The pH, ionic strength, and polarity of solvents are used to optimize the separation.
- Usually low pH with or without gradient of the organic phase is used with C18 or phenyl supports.

Ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS)

- The determination of vitamins in food represents a complex analytical problem.
- Vitamins naturally present in foods at very low levels and in very complex matrixes.
- Furthermore, vitamins are easily destroyed by strong acids or alkali; affected by many factors such as pH, heat, light, air, extraction solvent, and time; and also affected by other food components.
- Despite the development of several HPLC and LC–MS methods for vitamin analysis in food, there is still a need for better resolution and sensitivity when analyzing complex food matrices containing different vitamin derivatives.
- Owing to its high resolution and sensitivity, as well as its high sample throughput, UPLC is particularly attractive in the area of food analysis where sample matrices are very complex.
- The content of vitamin C in food commodities (the sum of the contents of l-ascorbic acid and dehydroascorbic acid) is used as an index of health-related quality of products,since, as compared with other beneficial compounds, it is more sensitive for degradation by processing and storage.
- Therefore, interest in the simultaneous analysis of these molecules has increased greatly in food analysis.
- The determination of total vitamin C content in several fruits and vegetables (lemons, passion fruits, papayas,strawberries, broccoli, green and red peppers) using UPLC-photodiode array (PDA)system.

Gas chromatography

- Gas chromatographic methods for assay of vitamin E were developed before the advent of LC.
- Vitamin E is a fat-soluble vitamin with several forms, but alpha-tocopherol is the only one used by the human body.
- Early methods were hampered by the inability of packed-column chromatography to resolve β- and γ-tocopherols and β- and γ-tocotrienols.
- In addition, packed-columnchromatography was labor-intensive and affected by interferences to both the tocopherols and the internal standard peaks, requiring saponification to reduce the interferences and the use of correction factors to correct for unremoved interferences.
- Development of capillary GC solved many of the problems associated with packed-column chromatography.
- Although LC is now universally accepted as an easy and accurate approach for quantitation of vitamin E homologs, GC methods provide an alternative approach for analysis of complex matrices.
- Such procedures usually rely on the linking of GC and mass spectrometry (GC-MS).

Liquid chromatography-mass spectrometry

- The feasibility of using reversed-phase liquid chromatography/diode array/tandem mass spectrometry (LC –DAD –MS/MS) for a rapid and comprehensive profiling of fat soluble vitamins and pigments in some foods of plant origin (maize flour, greenand golden kiwi) was evaluated.