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Vitamins and carotenoids. Their physiological functions and technological properties

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Significance and Functions of Vitamins

The essential character of vitamins for human life and their significance for health has long been known. The 13 organic substances which are referred to as vitamins have a multitude of physiological functions, most of which are as coenzymes in metabolic reaction pathways.

In recent years compelling scientific evidence has accumulated that a number of vitamins, i.e. A, C, E Folic acid and the provitamin fl-carotene, as well as other carotenoids have effects that go far beyond these classical and rather wellknown tasks.

I would like to discuss these vitamins and their physiological functions a little more in detail, then move on to the question how much vitamins we need, or better, how much is desirable, and lastly I want to look at them from a very practical point of view, namely what can the food industry do to contribute to a better vitamin supply of the general public.

Physiological Functions of Vitamin A

Vitamin A is essential for cell differentiation and normal growth, for development and differentiation of the epithelium. It plays a part in the synthesis of the male hormone testosterone and of glycoproteins. Without vitamin A we could not reproduce nor could we see. It is also important for a well functioning immune system.

Beside these «classical» functions, it has been discovered in recent years that vitamin A, owing to its differentiation regulating and tumor antipromoting properties can be indicated for an adjuvant cancer therapy.

Until recently it was also believed that vitamin A is an antioxidant vitamin. This, however, could not be confirmed. Today there is general agreement among scientists that vitamin A has no antioxidative potential.

Fl-carotene, however, has. This major representative of the large pigment group of the carotenoids is wellknown as a precursor of vitamin A and thus as provitamin A, however is nowadays more and more often referred to as a vitamin itself. It can act by two different mechanisms, namely by deactivating excited oxygen, also referred to as singlet oxygen, or as chain breaking antioxidant.

Deactivation of Singlet Oxygen by fl-carotene

Excited by light, certain molecules, symbolized as S for Photosensitizer on the slide, can be promoted to a higher energy level. For example, the photosensitizer S is promoted to the level 1 and/or 3. These excited molecules are highly reactive and will attack any suitable molecule available and react with it. A possible candidate for such a reaction is oxygen, symbolized as $3O_2$ which is triplet oxygen, means oxygen on its normal energetic level. The excited sensitizer imparts its energy to the oxygen and promotes it to the highly excited and reactive singlet level. In this state, oxygen reacts with everything, in vivo for instance with cell membrane proteins, DNA, phospholipids, fatty acids etc. This means in other words cell damage.

Owing to its long conjugated double bond system fl-carotene - and also other carotenoids - is capable of absorbing the energy from the singlet oxygen and releasing it as harmless heat energy.

Carotenoids as Antioxidants

The chain breaking properties of fl-carotene - or more general, of carotenoids - are also based on the structural element of the long conjugated double bond system, which is able of producing a resonance-stabilized carbon-centered radical.

Vitamin C is an antioxidant too. A multitude of biochemical functions are known and we can be sure, many are not known yet or, at least, not entirely understood. One example is the enhancement of our immune system. It is an old wisdom that vitamin C is good against common cold and many people are sure it helps them, however, from a purely scientific point of view this effect could not be proven yet.

One of the more recently discovered and certainly highly interesting functions of vitamin C is its role in the physiological antioxidant system, where it, for instance, reduces the superoxide anion-radical to form hydrogen peroxide.

Probably its most significant task in this antioxidant system is the regeneration of tocopherol which is - as you know - the biologically active form of vitamin E and the principal fat soluble antioxidant in our organism. As a result of its action in the cell membrane, vitamin E is oxidized to form chinoid structures. Would it not be regenerated, one molecule of vitamin E could only deactivate one radical which would be very inefficient.

The mode of action of vitamin E and C is shown on the next two slides.

The Pecking Order of Free Radicals

The phytyl tail of vitamin E is located in the lipid compartment and the chromane ring faces towards the aqueous compartment. The phenolic OH-groups of tocopherol are near the polar head groups of the phospholipids that compose the bilayer.

In step A an oxidizing radical X initiates a peroxidation process by abstracting a bis-allylic hydrogen, thereby forming a pentadienyl radical in the fatty acid tail of a phospholipid in presence of oxygen. This happens in step B. The fatty acid peroxide radical has a distinct dipole character and floats to the membrane-water-interphase where the proximity to the hydroxyl-group of tocopherol is sufficient to react with it to become a rather stable hydroperoxide. This happens in steps C and D. We have a tocopheryl radical now which is immediately recycled by vitamin C, symbolized as AscH minus in the scheme. The ascorbyl radical itself is recycled enzymatically subsequently.

The last step is then that the fatty acid hydroperoxide is reduced to the original fatty acid again by an enzyme system, e.g. glutathione peroxidase, phosopholipase A2 and others.

Pathways for the Oxidation and Regeneration of Vitamin E

From this slide it becomes obvious that vitamin C is not the only vitamin E-regenerating substance but also other reducing agents like glutathione and Ubiquinol do the same job.

This graph also gives an impression how sophisticated and balanced the physiological antioxidant system is and that all the individual antioxidants complement each other.

Another vitamin has enjoyed remarkable publicity in recent times. Folic acid. It has been proven to prevent or to drastically reduce the prevalence of Neural Tube Defects in neonatal children. Neural Tube Defects are very severe malformations which are usually fatal in the first few weeks of the child's life. Folic acid supplementation of 400 µg per day has been shown to significantly reduce the number of children with NTD. It is necessary, however, to supplement during the first month of pregnancy. Since women usually don't know that they are pregnant a few weeks after conception, it is advisable to assure the folic acid supply either by appropriate food selection or by supplementation, generally within childbearing age.

Vitamin requirements

How much of each vitamin do we really need or how much is desirable?

These are perhaps the most asked questions about vitamins. The right answer to them, however, is very difficult to find, and I don't hesitate to say that a general answer is probably not existing.

Nevertheless, health authorities all around the world have set up recommendations for daily vitamin intakes that are supposed to be more or less optimal for maintaining a well functioning organism.

Vitamin RDAs of Various International Nutr. Auth.

As you can see from this table, there are tremendous differences between the recommendations in different countries. Most extreme for vitamin K where the recommendations differ by a factor of 100. This reflects the uncertainty that is still existing about the real requirements for vitamins.

It can nowadays be suspected that the RDA-values for a number of vitamins in many countries are too low, which means below the optimal intake; undoubtedly they are all above the minimum requirement which is necessary to avoid clinical deficiency symptoms.

Particularly for the antioxidant vitamins C, E and ß-carotene, the RDA-values appear to be below the optimum. There is agreement in the scientific community that the intakes of the antioxidants should be considerably above even the highest existing RDA-values.

The highest RDA for vitamin C is 100 mg, the scientific recommendation is 100 - 150 mg.

The highest RDA for vitamin E is 50 mg, the scientific recommendation is 60 - 100 mg.

No official RDA exists for ß-carotene; the scientific recommendations vary strongly between 5 and 20 mg.

Particularly these examples show you that the official RDAs should be reconsidered and - very importantly - internationally harmonized.

Above I had raised the question «How much vitamins do we need?» The next question that usually follows is «Do we get enough?» It is obvious that, if we are not even sure how much we need as an optimum, we hardly can say whether we get enough. What we can say, however, is whether we get enough if we use the RDAs as the calculation basis. We also can calculate if we get enough of the antioxidants if we use the scientific recommendations that I quoted, as a basis.

It is certainly true that, on a worldwide basis, many people don't get as much of certain vitamins as they should get. It is a wellknown fact that an abundant vitamin A deficiency, particularly in small children, exists in many developing countries. UNICEF attempts to combat this problem by distributing vitamin A capsules; a few countries have taken the initiative themselves and enrich staple food like sugar with vitamin A.

I will come back to this topic a little later.

I mentioned folic acid and birth defects before. Folic acid is another vitamin which large parts of the population - even in developed industrialized countries - don't get enough of. This is particularly true for younger women at reproductive age.

As far as the antioxidant vitamins are concerned, we can assume that even most people are below the desirable supply, if we regard the intakes that are scientifically recommended as desirable.

The next slide gives you an impression how difficult it is to obtain these scientifically recommended daily amounts through normal foods.

What daily amounts of foods rich in antioxidant vitamins ...

Whereas it seems not unrealistic to get enough vitamin C through enough fruits and vegetables it seems quite unlikely to assure a sufficient supply of vitamin E and ß-carotene just through the normal diet.

It is obvious that the food industry is challenged here. The food industry obviously can play - and I would even say - has to play the leading role in the permanent process of improving and optimizing the general populations nutrition, and thus health. In this process, the science elaborates the knowledge and hence the basis for new ideas, the health- and nutrition politicians - and this is certainly the prerequisite for any progress - have to provide a liberal legislative frame that allows to implement innovative and creative ideas, and finally the food industry develops and supplies products based on the knowledge science has provided.

Foods are the natural sources of vitamins and micronutrients in general. What is more obvious than adding vitamins or other micronutrients like minerals to suitable foods, if an optimal supply of these nutrients is not assured through normal nutrition?

Fortification of Foods

With respect to vitamin fortification I subdivide foods into three groups according to the purpose of their vitaminization. This is shown on the next slide.

Vitamin Fortification of Foods

While the purpose of vitamin addition in group 1 is rather restoration than actual enrichment, and in group 2 a necessity that is legally compulsory anyway, group 3 represents the area where the food industry can be creative and develop novel, interesting and well accepted product concepts.

Let me move on now to some technological aspects.

In order to utilize and process vitamins in foods, some knowledge about their stabilities is necessary. Since vitamins are chemically entirely different entities, their stabilities are different as well. The next two slides show in a qualitative manner to which influences the individual vitamins are particularly susceptible.

Factors affecting the stability of the fat soluble vitamins

Vitamin A is one of the most unstable vitamins. Retinol, the alcohol-form of vitamin A is the least stable form. An increase in stability can be obtained by esterifying retinol with fatty acids. Hence, in the food industry exclusively the esters vitamin A-palmitate and vitamin A-acetate are used.

Vitamin A can be stabilized with phenolic antioxidants like tocopherol, BHT or BHA. However, still in these stabilized forms, vitamin A has to be handled with care and overages to compensate for losses are mostly inevitable.

Also vitamin D2 and D3 are sensitive against oxygen, light, humidity and heat but to a distinetty lesser extend than vitamin A.

The naturally occurring vitamin E active compounds, the tocopherols, are extremely unstable in the presence of oxygen. Oxidative decomposition is promoted by light and heat. Tocopherols, particularly the synthetic DL-alpha-tocopherol, are therefore used in the food industry almost exclusively as highly efficient, fat-soluble antioxidants, but practically never as vitamins in their own right.

The reactive centre in the tocopherol molecule is a phenolic hydroxyl group that can be esterified and thus protected. Accordingly, tocopheryl acetate, which is better known as vitamin E acetate, is used exclusively as a source of vitamin E. This derivative is very stable and is one of those vitamins that give rise to very few problems.

Very little data are available on vitamin-K-stability. Only a certain sensitivity to light is known. Generally, vitamin K seems to be one of the more stable vitamins.

Factors affecting the stability of the water soluble vitamins

Among the water soluble vitamins B1, C and folic acid are the problematic ones with regard to stability.

Thiamin is very heat sensitive. The higher the pH-value the faster the degradation proceeds.

Ladies and Gentlemen, for time reasons I cannot discuss the remaining vitamins individually.

I want to stress, however, that all vitamins in a pure state, at appropriate storage - and packaging conditions, possess a considerable stability over quite a long time. «Appropriate storage and packaging» means cool, dry, air- and humidity-proof.

You will remember the 3 categories of vitaminized food I had defined a little while ago. I believe for this region, Latin America, category 1, fortified staple food, is the most relevant and interesting group. Particularly the enrichment with vitamin A makes a lot of sense, as vitamin A deficiency is the most abundant vitamin deficiency.

Sugar is a very suitable carrier for vitamin A. Available and affordable for practically everyone, it is consumed every day and can assure the vitamin A supply of the general public on a constant basis.

The enrichment process is easy.

Enrichment of Sugar With Vitamin A

It was developed by INCAP and subsequently used, and is still used, in Guatemala to fortify the entire sugar production.

First a sugar / vitamin A / antioxidant premix is prepared which is subsequently added to the bulk sugar. A vitamin A-palmitate dry powder with a potency of 250,000 i.u./g is used. The antioxidant protects the vitamin so that a relatively small overage of only 10 % is necessary.

A second example for a suitable staple food is vegetable oil. In most countries oil is consumed by everyone. It has the advantage that the fortification with fat soluble vitamins like vitamin A, but possibly also D and/or E is extremely simple from a technological point of view. The stability of vitamin A in oil is very good, even when the oil is used for cooking and frying.

Quantitative data is available from work that has been done at the University of Sao Paulo as well as from our own labs at BASF in Germany.

Ladies and Gentlemen, unfortunately, for time reasons, I can't discuss any more individual foods. I just want to summarize that there are countless possibilities for the enrichment of practically all kinds of foods in order to improve the vitamin supply of the population which is far away from being optimal, not only, but particularly in developing countries.

From a technological point of view the fortification of a certain food is not always a simple task, but this is exactly where we from BASF offer our help.