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Food products — Guidance on how to express vitamins and their vitamers content

Produits alimentaires — Lignes directrices pour exprimer les teneurs en vitamines et en leurs vitamères

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Vitamins can be naturally found in foods in different molecular forms. In more elaborated food products, vitamins can be used for fortification by adding several molecular forms with different levels of vitamin activity. There are regulations governing the addition of vitamins in food products. The authorized compounds for fortification depend on the type of food. Regulation deals with, for instance baby food or food supplements. The main problem is that the vitamin activities of the authorized compounds are not clearly described.

At the same time, it is not mandatory to list the chemical name of the compound used for food fortification purposes according to food labelling regulations. For example, vitamin E can be written in the list of ingredients without knowing if it is D-alpha tocopherol or D,L-alpha tocopherol, even though each molecular form presents different vitamin E activity.

ISO and CEN analytical standards express results in mass units related to the vitamin standard used for quantification. As expression in specific units of vitamin activity can be linked to regional/national regulatory requirements, analytical methods do not give guidance for this conversion. This document proposes ways to express the vitamin content in order to facilitate harmonization between different laboratories and also to reduce misunderstanding of the results expressed in vitamin content and in some cases in the vitamin activity of vitamers.

Food products — Guidance on how to express vitamins and their vitamers content

1 Scope

This document provides guidelines on:

— how to express vitamin quantity,

— the expression of different molecular forms in appropriate units,

— and in some cases, vitamin activity, according to vitamers present or used in food products, in addition to the quantitative content determination obtained from ISO and CEN analytical standards.

It provides information to be used as a basis for discussion between stakeholders and food control laboratories. It is not intended to be prescriptive or exhaustive.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
vitamer
any of a number of chemical compounds of a particular vitamin, generally having a similar molecular structure, each of which shows vitamin activity in a vitamin-deficient biological system

Note 1 to entry: See Reference [1].

4 Guidelines per vitamins

4.1 General

This document begins by addressing vitamin E, as this vitamin is considered to be the most complex in terms of variation of possible units of measurement. Vitamin E is followed by the other fat-soluble vitamins and water-soluble vitamins respectively.

To include the diversity of available conversion factors, regulations in different countries/regions are evaluated, including the European Union (EU), Regulations of the United States Food and Drug Administration (USFDA), United States of America (USA), National Standard (GB) of China, Food Standards Australia/New Zealand (FSANZ). In addition, standards of global organizations (CODEX[2]) are included.

4.2 Vitamin E

Vitamin E is a fat-soluble vitamin. Vitamin E is mainly found in the fat of the different food products as tocopherols and tocotrienols esters.

Food regulations of many countries authorize the fortification of different kind of food products with molecules having a vitamin E activity. The names of the authorized molecular forms to add are generally linked to the type of food product. Table 1 gives an example of vitamin E compounds permitted for use for food fortification according to EU regulations, depending on the product type.

Table 1 — Example of authorized vitamin E compounds for food fortification in the EU according to products concerned

Product type	Molecular form	Molecular mass (g/mol)	Examples of regulations
Food with a specific nutritional purpose	D-alpha-tocopherol	430,7	Regulation (EU) n° 609/2013[3]
	DL-alpha-tocopherol	430,7	
	D-alpha-tocopheryl acetate	472,8	
	DL-alpha-tocopheryl acetate	472,8	
	D-alpha-tocopheryl polyethylene glycol 1000 succinate		
Supplemented food	D-alpha-tocopherol	430,7	Regulation (EC) n°1925/2006[4]
	DL-alpha-tocopherol	430,7	
	D-alpha-tocopheryl acetate	472,8	
	DL-alpha-tocopheryl acetate	472,8	
	D-alpha-tocopheryl acid succinate	530,8	
Food supplements	D-alpha-tocopherol	430,7	Directive n°2002/46/EC[5]
	DL-alpha-tocopherol	430,7	
	D-alpha-tocopheryl acetate	472,8	
	DL-alpha-tocopheryl acetate	472,8	
	D-alpha-tocopheryl acid succinate	530,8	
	Mix of tocopherols and tocotrienols		

There is no global convention on how the amount of vitamin E should be expressed for labelling purposes. In Table 2 an overview of units of measurement for vitamin E is given according to several national/regional regulations and a CODEX standard.

Table 2 — Definition of vitamin E for labelling purposes in several standards/regulations

US (until 2019-12-31)	US (as from 2020-01-01)	EU	CODEX	China/Australia/New Zealand
IU[a]	mg alpha-tocopherol	mg alpha – TE[b]	mg alpha - TE	mg alpha – TE

[a] International Unit, a measure of biological activity, different for each substance.

[b] Tocopherol equivalent.

Table 2 *(continued)*

US (until 2019-12-31)	US (as from 2020-01-01)	EU	CODEX	China/Australia/New Zealand
1 IU = 0,67 mg for D-alpha tocopherol (natural)	1 mg vitamin E (as alpha-tocopherol) label claim = 1 mg of natural alpha-tocopherol (D-alpha-tocopherol)	1 mg alpha – TE = 1 mg D-alpha tocopherol	1 mg alpha – TE = 1 mg D-alpha tocopherol	1 mg alpha – TE = 1 mg D-alpha tocopherol
1 IU = 0,9 mg for D, L -alpha tocopherol (synthetic)	1 mg vitamin E (as alpha-tocopherol) label claim = 2 mg of synthetic alpha-tocopherol (D,L-alpha-tocopherol			

[a] International Unit, a measure of biological activity, different for each substance.

[b] Tocopherol equivalent.

The vitamin E unit of measurement is generally defined as 1 mg of D-alpha-tocopherol (natural form). There is currently no standard or official analytical method able to quantify this natural form specifically.

There are two main analytical approaches for quantifying vitamin E:

1) Saponification of tocopheryl esters to liberate them as free tocopherols. Solvent extraction and separation of the individual tocopherols by liquid chromatography. Quantification by fluorescence detection.

   An example is EN 12822[6], which includes a saponification and an extraction step. It enables the separation by a normal phase HPLC, and the quantification of the free alpha, beta, gamma and delta tocopherols using fluorescence detection. Tocotrienols are also separated but not quantified. Tocotrienols are not taken into account for the expression of the vitamin result. The D-tocopherols are not distinguished from the D,L-tocopherols.

2) Direct solvent extraction and separation of different esters and free tocopherols by liquid chromatography. Quantification by fluorescence detection.

   An example is ISO 20633, where the hydrophilic coating of fat micelles is hydrolysed by an enzyme. The hydrophobic contents of the micelles are then extracted into iso-octane. The extract is analysed by normal phase HPLC. Alpha-tocopherol and alpha-tocopheryl acetate are quantified using fluorescence detection.

By taking into account the molecular mass and the activity ratio of the D and the D,L the following values can be found[8]:

— 1 mg of vitamin E = 1 mg of D-alpha-tocopherol = 1 mg alpha-TE;

— 1 mg of vitamin E = 1,35 mg of D,L-alpha-tocopherol;

— 1 mg of vitamin E = 1,1 mg of D-alpha-tocopheryl acetate;

— 1 mg of vitamin E = 1,49 mg of D,L-alpha-tocopheryl acetate;

— 1 mg of vitamin E = 1,23 mg of D-alpha-tocopheryl acid succinate.

As mentioned above, there is no specific detection at the quantification step for the D-alpha tocopherol (natural form) and the D,L-alpha tocopherol (synthetic form) respectively. So, when a sample is analysed, and there is no specific information about the added form (D or D,L) on the label of the product, there

can be a misunderstanding about the true value of the vitamin E content. The difference can be a factor of 1,35 or higher.

Regarding the nutritional aspect, there is no general agreement about the activities of the other forms of tocopherols and tocotrienols.

When in the presence of certain amounts of beta and/or gamma and/or delta tocopherols, it is often underlined that the relative vitamin E activities should be included in the final result. The more common coefficients[8] are:

— 1 mg of D-beta-tocopherol = 0,5 mg of vitamin E;

— 1 mg of D-gamma-tocopherol = 0,1 mg of vitamin E;

— 1 mg of D-delta-tocopherol = 0,03 mg of vitamin E.

Tocotrienols are sometimes also taken into account by certain guidelines, such as the FAO/INFOODS guideline[9] as in the following example:

Vitamin E (mg/100 g) = alpha-tocopherol (mg/100 g) + 0,4 beta-tocopherol (mg/100 g) + 0,1 gamma-tocopherol (mg/100 g) + 0,01 delta-tocopherol (mg/100 g) + 0,3 alpha-tocotrienol (mg/100 g) + 0,05 beta-tocotrienol (mg/100 g) + 0,01 gamma-tocotrienol (mg/100 g)

Despite this fact, in most of the cases, only the alpha-tocopherol is considered.

At the session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (40^{th} Session, 5^{th} - 9^{th} December 2016, Hamburg, Germany), it was proposed to take only the alpha-tocopherol into account for labelling purposes [10]. In this case, the expression of vitamin E is very simple:

vitamin E = 1 mg of alpha-D-tocopherol = 1 mg RRR-alpha-tocopherol.

Taking into account most of these parameters, the vitamin E content can be calculated as follows:

a) If only D-alpha tocopherol (natural) is present in the product, the vitamin E content (expressed in D-alpha tocopherol equivalent) can be calculated as vitamin E (mg/100 g) = alpha-D-tocopherol

   or in some cases, e.g. for vegetable oils:

   vitamin E (mg/100 g) = alpha-tocopherol + 0,5 beta-tocopherol + 0,1 gamma-tocopherol +

   0,03 delta-tocopherol.

b) If D,L alpha tocopherol (synthetic) is present in the product, the alpha-tocopherol result is divided by 1,35 and the vitamin E content (expressed in D-alpha tocopherol equivalent) can be calculated as vitamin E (mg/100 g) = 0,74 alpha-tocopherol (mg/100 g)

   or in some cases:

   vitamin E (mg/100 g) = 0,74 alpha-tocopherol (mg/100 g) + 0,5 beta-tocopherol (mg/100 g) + 0,1 gamma-tocopherol (mg/100 g) + 0,03 delta-tocopherol (mg/100 g).

c) If the vitamin E molecular form is not known, it is recommended that the two former possibilities be studied. Follow-up action with the producer of the product can be necessary before a conclusion can be drawn.

d) Some methods do not involve a saponification step, like in ISO 20633. In these cases, the calculation is adapted as follows:

   vitamin E (mg/100 g) = D-alpha tocopherol (mg/100 g) + 0,909 D-alpha tocopherol acetate (mg/100 g).

In addition, it is important to consider that apart from adding vitamin E for food fortification purposes, it is common practice and permitted to add tocopherols as food additives (antioxidants) to food products, including:

— E 306: rich tocopherols extracts;

— E 307: alpha tocopherol;

— E 308: gamma tocopherol;

— E 309: delta tocopherol.

At the analysis step, this can lead to a difference between the vitamin E quantified value and the vitamin E labelled value because no test is capable of identifying the part of vitamin E added for nutritional purposes from the part added for antioxidation. In any case, the labelled vitamin E value should not include the tocopherol forms added as antioxidants.

4.3 Vitamin A

Vitamin A is a fat-soluble vitamin. Vitamin A is mainly found in the fat of the different food products as retinol esters.

The food regulations of many countries authorize the fortification of different kinds of food products with molecules having a vitamin A activity. The names of the authorized molecular forms to add in are generally linked to the type of food product. Table 3 gives an example of vitamin A compounds permitted for use for food fortification according to EU regulations, depending on the product type.

Table 3 — Example of authorized vitamin A compounds for food fortification in the EU according to products concerned

Product type	Molecular form	Molecular mass (g/mol)	Examples of regulations
Food with a specific nutritional purpose	Retinol	286,5	Regulation (EU) n° 609/2013
	Retinyl acetate	328,5	
	Retinyl palmitate	524,9	
	Beta carotene	536,9	
Supplemented food	Retinol	286,5	Regulation (EC) n° 1925/2006
	Retinyl acetate	328,5	
	Retinyl palmitate	524,9	
	Beta carotene	536,9	
Food supplements	Retinol	286,5	Directive n°2002/46/EC
	Retinyl acetate	328,5	
	Retinyl palmitate	524,9	
	Beta carotene	536,9	

There is no global convention on how the amount of vitamin A should be expressed for labelling purposes. In Table 4 an overview of units of measurement for vitamin A is given according to several national/regional regulations and a CODEX standard.