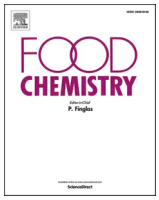
Food Chemistry 451 (2024) 139383

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Review

Limitations of current analytical reference methods to determine vitamins in foods: Challenges to support regulatory compliance and nutritional composition data

Erik Konings [a,*], Brendon David Gill [b], Jette Jakobsen [c], George Joseph [d], Esther Campos-Giménez [e], Jean-Luc Deborde [f], Wei Wang [g], Ruben Buis [h], Harvey Indyk [b]

[a] Société des Produits Nestlé S.A., Nestlé Institute of Food Safety and Analytical Sciences, EPFL Innovation Park, Bâtiment G, 1015 Lausanne, Switzerland
[b] Fonterra Co-operative Group Limited, P.O. Box 7, Waitoa 3380, New Zealand
[c] National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark
[d] AsureQuality, PO Box 41, Shortland Street, Auckland 1140, New Zealand
[e] Société des Produits Nestlé S.A., Nestlé R&D Konolfingen, Nestlé-Strasse 3, 3510 Konolfingen, Switzerland
[f] Laboratoire de Strasbourg (SCL), 13, chemin du routoir 67400 Illkirch-Graffenstaden, Strasbourg, France
[g] Société des Produits Nestlé S.A., Nestlé Shanghai, 1600 Zhongshan West Rd, Shanghai, China
[h] Van Hall Larenstein University of Applied Sciences, Leeuwarden, The Netherlands

ARTICLE INFO

Keywords:
Food
Analysis
Reference
Harmonisation
Methods
Vitamers

ABSTRACT

Foods are analysed for their vitamin content to support the verification of regulatory compliance or to generate food composition data. Many international reference methods for the analysis of vitamins in foods originate from the 1990s. Advances in nutrition science and analytical technology and the continuing evolution of statutory regulations necessitate the need of new or supplementary regulatory standards. We have evaluated recent developments in these areas and conclude that most current international reference methods are no longer fit-for-purpose to accurately determine vitamin content in foods and food supplements. We have made recommendations to consider new and/or updated reference methods and regulatory standards for the analysis of vitamins A, D, E, K, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C and carotenoids in foods and food supplements. This area of nutrients may benefit from globally harmonised definitions specifying what compounds to include or exclude for analysis, and applicable bioactivity factors.

1. Introduction

Vitamins are critical for normal cell function, growth, and development, and since mammalian cells cannot, in general, biosynthesise them, they are essential components of the diet. The quantification of the total amounts of individual vitamins in foods, i.e., vitamins both naturally present and added to foods to improve or maintain their nutritional quality, is challenging. Developments in nutrition research, changes in regulations, ambiguity over which compounds to include or how to adequately quantify a vitamin contribute to these challenges. An ISO Technical Report (ISO, 2021) gives guidance on the multiple options to express vitamins on food product labels that accounts for different molecular forms and regulatory requirements.

There are generally three sectors interested in data on the vitamin content of foods and food supplements. Firstly, the food industry needs data to ensure safety and quality of their products in accordance with regulatory requirements and to ensure nutritional claims are met. Based on recipe calculations involving natural levels in ingredients and added amounts of vitamins, producers know the forms and quantities of vitamins in their products. Secondly, nutrition researchers and risk assessors use food consumption data to determine dietary intake (Castanheira, Saraiva, Rego, & Ollilainen, 2016). For this group it is important to know what value to assign to total vitamin amount, how it is determined, and how it should be expressed. This group relies heavily on available reference methods of analysis. Reliable analytical methods are essential for population-based policies related to Recommended Daily

* Corresponding author.
E-mail addresses: erik.jmkonings@outlook.com (E. Konings), brendon.gill@fonterra.com (B.D. Gill), jeja@food.dtu.dk (J. Jakobsen), george.joseph@assurequality.com (G. Joseph), esther.campos-gimenez@rdls.nestle.com (E. Campos-Giménez), deborde.jean-luc@wanadoo.fr (J.-L. Deborde), wei.wang@cn.nestle.com (W. Wang), ruben.buis@hvhl.nl (R. Buis), indykharvey@gmail.com (H. Indyk).

https://doi.org/10.1016/j.foodchem.2024.139383
Received 26 February 2024; Received in revised form 11 April 2024; Accepted 13 April 2024
Available online 16 April 2024

Allowances, total diet surveys, as well as supporting clinical testing. Thirdly, enforcers of regulations generally have no information on the composition of a food product. They need robust methods to be able to quantify vitamin content (natural + added) to verify label declarations and nutritional claims. A complicating factor is that some vitamins, such as riboflavin, ascorbic acid and α-tocopherol, can be added to foods both as nutrients and as functional additives, which makes the verification of labelled nutritional amounts difficult, if not impossible.

Multiple horizontal European standards for the determination of vitamins in foods are published by the European Committee for Standardisation (CEN) and originate from the 1990s. Blake and Konings (2005) listed the available reference methods for vitamins in foods published by AOAC INTERNATIONAL, CEN and the International Organisation for Standardisation (ISO), which have not changed significantly since then. The scope of other more recently published reference methods for vitamins by AOAC INTERNATIONAL, ISO and the International Dairy Federation (IDF) is restricted to infant formula and adult nutritionals. Several of these CEN, AOAC, ISO and IDF reference methods are included in Codex standard CXS-234 on recommended methods of analysis and sampling (Codex, 1999) to verify Codex provisions.

Scientific knowledge on the presence and bioavailability of certain vitamin compounds has evolved, and this may have an impact on the total vitamin content quantified in a food product. Recently for example, Jakobsen, Melse-Boonstra, and Rychlik (2019) stated that there are no standardised methods for the quantification of the vitamers of vitamin A, vitamin D, and folate in foods. In addition, regulatory requirements, including newly approved compounds for some vitamins, have changed since the publication of the above-mentioned reference methods.

This paper elaborates on the most important recent and relevant developments in science, technology, and regulatory requirements that will necessitate updated or new analytical reference methods that will ensure accurate compositional data for regulatory compliance verification or support dietary recommendations.

2. Developments contributing to limitations in current reference methods

2.1. Vitamin A and carotenoids

Vitamin A is a fat-soluble vitamin obtained from the diet either as preformed vitamin A (mainly retinol and retinyl esters) in foods of animal origin or as provitamin A carotenoids in plant-derived foods, primarily in oils, fruits and vegetables (Meléndez-Martínez et al., 2022). The term vitamin A comprises retinol and a family of naturally occurring molecules associated with biological activity such as retinal, retinoic acid, retinyl esters, as well as provitamin A carotenoids that are dietary precursors of retinol (EFSA, 2015a).

Among approximately 600 carotenoids in nature, about 50 possess provitamin A activity in varying degrees of potency, but food composition data are only readily available for β-carotene, α-carotene and β-cryptoxanthin (Ball, 2006; NHMRC, 2017). The predominant isomer of retinol is all-*trans* retinol, which possesses 100% vitamin A bioactivity, while other isomers exhibit lower bioactivity. In foods, vitamin A can exist in several isomeric forms and as esters. Fresh and processed fruits and vegetables are the main contributors to the dietary intake, with β-carotene, α-carotene and β-cryptoxanthin being the most abundant provitamin A carotenoids (Ball, 2006). Dairy products may also have an important contribution to the total dietary β-carotene intake. Currently, there is no globally harmonised way to express vitamin A activity of carotenoids. Some regulations include β-carotene and other provitamin A carotenoids to express a total amount of vitamin A. Some regulations require the calculation of Retinol Equivalents (RE) with the inclusion of carotenoid contents for labelling purposes of vitamin A. Recently, the U.S Food and Drug Administration (USFDA) introduced a new unit to express vitamin A activity: retinol activity equivalents (RAE), which includes the major provitamin A carotenoids (USFDA, 2016; USFDA, 2019). In addition, a differentiation in bioactivity of supplemental and dietary β-carotene is made.

Table 1
Overview of units of expression and conversion factors according to global and regional regulations for vitamin A including carotenoids[a].

Source and Authority	Expression / calculation
Pre-formed retinol and retinyl esters	
Codex	μg RE or RAE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87)
USFDA	μg RAE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87)
EU	μg vitamin A = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87)
CHINA GB	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87)
FSANZ	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87)
Provitamin A carotenoids (foods)	
Codex	μg RE = (μg β-carotene /6) + (μg other provitamin A carotenoids /12) μg RAE = (μg β-carotene /12) + (μg other provitamin A carotenoids /24)
USFDA	μg RAE = (μg supplemental β-carotene /2) + (μg supplemental α-carotene and β-cryptoxanthin /4) + (μg dietary β-carotene /12) + (μg dietary α-carotene and β-cryptoxanthin /24)
EU	No scientific consensus
CHINA GB	μg RE = (μg β-carotene /6) + (μg other provitamin A carotenoids /12)
FSANZ	μg RE = (μg synthetic all trans β-carotene /6) + (μg total dietary α-carotene, β-carotene, β-cryptoxanthin and other provitamin A carotenoids like β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester /12)
Infant formula and formula for older infants and young children	
Codex	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87) (carotenoids excluded)
USFDA	μg RAE = μg retinol + (μg retinyl palmitate x 0.55) + (μg retinyl acetate × 0.87) + (μg supplemental β-carotene /2) + (μg supplemental α-carotene and β-cryptoxanthin /4) + (μg dietary β-carotene /12) + (μg dietary α-carotene and β-cryptoxanthin /24)
EU	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87) carotenoids excluded
CHINA GB	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87) carotenoids excluded
FSANZ	μg RE = μg retinol + (μg retinyl palmitate × 0.55) + (μg retinyl acetate × 0.87) + (μg synthetic all trans β-carotene /6) + (μg total dietary α-carotene, β-carotene, β-cryptoxanthin and other provitamin A carotenoids like β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester /12)

[a] Abbreviations are: RE, retinol equivalents; RAE, retinol activity equivalents.

Table 1 includes the conversion factors used in global standards and regulations including Codex standards (Codex Stan 72–1981; Codex Stan 2–1985 and Codex Stan 156–1987: Codex, 1981, 1985 and Codex, 1987, respectively) and regional regulations in the USA (USFDA, 2016; USFDA, 2019), European Union (EU) regulations (regulations 2002/46, 1169/2011, 2016/127: EU, 2002, 2011, 2016, respectively), China national standards (GB) (GB/Z 21922–2008, GB 25596–2010, GB 28050–2011, GB 14880–2012, GB 29922–2013, GB 10765–2021, GB 10766–2021, GB 10767–2021: GB, 2008, 2010, 2011, 2012, 2013, 2021a, 2021b, 2021c, respectively), Food Standards Australia New Zealand (FSANZ) Schedules (FSANZ, 2018a, 2018b, 2018c, 2018d) and Nutrient Reference Values (NRV) for Australia and New Zealand (NHMRC, 2017).

The retinol equivalent factors in the FSANZ Schedules are specific for carotenoids that are being added to foods. The retinol activities of carotene forms like β-apo-8′-carotenal and β-apo-8′-carotenoic acid ethyl ester that are listed in general pharmacopoeias are included in FSANZ Schedules, whereas these forms are excluded in NHMRC NRV. The factors used in the NHMRC NRV are for carotenoids naturally occurring

or fortified in foods and are in line with the most recent decision of the FAO (FAO/WHO, 2001).

It should be noted that there are no carotenoid conversion factors to express vitamin A for European labelling requirements (EU 1169/2011: EU, 2011). The physiological equivalence between provitamin A carotenoids and retinol is an issue on which a scientific consensus has not yet been established (Dias et al., 2021). However, the European Food Safety Authority (EFSA) concluded in 2015 that 1 μg of retinol, or 6 μg of β-carotene, or 12 μg of other provitamin A carotenoids is equal to 1 μg RE (EFSA, 2015a).

In natural foodstuffs, the all-*trans* retinol form is frequently accompanied by smaller amounts of the less potent 13-*cis* retinol that has a 75% vitamin A activity (Ames, 1966; Weiser & Somorjai, 1992). Some current global reference methods for the determination of vitamin A in foods include both all-*trans* and the 13-*cis* retinol forms in the quantification of vitamin A (AOAC INTERNATIONAL, 2023a; CEN, 2009a; ISO, 2015a). However, historically the 13-*cis* form was not always considered for inclusion, which may result in differences in reported vitamin A amounts, dependent on the analytical method used. In addition, lack of harmonised definitions of vitamin A adds to confusion whether the 13-*cis* form should be included, and which bioactivity factor should be applied. Codex standard CXS-72 on infant formulas and formulas for special medical purposes intended for infants expresses 1 μg RE as 1 μg all-*trans* retinol and specifies that retinol contents shall be provided by preformed retinol, while any contents of carotenoids should not be included in the calculation and declaration of vitamin A activity (Codex, 1981). China national standards for infant formula and formula for older infants and young children (GB, 2010, 2013, 2021a, 2021b, 2021c) refer to all-*trans* retinol as the compound to be considered. However, newer China standards for all foods including infant formula (GB 5009.82–2016: GB, 2016a) do not distinguish between the all-*trans* and 13-*cis* form. Depending on fortification and processing conditions, the amount of 13-*cis* retinol can be 10–30% of total retinol (Panfili, Manzi, & Pizzoferrato, 1998). AOAC has published Standard Method Performance Requirements (SMPR®) where all-*trans* and 13-*cis* retinol are included in the applicability statement for an AOAC Official Method for the determination of vitamin A in infant formula and adult nutritionals (AOAC INTERNATIONAL, 2023b).

It is recommended that future reference methods that are developed and/or validated for vitamin A in foods shall include the quantification of both all-*trans* and 13-*cis* retinol. In addition, from a global perspective, a reference method for the determination of provitamin A carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin in foods should be considered.

In addition to provitamin A carotenoids, certain foods also provide carotenoids that are important in promoting and maintaining human health (e.g., lutein, zeaxanthin, astaxanthin, and cantaxanthin (Meléndez-Martínez et al., 2022). From all the carotenoids present in the diet, only six major ones are found in human plasma and milk: α-carotene, β-carotene, lycopene, β-cryptoxanthin, zeaxanthin, and lutein (Hostetler, 2017; Meléndez-Martínez et al., 2022) with lutein, β-carotene and lycopene the most common in human milk and lutein and β-carotene most commonly added to infant formula and adult nutritionals (Hostetler, 2020). This was the rationale for the development of an AOAC and ISO standards for the determination of these carotenoids in these products (AOAC INTERNATIONAL, 2023c; ISO, 2020a).

With an increased awareness on health-based products it is expected that the market for carotenoid-based health supplements will grow in the coming years (Allied Market Research, 2023). Lutein, β-carotene, lycopene, astaxanthin and zeaxanthin and mixtures of these carotenoids are popular among consumers, with zeaxanthin supplements typically taken to support eye health. Two separate specifications for zeaxanthin (synthetic) and for zeaxanthin rich extract from *Tagetes erecta* are approved by the Joint Expert Committee on Food Additives (JECFA, 2006). Both sources can be used either as a nutritional supplement or as colouring agents in a wide range of foods. In 2013 the European Commission authorised the synthetic form as a novel food ingredient and needs as such to be labelled as "synthetic" (EU, 2013a). Obviously, there is a significant price difference between the synthetic and botanical source of zeaxanthin; requiring analytical methods developed for the purpose of confirming analyte origin. The botanical source of zeaxanthin is exclusively present as the (3*R*, 3′*R*) enantiomer, whereas synthetic zeaxanthin occurs as the *meso* or (3*R*, 3′*S*) isomer. To distinguish between the two sources, the use of chiral column chromatography is necessary. Because there were health concerns with the *meso*-form before its authorisation as a novel food ingredient, the French authorities developed an analytical method to separate and quantify the two zeaxanthin isomers (Regnard, Arella, & Deborde, 2014).

In the wild, the flesh of salmon and trout can have a red/pink colour due to the carotenoid astaxanthin. Astaxanthin is an essential nutrient for salmon and is produced in natural waterways by algae, yeast and bacteria, all of which are in the food chain of salmonids. The ratio of optical isomers of astaxanthin indicates if the astaxanthin comes from chemical synthesis or natural sources such as shrimp or krill. Astaxanthin synthesised in nature occurs as the all-*trans* (3S, 3S) form, whereas synthetic astaxanthin is a mixture of the two optical isomers and the *meso* form at a ratio of 1:2:1 (3*R*, 3′*R*), (3*R*, 3′*S*) and (3*S*, 3′*S*) and can be used to distinguish wild from farmed salmon. CEN has published a technical specification (CEN, 2011a, 2011b) that includes a method for the determination of the enantiomer ratio of astaxanthin. However, additional validation data are needed to advance this method to a full reference method.

The European Union authorised the use of an astaxanthin-rich oleoresin from *Haematococcus pluvialis* algae as a novel food. The European Regulation EU 2023/1581 (EU, 2023a) sets maximum levels of this naturally occurring carotenoid in food supplements used by children, adolescents, and the general population. Currently no official method exists to enforce these maximum levels. In 2022, CEN issued a call for methods to standardise the determination of carotenoids in algae in Technical Committee 454: Algae and algae products.

2.2. Vitamin E

Vitamin E as an antioxidant, protects many other biochemicals from damage by reactive oxygen and other free radicals. It works closely with vitamin C in this respect, particularly in cell membranes, where ascorbic acid regenerates the α-tocopheryl radical to α-tocopherol. Rich food sources of vitamin E are oils extracted from cereal grains, nuts, beans, and seeds. Cereal grain products, fish, meat, eggs, dairy products, and green leafy vegetables also provide significant amounts (Ball, 2006).

Vitamin E is represented traditionally as a group of 8 molecules in two classes: tocopherols and tocotrienols, which in food all act as antioxidants. Of these 8 molecules, however, only α-tocopherol is considered as a vitamin E source for nutritional labelling (Institute of Medicine Panel, 2000). Thus, for labelling of vitamin E, the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) agreed in 2016 to include only the α-tocopherol form (REP17/NFSDU, 2016), where 1 mg α-tocopherol is equivalent to 1 mg *RRR*-α-tocopherol (Codex, 2017). α-Tocopherol contains three asymmetric carbon atoms, allowing for 8 optical isomers (Jensen & Lauridsen, 2007). While only *RRR*-α-tocopherol is present in nature, synthetic α-tocopherol occurs as a racemic mixture (all-*rac*-α-tocopherol). In its 2015 scientific opinion, EFSA stated that only the naturally occurring *RRR*-α-tocopherol should be considered as vitamin E (EFSA, 2015b). EFSA endorsed the Institute of Medicine Panel definition that the difference in relative activity of all-*rac*-α-tocopherol compared with *RRR*-α-tocopherol is 50% and defined 1 mg all-*rac*-α-tocopherol equal to 0.5 mg *RRR*-α-tocopherol and that 1 IU all-*rac*-α-tocopherol (or corresponding esters) as equal to 0.45 mg 2*R*-stereoisomeric forms of α-tocopherol and 1 IU *RRR*-α-tocopherol (or corresponding esters) as equal to 0.67 mg 2*R*-α-tocopherol (Institute of Medicine Panel, 2000). EFSA's opinion has been implemented in specific European regulations 2002/46/EC (EU, 2002) and EU 2016/127 (EU,

Table 2
Overview of units of expression and conversion factors according to global and regional regulations for vitamin E.

Source and Authority	Expression / calculation
Foods	
Codex	1 mg α-tocopherol = 1 mg *RRR*-α-tocopherol (D-α-tocopherol)
USFDA	1 mg vitamin E (as α -tocopherol) label claim = 1 mg of natural α-tocopherol (D-α-tocopherol) = 2 mg all-*rac*-α-tocopherol
EU	mg vitamin E
CHINA GB	1 mg α-TE (α-tocopherol equivalent) = 1 mg D-α-tocopherol α-tocopherol equivalent = α-tocopherol +0.5 β-tocopherol +0.1 γ-tocopherol +0.3 tocotrienol
FSANZ	Conversion factors for all natural forms not provided. 1 μg α-TE = • 1.36 DL-α-tocopherol • 1.10 D-α-tocopheryl acetate • 1.49 DL-α-tocopheryl acetate • 1.23 D-α-tocopheryl acid succinate
Infant formula, and formula for older infants and young children	
Codex	1 mg α-TE (α-tocopherol equivalent) = 1 mg D-α-tocopherol
USFDA	1 mg vitamin E (as α-tocopherol) label claim = 1 mg of natural α-tocopherol (D-α-tocopherol) = 2 mg all-*rac*-α-tocopherol
EU	mg α-tocopherol, based on vitamin E activity of *RRR*-α-tocopherol
CHINA GB	1 mg α-TE = • 1/0.74 = 1.35 mg DL-α-tocopherol • 1/0.5 = 2 mg β-tocopherol • 1/0.1 = 10 mg γ-tocopherol • 1/0.01 = 100 mg δ-tocopherol • 1/0.3 = 3.3 mg tocotrienol
FSANZ	1 μg α-TE = • 1.36 DL-α-tocopherol • 1.10 D-α-tocopheryl acetate • 1.49 DL-α-tocopheryl acetate • 1.23 D-α-tocopheryl acid succinate
Food supplements	
Codex	1 mg α-TE (α-tocopherol equivalent) = 1 mg D-α-tocopherol
USFDA	1 mg vitamin E (as α-tocopherol) label claim = 1 mg natural α-tocopherol (D-α-tocopherol) = 2 mg all-*rac*-α-tocopherol
EU	1 mg α-TE = 1 mg of D-α-tocopherol
CHINA GB	• 1 mg DL-α-tocopherol = 0.74 mg D-α-tocopherol • 1 mg D-α-tocopherol acetate = 0.91 mg D-α-tocopherol • 1 mg DL-α-tocopherol acetate = 0.67 mg D-α-tocopherol • 1 mg vitamin E calcium succinate (natural type) = 0.78 mg D-α-tocopherol • 1 mg vitamin E calcium succinate (synthetic) = 0.57 mg D-α-tocopherol • 1 mg D-α-tocopherol succinate = 0.81 mg D-α-tocopherol • 1 mg DL-α-tocopherol succinate = 0.60 mg D-α-tocopherol
FSANZ	1 μg α-TE = • 1.36 DL-α-tocopherol • 1.10 D-α-tocopheryl acetate • 1.49 DL-α-tocopheryl acetate • 1.23 D-α-tocopheryl acid succinate

2016), which authorise the fortification of different kinds of food products with vitamins. An overview of units of expression and their definition of vitamin E as per Codex standards (Codex, 1981, 1985, 1987) and regional regulations in the US (USFDA, 2016, 2019), Europe (EFSA, 2015b; EU, 2002, 2011, 2016), China (GB, 2008, 2010, 2011, 2012, 2013, 2021a, 2021b, 2021c) and Australia/New Zealand (FSANZ, 2018a, 2018b, 2018c, 2018d) is shown in Table 2.

The definitions for all the units of expression of vitamin E as shown in Table 2 mean that compounds to be included as vitamin E need to be corrected for their biological activity. If a product contains both natural tocopherol sources and synthetic tocopherol or its esters, it is necessary to apply the correct factors to yield an accurate result. For fortification purposes, manufacturers usually include all-*rac*-α-tocopheryl acetate or all-*rac*-α-tocopherol in their formulations. Additionally, *RRR*-α-tocopherol is present originating from natural oils as part of product ingredients. It should be noted that Codex guideline CXG 10–1979 (Codex, 1979) and European regulations EC 1170/2009 (EC, 2009) and EU 609/2013 (EU, 2013b), allow the use of D- and DL-α-tocopheryl acid succinate in addition to D- and DL-α-tocopherol, D- and DL-α-tocopheryl acetate and DL-α-tocopheryl polyethylene glycol-1000 succinate, mixed tocopherols and tocotrienols for fortification of foods and food supplements. Unfortunately, the detailed compositional information of a processed food is not typically available to analytical laboratories since only manufacturers possess recipe information.

Available international reference methods to determine vitamin E in foods are usually based on two approaches: (i) saponification of tocopheryl esters to liberate the free tocopherols followed by solvent extraction and separation of the individual tocopherols by HPLC and quantification by fluorescence detection, with an example of such a reference method being EN 12822:2014 (CEN, 2014a), and (ii) direct solvent extraction and simultaneous quantitative determination of vitamin E as α-tocopherol and α-tocopheryl acetate (e.g., AOAC and ISO methods for infant formula and adult nutritionals: AOAC INTERNATIONAL, 2023a; ISO, 2015a). With this approach it is assumed that α-tocopherol originates from a natural source, such as vegetable oils added as an ingredient, and α-tocopheryl acetate from a synthetic source used for fortification.

While not commonly used, the α-tocopherol succinate ester is a permitted form of vitamin E for fortification; however, it is not measured and/or validated by these methods.

Of nutritional significance is that neither of these commonly used analytical approaches distinguishes between *RRR*-α-tocopherol and the all-*rac*-α-tocopherol respectively. Recently, Gill and Indyk, 2020 published a suitable application where both *RRR*-α-tocopherol content and total α-tocopherol can be assayed for routine compliance testing. This method uses saponification as part of sample preparation, allowing for the measurement of endogenous α-tocopherol and α-tocopherol ester forms subsequent to HPLC separation utilising a chiral column and fluorescence detection. By calculating contributions of each α-tocopherol stereoisomer, the method is capable of distinguishing between natural and synthetic sources. A future compendial reference method based on this analytical approach is therefore required that can distinguish between natural and synthetic sources of α-tocopherol and thereby enable regulatory compliance verification.

2.3. Vitamin D

Vitamin D is represented by two fat-soluble compounds, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) and their respective 25-hydroxy vitamin D metabolites. The major food sources for naturally occurring vitamin D_3 include flesh of fatty fish and fish liver oils, while smaller amounts are found in egg yolks, milk, dairy products and beef liver, with vitamin D_2 present predominantly in fungi. 25-hydroxy metabolites of vitamin D are present in some foods of animal origin; however, information on amounts in most other types of food is lacking (Jakobsen et al., 2019; Jakobsen & Christensen, 2021). EFSA has recently set a factor of 2.5 for the bioactivity of 25-hydroxy-vitamin D_3 as compared to vitamin D_3 (EFSA, 2023). Additional sources of dietary vitamin D are fortified foods such as milk, margarine and/or butter, breakfast cereals and dietary supplements. Currently, vitamin D_3 and vitamin D_2 may be added to both foods and food supplements. Recent new European Union Regulation EU 2023/4 (EU, 2023b) authorised the use of vitamin D_2 mushroom powder as a novel food that stipulates its incorporation in a wide range of food categories compliant with maximum levels of vitamin D_2. Before the introduction of this regulation, vitamin D_3 was mainly used in Europe to fortify foods or applied in supplements. It should be noted that with the current European Standard (CEN, 2009b) for the determination of vitamin D in foods, only vitamin D_2 or vitamin D_3 can be quantified, wherein one of the compounds is used as an analytical internal standard in the presence of the other compound. Consequently, this method cannot be applied if both compounds are present in a food, a limitation given that nutrition labelling provisions, as according to Codex (Codex, 1985) or European regulation (EU, 2011), shall be based on total vitamin D present when

added to the food.

Recently, following an EFSA Scientific opinion (EFSA, 2018), new regulatory limits for vitamin D in infant formula were established by the European Commission with the Commission Delegated Regulation EU 2019/828 (EU, 2019) introducing a vitamin D interval range of 2 to 2.5 μg 100 $kcal^{-1}$. Available AOAC, CEN and ISO global reference methods (AOAC INTERNATIONAL, 2023d; CEN, 2009b; ISO, 2018a), able to determine either vitamin D_2 and/or vitamin D_3, have been assessed on their fitness for purpose to verify regulatory compliance (Gilliland et al., 2022; Konings et al., 2021). The assessment illustrates the high probability that a compliant product, with a true vitamin D level within the EU regulatory range, would fail to meet the revised regulatory requirements due to analytical method variability alone. Based on this assessment, it can be concluded that current, state-of-the-art methods cannot consistently verify product compliance for vitamin D with European regulatory requirements.

An additional challenge in vitamin D analysis in foods is that it exists in thermal equilibrium with the previtamin D conformer, and the simultaneous measurement of vitamin D and previtamin D is necessary to obtain an accurate and reliable estimate of its content in foods. Depending on storage conditions, foods containing vitamin D will have a significant contribution of previtamin D. Analytical methods typically use calciferol internal standards and are susceptible to analytical bias due to differences in the proportion of previtamin D in the sample and in the internal standard. To overcome this problem, a sufficiently high heat treatment during saponification is necessary to ensure that both the internal standard and sample analyte reach equilibrium prior to analysis (Gill et al., 2020).

If possible, a fit-for-purpose method applicable to the relatively narrow regulatory range for vitamin D in infant formula must be developed. Furthermore, to estimate the total vitamin D content in foods, a future reference method needs to include the 25-hydroxy vitamin D metabolites in addition to parent vitamin D_2 and vitamin D_3.

2.4. Vitamin K

Vitamin K is represented by phylloquinone (vitamin K_1), found in highest levels in certain green vegetables and vegetable oils, and the menaquinones (vitamin K_2, MK-n) found in animal foods such as meat and eggs (MK-4), but primarily in fermented foods such as cheeses and natto that contain MK-5 to MK-10. Naturally occurring phylloquinone has the C-2′ double bond in the *trans* configuration. Synthetic phylloquinone, used for fortification of predominantly infant formula, typically contains both the *trans* and *cis* isomers, but only the *trans* isomer is responsible for the vitamin's biological activity (Woollard, Indyk, Fong, & Cook, 2002). Differences between distribution of vitamin K vitamers following dietary intake have been observed in serum (Sato, Schurgers, & Uenishi, 2012; Schurgers & Vermeer, 2002).

Dihydro-K_1 is present in foods containing hydrogenated vegetable oils and contributes to physiologically active vitamin K in the diet (Dumont, Peterson, Haytowitz, & Booth, 2003). Fortification of foods with vitamin K is uncommon, except for infant formula to which synthetic preparations of phylloquinone are added. There are several regulations allowing the fortification of foods and food supplements with vitamin K, primarily as phylloquinone, but menaquinone-7 (MK-7) and menaquinone-6 (MK-6) forms are also approved in foods for special medical purposes and total diet replacement for weight control by European regulations (EC, 2009; EU, 2013b).

The European standard for the analysis of vitamin K in foods (CEN, 2003a) enables the quantification of total *cis*- and *trans*-vitamin K_1 and also enables the quantification of MK-4 and MK-7. However, an inter-laboratory study for the quantification of MK-n is still needed to demonstrate that the method is fit for purpose to quantify these compounds. Recently, quantification of vitamin K_1 and MK-4 to MK-10 in food achieved satisfactory results when comparing two detection methods, fluorescence and mass spectrometry (MS) (Jensen, Rød, Ložnjak Švarc, Oveland, & Jakobsen, 2022). A method for the determination of total vitamin K_1 in infant formula and adult nutritionals has been published by AOAC and ISO (AOAC INTERNATIONAL, 2023e; ISO, 2019). This method can distinguish between the bioactive *trans*- and non-bioactive *cis*-phylloquinone, but does not allow the measurement of MK-n. However, global regulatory requirements do not make any distinction between the geometric isomers. From the validation data of this method, based on 13 different fortified infant formula and adult nutritionals including four placebo materials, it became clear that approximately 12% of the total phylloquinone amount is *cis*-phylloquinone. Most of this amount (9%) comes from the premix used for fortification and the remaining fraction (3%) comes from the ingredients used for these products. The products analysed represent most matrices on the global market.

To enable verification of the regulatory requirements and information in food composition tables for vitamin K a reference method is needed to quantify the amount of phylloquinone and the menaquinones, as well as dihydro-K_1. Future regulatory requirements should consider the different bioactivities of *cis*- and *trans* vitamin-K_1 and the individual menaquinones.

2.5. Vitamin B_1

All plants contain primarily non-phosphorylated thiamine (vitamin B_1), while animal products contain thiamine mainly as phosphorylated forms (Gropper, Smith, & Carr, 2021). Thiamine diphosphate is involved as a coenzyme for enzymes in carbohydrate and branch-chained amino acid metabolism.

There are several international reference methods for the quantification of vitamin B_1 in foods, although only a few international reference methods are validated for the determination of vitamin B_1 in infant formula. In general, these methods liberate thiamine in its free form prior to measurement. An early fluorometric AOAC Official Method 942.23 (AOAC INTERNATIONAL, 2023f) cannot now be considered as fit for purpose. Currently, the most commonly applied procedure to determine vitamin B_1 uses HPLC with direct quantification either by mass spectrometry, or by fluorometry after a derivatisation step. A European standard, EN 14122:2014 (CEN, 2014b) for the determination of vitamin B_1 in foods provides an option to use either pre- or post-column derivatisation following enzymatic treatment. Usually, the vitamin B_1 content typically consists of thiamine and its phosphate derivatives. However, the use of a post-column derivatisation method to determine thiamine can show two peaks; one for thiamine and the other for the metabolite 2-(1-hydroxyethyl)thiamine (HET), which has equal bioactivity to thiamine (Jakobsen, 2008; Takashi, Yukiko, Kohei, Mari, & Kaname, 1990; Takashi, Yukiko, Kohei, Masako, & Kaname, 1991). If the pre-column derivatisation technique is used, the two compounds thiamine and HET result in two different derivatives that co-elute.

The relative content of HET compared with thiamine is dependent on the sample type. In meat and liver, the content of HET varied between 7 and 23%, in yeast the content was 3.8%, while in white cabbage, broccoli, oat flour, infant formula, milk powder and wheat the content was below 2% (Jakobsen, 2008). For the quantitative HPLC determination of vitamin B_1 in foods by post-column derivatisation, it is therefore recommended to include separate quantitative determination of thiamine and HET when analysing meat samples, and to monitor for HET for other foods. A future reference method should include the validation of the quantification of HET in addition to thiamine.

The largest component of vitamin B_1 in infant formula is from fortification, with thiamine chloride hydrochloride, thiamine hydrochloride and thiamine mononitrate as allowed sources (Codex, 1979; EC, 2009). In food supplements, thiamine monophosphate chloride and thiamine pyrophosphate chloride are also allowed as a vitamin B_1 source (EC, 2009). As some reference methods were developed prior to authorisation of certain vitamin B_1 sources, it is important to include these compounds in the validation of future reference methods. AOAC

Official Method 986.27 for the determination of vitamin B_1 in milk-based infant formula (AOAC INTERNATIONAL, 2023g) originates from the 1980's and is based on fluorometry. This method was considered not fit-for-purpose and was superseded in 2015 with a multi B-group vitamin method (AOAC INTERNATIONAL, 2023h; ISO, 2020b) based on LC-MS/MS, which will be described in more detail in the next paragraph on vitamin B_2.

A confusion with the expression of vitamin B_1 is related to the chemical form and related molecular weights (ISO, 2021). There is no globally harmonised convention how the amount of vitamin B_1 on the nutrition label is expressed. Since thiamine ($C_{12}H_{17}N_4OS$, molecular weight: 265.37 g mol^{-1}), thiamine chloride ($C_{12}H_{17}ClN_4OS$, molecular weight: 300.82 g mol^{-1}) and thiamine chloride hydrochloride (molecular weight: 337.27 g mol^{-1}) are all considered as vitamin B_1, this is problematic for enforcement laboratories verifying label declarations.

2.6. Vitamin B_2

Vitamin B_2 (riboflavin) is water-soluble and is biologically active in the form of two flavin coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), participating in oxidation-reduction reactions in numerous metabolic pathways. Riboflavin can be found in a wide range of food products, mainly in its phosphorylated forms, and rich sources include liver, kidney, eggs, meat, milk and cheese (Ball, 2006).

The forms of vitamin B_2 allowed for fortification of foods and food supplements include riboflavin and FMN sodium (Codex, 1979; EC, 2009). A horizontal European standard, originating from the 1980's to determine total vitamin B_2 in foods is EN 14152:2014 (CEN, 2014c) that includes an extraction of riboflavin after combined acid hydrolysis and enzymatic treatment, with riboflavin separated by HPLC and quantified by fluorometry. With current analytical technology, it is more efficient to determine multiple B-vitamins in a single analysis using MS/MS detection. AOAC 2015.14 or its equivalent ISO 21470:2020 (AOAC INTERNATIONAL, 2023h; ISO, 2020b), for example, specify a method for the simultaneous quantitative determination of four water-soluble vitamins in infant formula and related nutritional products, including relevant forms of vitamins B_1, B_2, B_3 and B_6 by enzymatic digestion and UHPLC-MS/MS quantification, with enhanced sensitivity and specificity. Although these reference methods are not intended to be used on products where these vitamins have not been added, with some adaptations and new validation rounds, they may form the basis as a future reference method for application to foods.

2.7. Vitamin B_3

In the labelling of food products, vitamin B_3 is often described as niacin. The vitamin activity is linked to two different molecules: nicotinic acid and nicotinamide (where an -NH_2 replaces the -OH of the carboxylic group) and both forms are allowed for fortification in foods and food supplements (Codex, 1979; EC, 2009). Some food standards and regulations express the amount in niacin equivalents (NE), which includes tryptophan, where 1 mg NE is 1 mg niacin or 60 mg tryptophan (Codex, 1985; USFDA, 2016).

Rich sources of vitamin B_3 include animal tissues, particularly as liver, muscles, kidney as well as cereals. In animal tissues, vitamin B_3 is mainly present as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), while in cereals vitamin B_3 is mainly present as esters with polysaccharides (niacytin) or is bound to polypeptides and glycopeptides (niacinogenes) (Chamlagain, Rautio, Edelmann, Ollilainen, & Piironen, 2020). This is important when considering the analysis of natural niacin present in food, as nicotinic acid and nicotinamide must both be released from NAD and NADH by the analytical procedure.

In the current European Standard EN 15652:2009 (CEN, 2009c) three method options are available. Option A is using an acid treatment in the sample preparation, option B uses an enzymatic treatment, and option C incorporates an alkaline treatment. In options A and B, nicotinic acid and nicotinamide are separated and quantified. For these two options, vitamin B_3 expressed as nicotinic acid and gives similar results, although it should be noted that a small amount of nicotinamide is transformed into nicotinic acid with the acid treatment. Typically, option A is faster than option B; however, if the exact amount of each form needs to be quantified, option B must be used. With option C, nicotinamide is transformed into nicotinic acid and the results are the same as with options A and B, except for cereals where the natural amount of vitamin B_3 is increased by the alkaline treatment. Chamlagain et al. (2020) challenged that this treatment may reflect total niacin rather than actual bioaccessible niacin.

With recent modifications of the European regulations, it seems important to test the applicability of the current CEN standard (CEN, 2009c) on two newly permitted forms of vitamin B_3 that can be used in food supplements and foods. European Regulation (EC, 2009) authorised the addition of inositol hexanicotinate in food supplements and more recently, European Regulation 2020/16 (EU, 2020) authorised the use of nicotinamide riboside chloride as a novel food in food supplements with maximum levels for the general population and pregnant and lactating women. European Regulation EU 2023/1065 (EU, 2023c) very recently also authorised the use of nicotinamide riboside chloride in foods as a source of niacin.

It may be possible that with sample preparation option A (acid hydrolysis) of EN 15652:2009 (CEN, 2009c), vitamin B_3 from these two new forms can be released when used in foods and food supplements. With option B, the nicotinamide is released from the riboside chloride with the action of the enzyme NAD^+ glycohydrolase by breaking the N-ribosidic bond (Apitz, Mickelson, Shriver, & Cordes, 1971). Where inositol hexanicotinate is used as vitamin B_3 source in fortified foods, it may be more difficult to release all nicotinate moieties during analysis.

A technical challenge when analysing vitamin B_3 in food supplements relates to the considerable difference in maximum amounts that are allowed to be used for nicotinamide and nicotinic acid, respectively. The tolerable upper intake level for nicotinamide is between 70 and 100 times higher as compared with nicotinic acid (EFSA, 2006). It is therefore important that nicotinamide and nicotinic acid forms are quantified and reported separately in food supplements.

2.8. Vitamin B_5

Vitamin B_5, pantothenic acid, a component of both coenzyme A and acyl carrier protein, is involved in multiple metabolic reactions related to the release of energy from carbohydrates, fats, and amino acids. Pantothenic acid is readily available in foods from animal and plant origin with the highest amounts in liver, kidney, yeast, egg yolk, and broccoli. Although also available in foods in its free form, the release of the bound forms is required for the quantification of total pantothenic acid in foods. Acid or alkaline hydrolysis conditions are not suitable to release this compound due to its lability at either acid or basic pH. Therefore, a combination of enzymatic treatments is generally used to liberate pantothenic acid before further quantification (Andrieux, Fontannaz, Kilinc, & Campos-Giménez, 2012).

AOAC 992.07 (AOAC INTERNATIONAL, 2023i) for the determination of pantothenic acid is based on a microbiological turbidimetric method and validated for milk-based infant formula. This method was replaced with AOAC 2012.16 and ISO 20639:2015 using UHPLC-MS/MS (AOAC INTERNATIONAL, 2023j; ISO, 2015b). Comparison of results between total and free pantothenic acid showed that the analysis of free pantothenic acid alone gave a good estimation of total pantothenic acid in the range of fortified products analysed. The method provides reliable free pantothenic acid results in a wide range of fortified foods including infant and adult nutritionals, cereal products and beverages, and shows good correlation with the microbiological method AOAC 992.07 (Andrieux et al., 2012). LC-MS/MS methodology is a more selective,

faster, and more robust alternative to microbiological determination and the development and validation of a reference LC-MS/MS method for foods is recommended (Andrieux et al., 2012). It should be noted however, that pantothenol, the alcohol analogue of pantothenic acid, is also allowed as source for the fortification of foods, food supplements, infant formula and follow on formula, processed cereal-based food and baby food, food for special medical purposes and total diet replacements for weight control (Codex, 1979; EC, 2009; EU, 2013b), and should be considered when determining total pantothenic acid in future reference methods.

2.9. Vitamin B_6

There are six vitamers of vitamin B_6: pyridoxine (pyridoxol), pyridoxal and pyridoxamine and their corresponding phosphorylated derivatives pyridoxine 5′-phosphate, pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate, with the vitamin activity of each being identical (Ball, 2006). Vitamin B_6 as pyridoxal 5′-phosphate serves as a cofactor for enzymes in many metabolic reactions.

Vitamin B_6 can be found in many food products either free or as phosphorylated forms, with the highest amounts found in dry yeast and wheat germ. However, for a human diet, animal sources such as meat, and fish as well as fruits, vegetables and cereals are important sources (Ball, 2006). Vitamin B_6 is mainly present as pyridoxine in plants and as pyridoxal and pyridoxamine in animal tissue, often bound to proteins and carbohydrates. In some vegetables vitamin B_6 can also be present as glucosides (Kall, 2003), but the bioavailability of these glucoside forms are regarded as lower (Gregory, 1997; Gregory et al., 1991).

To quantify vitamin B_6 in food products, it is therefore necessary to include an acid hydrolysis and an enzymatic step with a phosphatase as part of the analytical procedure to liberate the three bound forms. This drives the choice for an analytical method to either quantify the three forms separately, or chemically transform to a single vitamer form to determine an aggregate total vitamin B_6. In addition, to quantify the vitamin B_6 glucosides the analytical procedure must include an enzymatic step with glucosidase. There are two European Standards to determine vitamin B_6 in foods. EN 14663:2005 (CEN, 2005) includes an extraction of total vitamin B_6 after an acid hydrolysis and an enzymatic treatment with phosphatase, with pyridoxine, pyridoxamine and pyridoxal subsequently separated by HPLC and quantified by fluorometry. An additional optional enzymatic step with glucosidase has also been included to liberate pyridoxine glucosides. EN 14164:2014 (CEN, 2014d) includes an extraction of vitamin B_6 after an acid hydrolysis and an enzymatic treatment. Pyridoxamine and pyridoxal are both subsequently chemically transformed to pyridoxine, which is separated by HPLC and quantified by fluorometry.

In addition to pyridoxine hydrochloride and pyridoxine 5′-phosphate, European regulations have authorised the use of pyridoxine dipalmitate as a source to fortify foods (EC, 2009) in particular foods for special medical purposes, processed cereal-based food and baby food and total diet replacement for weight control (EU, 2013b). This source of vitamin B_6 is not captured with the current reference methods to quantify vitamin B_6 in foods.

2.10. Vitamin B_7

Biotin (vitamin B_7), initially named as vitamin H, or in some countries as vitamin B_8, is an essential micronutrient for all mammals because of its role as a cofactor for carboxylase enzymes. Human cells have four biotin-dependent carboxylases that catalyse key reactions in gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Biotin has three asymmetric carbons, and can therefore exist as eight potential stereoisomers, but only the D-biotin form is both biologically active and found in nature and is the only form of the parent vitamin that requires consideration. Biotin is widely distributed in the human diet, either mostly free in the case of milk, vegetables and fruit, or partly covalently protein-bound as biocytin (ε-N-biotinyl-L-lysine) in animal tissues and plant seeds (Eitenmiller, Landen Jr., & Ye, 2008; Woollard & Indyk, 2013).

Current global and international reference methods (EU, CEN, Codex, ISO, AOAC, GB, FSANZ) do not adequately define this micronutrient, leaving a gap as to whether the bioavailable biocytin form should be included from a compliance perspective. All current regulatory standards define this vitamin simply as biotin, and most do not reference its stereoisomeric form. Further, the presence of protein-bound biocytin in a food will lead to an underestimate of bioavailable biotin if excluded from analysis.

For an estimation of total biotin in food, a proteolytic step with biotinidase is required to specifically release biotin from any biocytin present in the food, which may then be included with free biotin in the analysis. Prior to end-point analysis, the variation in extraction protocols found in international reference methods related to the analysis of biotin in foods is a challenge, partly due to issues related to availability and specific conditions required for biotinidase (Eitenmiller et al., 2008; Woollard & Indyk, 2013). Variability of vitamin stability and recovery as a result of variations in sample preparation can have a greater significance on the result than the actual end-point detection technique and standardisation of sample preparation across methods is therefore needed to reduce food compositional data variability. Irrespective of whether free or total biotin is estimated, analytical methods report content as biotin. For those analytical methods in which biocytin is included, its bioavailability is accounted for as follows: 1 μg D-biocytin = 0.656 μg D-biotin due to the difference in molecular weight.

Analytical techniques utilised for the determination of biotin content can be categorised as microbiological, biological (no longer used), chromatographic, or biospecific ligand-binding, and have been comprehensively reviewed (Eitenmiller et al., 2008; Woollard & Indyk, 2013). Although the microbiological assay (GB 5009.259–2016) remains the reference method for foods (GB, 2016b), chromatographic methods with MS, UV or fluorescence detection are increasingly utilised (AOAC INTERNATIONAL, 2023k, 2023l; CEN, 2009d; Gill, Saldo, Wood, & Indyk, 2018; Woollard & Indyk, 2013). However, among current global reference methods, whether biocytin-derived biotin is included in the reported measurement is not always apparent, nor consistent. In contrast to most foods, this issue is less significant in the case of infant formulas, where the overwhelming majority of biotin is present in its free supplemental form.

While existing global reference methods target biotin as measurand, they are ambivalent and inconsistent regarding the inclusion of biocytin in the determination of biotin content of a food (AOAC INTERNATIONAL, 2023k, 2023l; CEN, 2009d; GB, 2016b; ISO, 2020c). Codex has adopted the AOAC and its equivalent ISO standard and CEN methods as Type II and III for foods respectively for special dietary uses (Codex, 1999). Depending on the reference method used, inherent differences in analytical results can therefore be expected.

In summary, the gap that currently exists is due to the absence of a more specific definition of biotin, generating confusion, primarily regarding either whether biocytin should be included in an estimate of total biotin, or to measure free biotin alone, the latter resulting in an underestimate of nutritionally available content. For this reason, regulatory standards should be revised and preferably harmonised globally to define the specific forms of biotin to be determined during analysis.

2.11. Vitamin B_9

Folic acid (vitamin B_9) is the synthetic form of folate used as a reference compound for a group of folate vitamers that have vitamin activity. 5-Methyltetrahydrofolate and 5-formyltetrahydrofolate are the main vitamers that occur naturally in foods, whether in a free form or as polyglutamates, while folic acid is the primary form used for food fortification. Folic acid is generally not present in nature, or present at very low levels from oxidation of other vitamers. To account for

Table 3
Overview of units of expression and conversion factors according to global and regional regulations for folate.

Source and Authority	Expression / calculation
Foods	
Codex	1 μg dietary folate equivalents (DFE) • 1 μg food folate • 0.6 μg folic acid added to food or as supplement consumed with food • 0.5 μg folic acid as supplement taken on an empty stomach
USFDA	1 μg dietary folate equivalents (DFE) = μg naturally occurring folate + (1.7 × μg folic acid)
EU	μg folic acid
CHINA GB	1 μg dietary folate equivalents (μg DFE) = naturally occurring folic acid in food (μg) + 1.7 × folic acid as supplement (μg)
FSANZ	μg folic acid
Infant formula and formula for older infants and young children	
Codex	μg folic acid/100 kcal/100 kJ
USFDA	DFE = dietary folate equivalents; 1 DFE = 1 μg naturally occurring folate = 0.6 μg folic acid.
EU	1 μg dietary folate equivalents (DFE) = 1 μg food folate = 0.6 μg folic acid from formula
CHINA GB	μg folic acid
FSANZ	μg folic acid

difference in bioavailability of supplemental versus natural occurring folate forms, the unit dietary folate equivalent (DFE) has been introduced. Table 3 gives an overview of units of expression and their definition as per Codex standards (1987; Codex, 1981, 1985) and regional regulations in the US (USFDA, 2016, 2019), EU (EC, 2009, EU, 2011, EU, 2016), China (GB, 2008, 2010, 2011, 2012, 2013, 2021a, 2021b, 2021c) and Australia/New Zealand (FSANZ, 2018a, 2018b, 2018c, 2018d).

Most global standards and regional regulations use DFE as the unit to express total folate content of foods, although general rules for nutrition labelling in the EU refer to folic acid in micrograms (EU, 2011). However, specific regulations authorising fortification of food products, such as infant formula, require the use of DFE to express folate content (EU, 2016). European regulation also allows the use of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt as a source of folate added for nutritional purposes to food supplements (EU Regulation 2015/414 (EU, 2015)), as well as the use of calcium-L-methylfolate as a source of folate in food for special medical purposes and total diet replacement for weight control (EU, 2013b).

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) proposed to use the same factor as for folic acid for conversion of 5-methyltetrahydrofolate into DFE for intakes <400 μg day^{-1}. As such intake levels are unlikely to be exceeded through fortified food consumption, the conversion factor of 1.7 relative to natural folate (NF) could be applied to 5-methyltetrahydrofolate added to foods and to food supplements providing <400 μg day^{-1}. At 400 μg day^{-1}, 5-methyltetrahydrofolate was found to be more bioavailable than folic acid and a conversion factor of 2 is proposed for this intake level and higher (EFSA, 2022).

To be able to verify compliance with the labelled values, an analytical method is needed that can distinguish between supplemental folic acid and/or 5-methyltetrahydrofolate and natural available folates. There is currently no European (CEN) standard available to support this requirement. The current European standard for the determination of folates in foods is based on a microbiological assay (CEN, 2003b) and is only able to assess total folate content. Recently an AOAC Official Method 2011.06 and equivalent ISO Standard 20,631:2023 have been established for determination of folate in infant formula and adult nutritionals (AOAC INTERNATIONAL, 2023m; ISO, 2023). This LC-MS/MS method quantifies folic acid as well as 5-methyl-tetrahydrofolate and 5-formyl-tetrahydrofolate, and has been single-laboratory and multi-laboratory validated for selected foods, infant formula and adult nutritionals (Bhandari, Gao, & Szpylka, 2018; Szpylka, DeVries, Cheney, & House, 2012). A suitable alternative LC-MS/MS method (Ložnjak Švarc et al., 2020b; Ložnjak, Striegel, Díaz De la Garza, Rychlik, & Jakobsen, 2020a)() for a broad range of food matrices is also standardised by NMKL (NMKL, 2020). The method includes a simplified enzymatic extraction procedure using a plant-based deconjugase and quantifies total folate including folic acid and naturally occurring food folates 5-methyltetrahydrofolate, 5-formylterahydrofolate, tetrahydrofolate, 10-formylfolic acid and 5,10-methenyltetrahydrofolate with satisfactory repeatability and reproducibility. To express total folate content in foods, it should be questioned if natural and supplemental 5-methyltetrahydrofolate can be distinguished analytically if the latter is used for fortification.

2.12. Vitamin B_{12}

Vitamin B_{12} is the term used to designate all compounds having the same biological activity as cyanocobalamin. It is present in nature as methylcobalamin, adenosylcobalamin and hydroxycobalamin, the latter originating mainly from the effect of light degradation. Food sources of vitamin B_{12} are restricted to those of animal origin including fish, meat, poultry, eggs, and dairy products (Ball, 2006; Watanabe, 2007). Cyanocobalamin, the most stable form, is used in fortification and supplements, although is only present in nature in trace amounts. Vitamin B_{12} cobalamin analogues, pseudo-vitamin B_{12}, are molecules that, structurally, are very similar to vitamin B_{12} and can also be found in nature. However, these are not utilised in the human body and may prevent uptake of physiologically active vitamin B_{12} (Carmel, Karnaze, & Weiner, 1988). These vitamin B_{12} analogues can be present in food supplements such as *Spirulina* or seaweeds (e.g., Nori, Wakame).

Microbiological methods such as AOAC 952.20 (AOAC INTERNATIONAL, 2023n), which are still considered as the reference method for foods and food supplements cannot distinguish vitamin B_{12} from pseudo-vitamin B_{12}. Consequently, certain foods may be claimed to be "rich in vitamin B_{12}", while in fact this may not be the case. Although chromatographic methods may be able to distinguish both forms, they have not included separation of pseudo-vitamin B_{12} from active forms as part of method validation. There is currently no European standard for the determination of vitamin B_{12} in foods. Recently, an AOAC Official Method 2011.10 and its equivalent ISO standard 20,634:2015 have been published for the determination of vitamin B_{12} in infant formula and adult nutritionals using HPLC-UV (AOAC INTERNATIONAL, 2023o; ISO, 2015c). In addition, AOAC published another HPLC-UV method (AOAC 2014.02; AOAC INTERNATIONAL, 2023p) for the determination of vitamin B_{12}, also for infant formula and adult nutritionals with different sample preparation, but equivalent results as compared with AOAC 2011.10. Future reference methods applicable to foods that are able to distinguish between vitamin B_{12} and its cobalamin analogues are therefore required.

2.13. Vitamin C

Vitamin C (ascorbic acid) is widely distributed in fresh fruits and vegetables and is an important antioxidant of reactive oxygen species such as singlet oxygen and plays a role as co-factor for metalloenzymes in the reduction phase (Gropper et al., 2021). The forms L-ascorbic acid and the oxidised form dehydroascorbic acid (DHAA) exhibit similar activity (Frikke-Schmidt, Tveden-Nyborg, & Lykkesfelt, 2016). However, D-isoascorbic acid named erythorbic acid is cheaper to manufacture than L-ascorbic acid and in some countries, it is legal to substitute erythorbate for ascorbate when, for technical reasons, the antioxidant capacity rather than the vitamin C activity is required. In some countries, erythorbate is not permitted as an antioxidant, and is also prohibited for use with raw and unprocessed meats. It should be noted that Codex Guidelines and European regulations (Codex, 1979; EC, 2009; EU, 2013b) allow the use of L-ascorbyl 6-palmitate as a source for vitamin C in addition to L-ascorbic acid and ascorbate salts. Although

ascorbyl palmitate does not occur in nature, it does exhibit the full antiscorbutic activity of ascorbic acid.

When analysing vitamin C in food products it is necessary to discriminate between L-ascorbic acid and D-isoascorbic acid and to include their dehydro-forms. Both ascorbic and dehydroascorbic acids should be quantified when estimating the total vitamin C activity of a food product. One of the significant challenges with any analytical method for the determination of vitamin C is the need to stabilise the very labile vitamin, both during extraction and subsequent measurement. Existing reference methods to determine vitamin C in foods can be divided into three categories:

(1) Titration with 2,6-dichlorophenolindophenol. The advantage of these methods is that they are cheap, rapid and therefore suitable for quality control in a factory environment. The disadvantages are that (i) these methods do not include dehydroascorbic acid, (ii) D-isoascorbic acid will react in the same manner, and (iii) these methods are vulnerable to potential interferences from pigmented and reducing sample components.
(2) Fluorometric methods. These are based upon oxidation of ascorbic acid to dehydroascorbic acid and reaction with *o*-phenylene diamine (OPDA) to form a quinoxaline. Advantages are that these methods include dehydroascorbic acid and can be applied to coloured extracts and samples containing reducing substances, although suitability to green leafy vegetables has been questioned (Wills, Wimalasirl, & Greenfield, 1983). A disadvantage is that D-isoascorbic acid will react in the same manner.
(3) HPLC based methods. These methods are based on acid extraction followed by chromatographic separation. For example, Kall and Andersen (1999) described a method for simultaneous determination of ascorbic acid and isoascorbic acid detected by UV, after which dehydroascorbic acid and dehydroisoascorbic acid were quantified following post-column derivatisation with OPDA and fluorescence detection.

There is no European standard for the determination of vitamin C in foods. Until 2017, AOAC 967.21 (AOAC INTERNATIONAL, 2023q), a titrimetric method applicable to determination of reduced ascorbic acid in citrus and tomato juices, was considered as a reference method (Type II) for the determination of vitamin C in infant formula by Codex. A method for the quantification of ascorbic acid and isoascorbic acid in fortified foods including infant formula and nutritional products (Fontannaz, Kilinc, & Heudi, 2006) was the basis for a recent AOAC official method and ISO standard for vitamin C in infant formula and adult nutritionals (AOAC INTERNATIONAL, 2023r; Campos-Giménez & Martin, 2017; ISO, 2018b). Using this method, applicability to a wider scope of matrices could be demonstrated for a future reference method applicable to foods. However, currently no reference method exists to quantify ascorbyl palmitate when used as a vitamin C source.

3. Recommendations

Existing reference methods for the determination of vitamins in foods need to be updated/renewed because of:

- Lack of up-to-date reference methods validated for foods in general
- New units of expression of vitamin amounts
- Newly authorised molecules for fortification
- Newly identified compounds with or without vitamin activity
- Advanced analytical technology, e.g. MS/MS as more selective, faster and robust alternative to microbial determination
- Simultaneous multi-vitamin analysis possible to save time and costs

For each of the vitamins the main recommendations for the development of new reference methods and/or other actions are summarised

Table 4
Most important recommendations and needs driven by limitations in current reference methods.

Vitamin	Recommendations/needs	Limitation
Vitamin A (and carotenoids)	Reference methods for the determination of provitamin A carotenoids including α-carotene, β-carotene, and β-cryptoxanthin in foods.	New expression of vitamin A in label requirements: REA (Retinol Activity Equivalent).
	Reference methods carotenoids in food supplements.	Need for chiral column chromatographic methods able to confirm economic motivated adulteration e.g. botanical versus synthetic zeaxanthin, or claims on "wild salmon".
	Propose globally harmonised definition of vitamin A.	Regulatory definitions of vitamin A are focused on "*all-trans*" retinol only, whereas 13-*cis* retinol has 0.75 vitamin A activity as compared to "*all-trans*".
Vitamin E	Reference methods using chiral column chromatography that can distinguish between natural and synthetic sources of α-tocopherol.	To express vitamin E correctly on a product label requires the use of different conversion factors for natural and synthetic sources of α-tocopherol.
	Validate future reference methods for all sources allowed for food fortification.	Current reference methods are not validated for tocopheryl succinate and tocopheryl polyethylene glycol-1000 succinate.
Vitamin D	Future reference method including the 25-hydroxy-vitamin D metabolites in addition to parent vitamin D_2 and vitamin D_3.	25-Hydroxy-vitamin D_3 has 2.5 times more bioactivity as vitamin D_3 and contributes significantly to vitamin D intake. Reference methods are lacking.
	Fit for purpose method applicable to demonstrate compliance with narrow European regulatory range for vitamin D in infant formula.	Precision of current reference methods too high.
Vitamin K	Propose globally harmonised definition for vitamin K_1 excluding *cis*-vitamin K_1.	*cis*-Vitamin K_1 has no vitamin K activity and currently considered as part of this vitamin.
	Reference methods for the determination of phylloquinone and the menaquinones as well as dihydro-K_1.	Lack of reference methods to determine these compounds in foods.
Vitamin B_1	Consider thiamine metabolite 2-(1-hydroxyethyl)thiamine (HET) as vitamin B_1.	HET has equal bioactivity as compared to vitamin B_1, currently not included and validated in reference methods, while present in foods.
	Propose globally harmonised convention how to express vitamin B_1 on product label.	Vitamin B_1 can be expressed as thiamine, thiamine chloride or thiamine chloride hydrochloride. This is confusing for enforcers of label claims.
Vitamin B_2	Reference methods for simultaneous quantitative determination of multiple B-vitamins including B_1, B_2, B_3, B_6 in foods by LC-MS/MS.	This would increase specificity, sensitivity, efficiency and reduce costs as compared to individual LC-FLD and/or microbial assays.
Vitamin B_3	Include newly authorised molecules to fortify foods and food supplements in Europe: inositol hexanicotinate and nicotinamide riboside chloride in future validated reference methods.	New molecules not included/ validated with current available reference methods.

(*continued on next page*)

Table 4 (*continued*)

Vitamin	Recommendations/needs	Limitation
Vitamin B_5	Replace current microbial assays for foods with LC-MS/MS and include authorised molecule pantothenol in validation of fortified foods and food supplements.	LC-MS/MS technology makes method more selective, faster and robust as compared to microbial assays. Current reference methods not validated for pantothenol as vitamin B_5 source in foods and food supplements.
Vitamin B_6	European regulation authorises the use of pyridoxine dipalmitate in foods and food supplements.	Current reference methods do not include this vitamin B_6 molecule.
Vitamin B_7	Propose globally harmonised definition which includes biocytin.	Current reference methods ambivalent and inconsistent regarding inclusion of biocytin.
Vitamin B_9	Replace current microbial reference methods with LC-MS/MS enabling the individual quantification of folate vitamers.	Total folate in foods is generally expressed as Dietary Folate Equivalents which require the use of different conversion factors for natural and synthetic sources of folate. Microbial methods are not able to distinguish between natural and synthetic sources of folate.
Vitamin B_{12}	Reference methods applicable to foods and able to distinguish between vitamin B_{12} and analogues.	Microbial methods still considered as reference for foods. They cannot distinguish vitamin B_{12} from pseudo-B_{12}.
Vitamin C	Reference methods for vitamin C in foods.	No fit for purpose reference methods validated for foods in general.

in Table 4.

As new analytical method development is time consuming and costly, an alternative first step could be to establish method performance requirements for the determination of relevant vitamins in foods. However, trueness is an important criterion typically not included in method performance requirements and the availability of (certified) reference materials is therefore extremely important. If not available, the creation of these materials could ideally be aligned with developing reference methods.

4. Conclusions

Existing reference methods for the determination of vitamins in foods originate from the 1990s, with more recently established reference methods between 2010 and 2021 specifically scoped for infant formula and adult nutritionals. Developments in science, technology and regulatory requirements have identified the need to consider new global reference methods applicable to vitamins and carotenoids to enable the verification of regulatory compliance and determination of food composition.

This area of nutrients may benefit from globally harmonised definitions on what compounds to include or exclude for analysis and their corresponding bioactivity factor where applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Erik Konings: Writing – review & editing, Writing – original draft, Conceptualization. **Brendon David Gill:** Writing – review & editing, Writing – original draft, Conceptualization. **Jette Jakobsen:** Writing – review & editing, Writing – original draft. **George Joseph:** Writing – review & editing, Writing – original draft. **Esther Campos-Giménez:** Writing – review & editing, Writing – original draft. **Jean-Luc Deborde:** Writing – review & editing, Writing – original draft. **Wei Wang:** Writing – review & editing. **Ruben Buis:** Writing – review & editing. **Harvey Indyk:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Allied Market Research. (2023). Carotenoids market. Carotenoids Market Size, Share, Growth | Forecast Report, 2031. alliedmarketresearch.com.

Ames, S. R. (1966). Methods for evaluating vitamin A isomers. *Journal of AOAC International, 49*, 1071–1078. https://doi.org/10.1093/jaoac/49.5.1071

Andrieux, P., Fontannaz, P., Kilinc, T., & Campos-Giménez, E. (2012). Pantothenic acid (vitamin B5) in fortified foods: Comparison of a novel ultra-performance liquid chromatography-tandem mass spectrometry method and a microbiological assay (AOAC Official Method℠ 992.07). *Journal of AOAC International, 95*, 143–148. https://doi.org/10.5740/jaoacint.10-333

AOAC INTERNATIONAL. (2023a). Official method 2012.10. Determination of vitamins E and a in infant formula and adult nutritionals. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL, & Latimer, G. W., Jr. (2023b). SMPR 2011.003. Standard method performance requirements for vitamin A in infant formula and Adult/pediatric nutritional formula. In *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023c). Official method 2016.13. Lutein, β-carotene, and lycopene in infant formula and adult nutritionals reversed-phase ultra-high-performance liquid chromatography (UHPLC). In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023d). Official method 2016.05. Analysis of vitamin D2 and vitamin D3 in fortified milk powders, infant formulas, and adult/pediatric nutritional formulas. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023e). Official method 2015.09. Trans and total (cis+trans) vitamin K1 in infant, pediatric, and adult nutritionals. HPLC with fluorescence detection. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023f). Official method 942.23. Thiamine (vitamin B1) in human and pet foods: Fluorometric method. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023g). Official method 986.27. Thiamine (vitamin B1) in milk-based infant formula: Fluorometric method. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023h). Official method 2015.14. Simultaneous determination of total vitamins B_1, B_2, B_3, and B_6 in infant formula and related nutritionals: Enzymatic digestion and LC-MS/MS. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023i). Official method 992.07. Pantothenic acid in milk-based infant formula: Microbiological turbidimetric method. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023j). Official method 2012.16. Pantothenic acid (vitamin B5) in infant formula and adult/pediatric nutritional formula: Ultra-high-pressure LC-MS/MS method. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023k). Official method 2016.02. Total biotin in infant formula and adult/pediatric nutritional formulas. Liquid chromatography coupled with immunoaffinity column cleanup extraction. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023l). Official method 2016.11. Biotin in infant, pediatric and adult nutritionals. High-performance liquid chromatography and fluorescence detection. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023m). Official method 2011.06. Total folate in infant formula and adult nutritionals: Trienzyme extraction and LC-MS/MS Quantitation. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023n). Official method 952.20. Cobalamin (vitamin B12 activity) in vitamin preparations. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023o). Official method 2011.10. Vitamin B_{12} in infant formulas and adult nutritionals. High-performance liquid chromatography. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023p). Official method 2014.02. Vitamin B_{12} (cyanocobalamin) in infant formula and adult/pediatric nutritional formula. Liquid chromatography-ultraviolet detection. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023q). Official method 967.21. Ascorbic acid in vitamin preparations and juices: 2,6-Dichloroindophenol titrimetric method. In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

AOAC INTERNATIONAL. (2023r). Official method 2012.22. Vitamin C in infant formula and adult/pediatric nutritional formula liquid chromatography with ultraviolet detection (LC-UV). In G. W. Latimer, Jr. (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Gaithersburg, MD, USA: AOAC INTERNATIONAL.

Apitz, R., Mickelson, K., Shriver, K., & Cordes, E. H. (1971). Some properties of reactions catalyzed by pig brain NAD glycohydrolase. *Archives of Biochemistry and Biophysics, 143*, 359–364. https://doi.org/10.1016/0003-9861(71)90222-0

Ball, G. F. (2006). *Vitamins in foods. Analysis, bioavailability, and stability*. Boca Raton, FL, USA: CRC Press, Taylor & Francis Group.

Bhandari, S. D., Gao, M., & Szpylka, J. (2018). Total folate in infant formula and adult nutritionals by trienzyme extraction and LC-MS/MS quantitation: A multilaboratory testing study, Final Action 2011.06. *Journal of AOAC International, 101*, 1881–1894. https://doi.org/10.5740/jaoacint.18-0114

Blake, C. J., & Konings, E. J. M. (2005). General referee reports of committee on food nutrition for fat-soluble vitamins and water-soluble vitamins. *Journal of AOAC International, 88*(1), 325–330. https://doi.org/10.1093/jaoac/88.1.325

Campos-Giménez, E., & Martin, F. (2017). Vitamin C in infant formula and adult/pediatric nutritional formula by liquid chromatography with UV Ddetection: Collaborative study, Final Action 2012.22. *Journal of AOAC International, 100*, 139–144. https://doi.org/10.5740/jaoacint.16-0232

Carmel, R., Karnaze, D. S., & Weiner, J. M. (1988). Neurologic abnormalities in cobalamin deficiency are associated with higher cobalamin analogue values than are hematologic abnormalities. *Journal of Laboratory and Clinical Medicine, 111*, 57–62. https://doi.org/10.5555/uri:pii:0022214388901047

Castanheira, I., Saraiva, M., Rego, A., & Ollilainen, V. (2016). EuroFIR guidelines for assessment of methods of analysis: GAMA. *Food Chemistry, 193*, 82–89. https://doi.org/10.1016/j.foodchem.2015.03.104

CEN. (2003a). *Foodstuffs – Determination of vitamin K1 by high performance liquid chromatography. EN 14148:2003*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2003b). *Foodstuffs – Determination of folate by microbiological assay. EN 14131: 2003*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2005). *Foodstuffs – Determination of vitamin B6 (including its glycosylated forms) by HPLC. EN 14663:2005*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2009a). *Foodstuffs – Determination of vitamin A by high performance liquid chromatography. Part 1: Measurement of all-E-retinol and 13-Z-retinol. EN 12823–1: 2014*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2009b). *Foodstuffs – Determination of vitamin D by high performance liquid chromatography-measurement of cholecalciferol (D3) or ergocalciferol (D2). EN 12821: 2009*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2009c). *Foodstuffs – Determination of niacin by HPLC. EN 15652:2009*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2009d). *Foodstuffs – Determination of D-biotin by HPLC. EN 15607:2009*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2011a). *Foodstuffs – HPLC method for the determination of xantophylls in fish flesh – Part 1: Determination of astaxanthin and canthaxanthin. TS 16233–1:2011*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2011b). *Foodstuffs – HPLC method for the determination of xantophylls in fish flesh – Part 2: Identification of the enantiomer ratio of astaxanthin. TS 16233–2:2011*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2014a). *Foodstuffs – Determination of vitamin E by high performance liquid chromatography - measurement of α-, β-, γ- and δ-tocopherol. EN 12822:2014*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2014b). *Foodstuffs – Determination of vitamin B1 by high performance liquid chromatography. EN 14122:2014*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2014c). *Foodstuffs – Determination of vitamin B2 by high performance liquid chromatography. EN 14152:2014*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

CEN. (2014d). *Foodstuffs – Determination of vitamin B6 by high performance chromatography. EN 14164:2014*. Brussels, Belgium: European Committee for Standardisation. https://www.cen.eu/.

Chamlagain, B., Rautio, S., Edelmann, M., Ollilainen, V., & Piironen, V. (2020). Niacin contents of cereal-milling products in food-composition databases need to be updated. *Journal of Food Composition and Analysis, 91*, Article 103518. https://doi.org/10.1016/j.jfca.2020.103518

Codex. (1979). *Advisory lists of nutrient compounds for use in foods for special dietary uses intended for infants and young children. CAC/GL 10–1979. Codex Alimentarius International Food Standards*. Rome, Italy: Food and Agricultural Organisation of the United Nations/World Health Organisation, Codex Alimentarius.

Codex. (1981). *Standard for infant formula and formulas for special medical purposes intended for infants. Codex Stan 72–1981. Codex Alimentarius International Food Standards*. Rome, Italy: Food and Agricultural Organisation of the United Nations/World Health Organisation, Codex Alimentarius.

Codex. (1985). *Guidelines on nutrition labelling. CXG 2–1985. Codex Alimentarius International Food Standards*. Rome, Italy: Food and Agricultural Organisation of the United Nations/World Health Organisation, Codex Alimentarius.

Codex. (1987). *Standard for follow-up formula CXS 156–1987. Codex Alimentarius International Food Standards*. Rome, Italy: Food and Agricultural Organisation of the United Nations/World Health Organisation, Codex Alimentarius.

Codex. (1999). *Recommended methods of analysis and sampling Codex Stan 234–1999. Codex Alimentarius International Food Standards*. Rome, Italy: Food and Agricultural Organisation of the United Nations/World Health Organisation, Codex Alimentarius.

Codex. (2017). Report of the thirty-eighth session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. Hamburg, Germany 5–9 December 2016. fao.org/fao-whocodexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-38%252FReport%252FFINAL%252FREP17_NFSDUe.pdf.

Dias, M. G., Borge, G. I. A., Kljak, K., Mandić, A. I., Mapelli-Brahm, P., Olmedilla-Alonso, B., et al. (2021). European database of carotenoid levels in foods. Factors affecting carotenoid content. *Foods, 10*, Article 912. https://doi.org/10.3390/foods10050912

Dumont, J. F., Peterson, J., Haytowitz, D., & Booth, S. L. (2003). Phylloquinone and dihydrophylloquinone contents of mixed dishes, processed meats, soups and cheeses. *Journal of Food Composition and Analysis, 16*, 595–603. https://doi.org/10.1016/S0889-1575(03)00029-2

EC. (2009). Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. *Official Journal of the European Union, L314*, 36–42.

EFSA. (2006). Tolerable upper intake levels for vitamins and minerals. In *Scientific committee on food scientific panel on dietetic products, nutrition and allergies, February 2006*. Parma, Italy: European Food Safety Authority.

EFSA. (2015a). Scientific opinion on dietary reference values for vitamin a. *EFSA Journal, 13*, Article 4028. https://doi.org/10.2903/j.efsa.2015.4028

EFSA. (2015b). Scientific opinion on dietary reference values for vitamin E as α-tocopherol. *EFSA Journal, 13*, Article 4149. https://doi.org/10.2903/j.efsa.2015.4149

EFSA. (2018). EFSA panel on dietetic products, nutrition and allergies. Update of the tolerable upper intake level for vitamin D for infants. *ESFA Journal, 16*, Article 5365. https://doi.org/10.2903/j.efsa.2018.5365

EFSA. (2022). EFSA Panel on Nutrition, Novel Food Allergens (NDA). Scientific opinion on the conversion of calcium-L-methylfolate and (6S)-5-methyltatrahydrofolic acid glucosamine salt into dietary folate equivalents. *EFSA Journal, 20*(8), 56. https://doi.org/10.2903/j.efsa.2022.7452, 7452.

EFSA. (2023). EFSA Panel on Nutrition, Novel Foods and Food Allergens. Scientific opinion on the tolerable upper intake level for vitamin D, including the derivation of a conversion factor for calcidiol monohydrate. *EFSA Journal, 21*(8), 1–219. https://doi.org/10.2903/j.efsa.2023.8145

Eitenmiller, R. R., Landen, W. O., Jr., & Ye, L. (2008). Biotin. In *Vitamin analysis for the health and food sciences* (2nd ed., pp. 535–560). Boca Raton, FL, USA: CRC Press.

EU. (2002). Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. *Official Journal of the European Union, 45*, 51–57, 12.07.2002, L 183 https://faolex.fao.org/docs/pdf/eur37787.pdf.

EU. (2011). Regulation (EU) 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union, 54*, 18–63, 22.11.2011, L 304 https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:en:PDF#:~:text=This%20Regulation%20provides%20the%20basis,functioning%20of%20the%20internal%20market.

EU. (2013a). Commission Implementing Decision of 22 January 2013. Authorising the placing on the market of synthetic zeaxanthin as a novel food ingredient under Regulation (EC) 258/97 of the European Parliament and the Council. *Official Journal of the European Union, 24.1.2013, L21, 56*, 32–33. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2013:021:FULL&from=PT.

EU. (2013b). Regulation (EU) 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. *Official Journal of the European Union, 29.6.2013, L181, 56*, 35–56. /35 http://data.europa.eu/eli/reg/2013/609/oj.

EU. (2015). Commission Regulation (EU) 2015/414, amending Directive 2002/46/EC of the European Parliament and of the Council as regards (6S)-5-methyltetrahydrofolic acid, glucosamine salt used in the manufacture of food supplements. *Official Journal*

of the European Union, 58, 26–27, 13.3.2015, L68 https://faolex.fao.org/docs/pdf/eur142580.pdf.
EU. (2016). Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. *Official Journal of the European Union, 59*, 1–19, 02.02.2016, L 25 http://data.europa.eu/eli/reg_del/2016/127/oj.
EU. (2019). European Commission Delegated Regulation (EU) 2019/828 of 14 March 2019 amending Delegated Regulation (EU) 2016/127 with regard to vitamin D requirements for infant formula and erucic acid requirements for infant formula and follow-on formula. *Official Journal of the European Union, 62*, 12–14, 23.5.2019, L 137. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0828&rid=1
EU. (2020). Commission Implementing Regulation (EU) 2020/16 of 10 January 2020 authorising the placing on the market of nicotinamide riboside chloride as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470. *Official Journal of the European Union, 63*, 6–10, 13.1.2020, L 7 http://data.europa.eu/eli/reg_impl/2020/16/oj.
EU. (2023a). Commission Implementing Regulation (EU) 2023/1581 of 1 August 2023 amending Implementing Regulation (EU) 2017/2470 as regards the conditions of use of the novel food 'astaxanthin-rich oleoresin from *Haematococcus pluvialis* algae'. *Official Journal of the European Union, 2.8.2023, L194, 66*, 4–7. http://data.europa.eu/eli/reg_impl/2023/1581/oj.
EU. (2023b). Commission Implementation Regulation (EU) 2023/4 of 3 January 2023 authorising the placing on the market of vitamin D2 mushroom powder as a novel food and amending Implementing Regulation (EU) 2017/2470. *Official Journal of the European Union, 4.1.2023, L2, 66*, 3–8. http://data.europa.eu/eli/reg_impl/2020/16/oj.
EU. (2023c). Commission Regulation (EU) 2023/1065 of 1 June 2023 amending Annex II to Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards nicotinamide riboside chloride added to foods. *Official Journal of the European Union, 2.6.2023, L143, 66*, 6–8. http://data.europa.eu/eli/reg/2023/1065/oj.
FAO/WHO. (2001). *Human vitamin and mineral requirements. Report of a joint FAO:WHO expert consultation. Bangkok, Thailand*. Rome, Italy: Food and Agricultural Organization of the United Nations.
Fontannaz, P., Kilinc, T., & Heudi, O. (2006). HPLC-UV determination of total vitamin C in a wide range of fortified food products. *Food Chemistry, 94*, 626–631. https://doi.org/10.1016/j.foodchem.2005.02.012
Frikke-Schmidt, R., Tveden-Nyborg, P., & Lykkesfelt, J. (2016). L-Dehydroascorbic acid can substitute L-ascorbic acid as dietary vitamin C source in guinea pigs. *RedoxBiology, 7*, 8–13. https://doi.org/10.1016/j.redox.2015.11.003
FSANZ. (2018a). *Australia New Zealand Food Standards Code – Standard 2.9.1 – Infant formula products*. Wellington, New Zealand: Food Standards Australia New Zealand.
FSANZ. (2018b). *Australia New Zealand Food Standards Code—Schedule 1 – RDIs and ESADDIs*. Wellington, New Zealand: Food Standards Australia New Zealand.
FSANZ. (2018c). *Australia New Zealand Food Standards Code—Schedule 17 Vitamins and minerals*. Wellington, New Zealand: Food Standards Australia New Zealand.
FSANZ. (2018d). *Australia New Zealand Food Standards Code—Standard 1.1.2 Definitions used throughout the Code*. Wellington, New Zealand: Food Standards Australia New Zealand.
GB. (2008). *GB/Z 21922–2008: National Standard China. Fundamental terminology and definition of nutritional components in foods*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2010). *GB 25596–2010: National Food Safety Standard China: General standard for infant formula for special medical purposes*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2011). *GB 28050–2011: National Food Safety Standard China: General rules for nutrition labeling of prepackaged foods*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2012). *GB 14880–2012: National Food Safety Standard China for the use of nutritional fortification substances in foods*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2013). *GB 29922–2013: National Food Safety Standard China: General principles for formula foods of special medical purpose*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2016a). *GB 5009.82–2016: China National Food Safety Standard. Determination of vitamin A, D, E in foods*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2016b). *GB 5009.259–2016. China National Food Safety Standard. Determination of biotin in foods*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2021a). *GB 10765–2021: National Food Safety Standard China: Infant formula*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2021b). *GB 10766–2021: National Food Safety Standard China: Older infants formula*. Beijing, China: Bureau of Import and Export Food Safety.
GB. (2021c). *GB 10767–2021: National Food Safety Standard China: Older infants and young children formula*. Beijing, China: Bureau of Import and Export Food Safety.
Gill, B. D., Abernethy, G. A., Indyk, H. E., Wood, J. E., & Woollard, D. C. (2020). Differential thermal isomerization: Its role in the analysis of vitamin D3 in foods. *Journal of AOAC International, 103*, 563–569. https://doi.org/10.1093/jaocint/qsz001
Gill, B. D., & Indyk, H. E. (2020). Separation of *RRR*-α-tocopherol by chiral chromatography. *Journal of AOAC International, 103*, 1288–1292. https://doi.org/10.1093/jaoacint/qsaa055
Gill, B. D., Saldo, S., Wood, J. E., & Indyk, H. E. (2018). A Rapid Method for the Determination of Biotin and Folic Acid in Liquid Milk, Milk Powders, Infant Formula, and Milk-Based Nutritional Products by Liquid Chromatography–Tandem Mass Spectrometry. *Journal of AOAC International, 101*, 1578–1583. https://doi.org/10.5740/jaoacint.18-0065
Gilliland, D. L., Gill, B. D., Kissling, R. C., Starkey, D. E., Indyk, H. E., McMahon, A., et al. (2022). Assessment of regulatory compliance testing for vitamin D in infant formula – Impact of delegated regulation (EU) 2019/828. *Journal of AOAC International, 105* (5), 1360–1366. https://doi.org/10.1093/jaoacint/qsac060
Gregory, J. F. (1997). Bioavailability of vitamin B6. *European Journal of Clinical Nutrition, 51*, S43–S48.
Gregory, J. F., Trumbo, P. R., Bailey, L. B., Toth, J. P., Baumgartner, T. G., & Cerda, J. J. (1991). Bioavailability of pyridoxine-5′-β-D-glucoside determined in humans by stable-isotopic methods. *Journal of Nutrition, 121*, 177–186. https://doi.org/10.1093/jn/121.2.177
Gropper, S. S., Smith, J. L., & Carr, T. P. (2021). *Advanced nutrition and human metabolism (8th ed.)*. Boston, MA, USA: Cengage Learning.
Hostetler, G. L. (2017). Determination of lutein and β-carotene in infant formula and adult nutritionals by ultra-high-performance liquid chromatography: Single-laboratory validation, first action 2016.13. *Journal of AOAC International, 100*, 768–781. https://doi.org/10.5740/jaoacint.16-0386
Hostetler, G. L. (2020). Determination of lutein, β-carotene, and lycopene in Infant Formula and adult nutritionals by ultra-high performance liquid chromatography: Collaborative study, Final Action 2016.13 for β-carotene and lycopene only. *Journal of AOAC International, 103*, 818–832. https://doi.org/10.1093/jaocint/qsz017
Institute of Medicine Panel. (2000). *Dietary A, related C. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC, USA: National Academies Press.
ISO. (2015a). Infant formula and adult nutritionals – Determination of vitamin E and vitamin A by normal phase high performance liquid chromatography. ISO 20633: 2015.. Geneva, Switzerland: International Standardisation Organisation http://www.iso.org.
ISO. (2015b). *Infant formula and adult nutritionals – Determination of pantothenic acid by ultra high performance liquid chromatography and tandem mass spectrometry method (UHPLC-MS/MS). ISO 20639:2015*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2015c). *Infant formula and adult nutritionals – Determination of vitamin B12 by reversed phase high performance liquid chromatography (RP-HPLC). ISO 20634: 2015*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2018a). *Infant formula and adult nutritionals – Determination of vitamin D by liquid chromatography-mass spectrometry. ISO 20636:2018*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2018b). *Infant formula and adult nutritionals – Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U)HPLC-UV). ISO 20635:2018*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2019). *Infant formula and adult nutritionals – Determination of trans and total (cis + trans) vitamin K1 content – Normal phase HPLC. ISO 21446:2019*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2020a). *Infant formula and adult nutritionals – Determination of β-carotene, lycopene and lutein by reversed-phase ultra-high performance liquid chromatography (RP-UHPLC). ISO 23443:2020*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2020b). *Infant formula and adult nutritionals – Simultaneous determination of total vitamins B1, B2, B3 and B6. Enzymatic digestion and LC-MS/MS. ISO 21470:2020*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2020c). *Fortified milk powders, infant formula and adult nutritionals – Determination of total biotin by liquid chromatography coupled with immunoaffinity column clean-up extraction. ISO 23305 (2020). ISO/TC 34*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2021). *Food products – Guidance on how to express vitamins and their vitamers content. ISO TR 23034:2021*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
ISO. (2023). *Infant formula and adult nutritionals – Determination of total folates content by trienzyme extraction and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). ISO 20636:2023*. Geneva, Switzerland: International Standardisation Organisation. http://www.iso.org.
Jakobsen, J. (2008). Optimisation of the determination of thiamin, 2-(1-hydroxyethyl) thiamine, and riboflavin in food samples by use of HPLC. *Food Chemistry, 106*, 1209–1217. https://doi.org/10.1016/j.foodchem.2007.06.008
Jakobsen, J., & Christensen, T. (2021). Natural vitamin D in food: To what degree does 25-hydroxyvitamin D contribute to the vitamin D activity in food? *JBMR Plus, 2021*, Article e10453. https://doi.org/10.1002/jbm4.10453
Jakobsen, J., Melse-Boonstra, A., & Rychlik, M. (2019). Challenges to quantify total vitamin activity: How to combine the contribution of diverse vitamers? *Current Developments in Nutrition, 3*, Article nzz086. https://doi.org/10.1093/cdn/nzz086
JECFA. (2006). *Chemical and technical assessment (CTA). Zeaxanthin (synthetic) and zeaxanthin-rich extract*. Geneva, Switzerland: 67th Joint FAO/WHO Expert Committee on Food Additives.
Jensen, M. B., Rød, K. E., Ložnjak Švarc, P., Oveland, E., & Jakobsen, J. (2022). Vitamin K (phylloquinone and menaquinones) in foods – Cost-effective quantification by LC-ESI-MS/MS. *Food Chemistry, 385*, Article 132672. https://doi.org/10.1016/j.foodchem.2022.132672

Jensen, S. K., & Lauridsen, C. (2007). α-Tocopherol stereoisomers. *Vitamins and Hormones, 76*, 281–308. https://doi.org/10.1016/S0083-6729(07)76010-7

Kall, M. A. (2003). Determination of total vitamin B_6 in foods by isocratic HPLC: A comparison with microbiological analysis. *Food Chemistry, 82*, 315–327. https://doi.org/10.1016/S0308-8146(02)00568-X

Kall, M. A., & Andersen, C. (1999). Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroascorbic acid in food and biological samples. *Journal of Chromatography B, 730*, 101–111. https://doi.org/10.1016/S0378-4347(99)00193-0

Konings, E. J. M., Roux, A., Reungoat, A., Nicod, N., Campos-Giménez, E., Ameye, L., et al. (2021). Challenge to evaluate regulatory compliance for nutrients in infant formulas with current state-of-the-art analytical reference methods. *Food Control, 119*, Article 107423. https://doi.org/10.1016/j.foodcont.2020.107423

Ložnjak, P., Striegel, L., Díaz De la Garza, R.I., Rychlik, M., Jakobsen, J. (2020a). Quantification of folate in food using deconjugase of plant origin combined with LC-MS/MS: A method comparison of a large and diverse sample set. Food Chemistry, 305, Article 125450. https://doi.org/10.1016/j.foodchem.2019.125450.

Ložnjak Švarc, P., Oveland, E., Strandler, H. S., Kariluoto, S., Campos-Giménez, E., Ivarsen, E., et al. (2020b). Collaborative study: Quantification of total folate in food using an efficient single-enzyme extraction combined with LC-MS/MS. *Food Chemistry, 333*, Article 127447. https://doi.org/10.1016/j.foodchem.2020.127447

Meléndez-Martínez, A. J., Mandić, A. I., Bantis, F., Böhm, V., Borge, G. I. A., Brnčić, M., et al. (2022). Comprehensive review on carotenoids in foods and feeds: Status quo, applications, patents, and research needs. *Critical Reviews in Food Science and Nutrition, 62*, 1999–2049. https://doi.org/10.1080/10408398.2020.1867959

NHMRC. (2017). *Australian Government Department of Health and Ageing, New Zealand Ministry of Health. Nutrient reference values for Australia and New Zealand 2006, updated 2017*. Canberra, Australia: National Health and Medical Research Council.

NMKL. (2020). Total folate in food by LC-ESI-MS/MS. Quantification of 6 folate vitamers. NMKL 204. Bergen, Norway: Nordic-Baltic Committee on Food Analysis. www.nmkl.org.

Panfili, G., Manzi, P., & Pizzoferrato, L. (1998). Influence of thermal and other manufacturing stresses on retinol isomerization in milk and dairy products. *Journal of Dairy Research, 65*, 253–260. https://doi.org/10.1017/s0022029997002811

Regnard, D., Arella, F., & Deborde, J.-L. (2014). Développements de méthodes d'analyse et de dosage de quelques caroténoïdes par CLHP / UV. Application à la détection d'un composé non autorisé dans les compléments alimentaires à visée oculaire. *Société des Experts Chimistes de France.*, 1–9 (Revue 980-1er semestre: (in French).

REP17/NFSDU. (2016). *Report of the thirty-eight session of the Codex Committee on nutrition and foods for special dietary uses*. Germany: Hamburg.

Sato, T., Schurgers, L. J., & Uenishi, K. (2012). Comparison of menaquinone-4 and menaquinone-7 bioavailability in healthy women. *Nutrition Journal, 11*, 9–12. https://doi.org/10.1186/1475-2891-11-93

Schurgers, L. J., & Vermeer, C. (2002). Differential lipoprotein transport pathways of K-vitamins in healthy subjects. *Biochimica et Biophysica Acta - General Subjects, 1570*, 27–32. https://doi.org/10.1016/S0304-4165(02)00147-2

Szpylka, J., DeVries, J., Cheney, A., & House, S. (2012). Determination of total folates in infant formula and adult nutritionals by trienzyme extraction and UPLC-MS/MS quantitation: First Action 2011.06. *Journal of AOAC International, 95*, 1547–1554. https://doi.org/10.5740/JAOACINT.CS2011_06

Takashi, U., Yukiko, T., Kohei, M., Mari, T., & Kaname, K. (1990). Simultaneous determination of 2(1-hydroxyethyl)thiamine and thiamine in foods by high performance liquid chromatography with post-column derivatisation. *Vitamins (Japan), 64*, 379–385.

Takashi, U., Yukiko, T., Kohei, M., Masako, M., & Kaname, K. (1991). Distribution and stability of 2(1-hydroxyethyl)thiamine and thiamine in foods. *Vitamins (Japan), 65*, 249–256.

USFDA. (2016). *Updated nutrition facts label (81 FR 33742 at 33906-33916 and § 101.9 (21 CFR 101.9))*. Washington, DC, USA: United States Food & Drug Administration.

USFDA. (2019). *Guidance for industry: Converting units of measure for folate, niacin, and vitamins A, D, and E on the nutrition and supplement facts labels*. Washington, DC, USA: United States Food & Drug Administration Center for Food Safety and Applied Nutrition. Available from: www.fda.gov/regulatory-information. (Accessed 20 January 2022).

Watanabe, F. (2007). Vitamin B12 sources and bioavailability. *Experimental Biology and Medicine (Maywood, N.J.), 232*(10), 1266–1274. https://doi.org/10.3181/0703-mr-67

Weiser, H., & Somorjai, G. (1992). Bioactivity of cis and dicis isomers of vitamin a esters. *International Journal for Vitamin and Nutrition Research, 62*, 201–208.

Wills, R. B. H., Wimalasirl, P., & Greenfield, H. (1983). Liquid chromatography, microfluorometry, and dye-titration determination of vitamin C in fresh fruit and vegetables. *Journal of AOAC International, 66*, 1377–1379. https://doi.org/10.1093/jaoac/66.6.1377

Woollard, D. C., & Indyk, H. E. (2013). Biotin analysis in dairy products. In V. R. Preedy (Ed.), *Food and nutritional components in focus, no. 4, B vitamins and folate: Chemistry, analysis, function and effects* (pp. 377–395). London, UK: Royal Society of Chemistry.

Woollard, D. C., Indyk, H. E., Fong, B. Y., & Cook, K. K. (2002). Determination of vitamin K_1 isomers in foods by liquid chromatography with C_{30} bonded-phase column. *Journal of AOAC International, 85*, 682–691. https://doi.org/10.1093/jaoac/85.3.682