

Determination of Vitamin E in Cereal Products and Biscuits by GC-FID

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Abstract

A rapid, precise, accurate and low cost method for the determination of vitamin E (α -tocopherol) in cereal products and biscuits was developed. The uncertainty was calculated for the first time and the methods were performed in different cereal products and biscuits, characterized as “superfoods”. The limits of detection and quantification were calculated. Accuracy and precision were estimated using the certified reference material FAPAS T10112QC and the determined values were in good accordance with the certified values. The health claims according to the daily reference values for vitamin E were calculated and the results proved that the majority of the samples examined showed %daily value higher than 15%.

Keywords: Vitamin E; GC-FID; Uncertainty; Cereal products; Reference daily value; Health claim

1. Introduction

Vitamin E is a fat soluble vitamin found in many foodstuffs, such as cereals, eggs, olive oils, and vegetables. Vitamin E occurs in many different forms (α -, β -, γ - and δ -tocopherol and α -, β -, γ - and δ -tocotrienols) and has many health benefits and is mostly used for treating and preventing heart diseases [1,2]. It is a well known antioxidant preventing different types of cancer, such as lung, oral cancer and other. It is also believed to help patients with Alzheimer disease and other types of diseases related to the nervous system. According to the European Regulation 1169/2011 on the provision of food information to consumers there is a daily reference intake for vitamin E equal to 12 mg per 100 g. As a rule to decide if a food has a significant amount of vitamin E, it must contain the 15% of the reference dose per 100 g or 1.8 mg per 100 g [3].

Nowadays, there is a great interest for functional foods, commonly known as “superfoods”. There is no clear definition about “superfoods”, and is a term used for foods that combine the nutritional health benefits and disease preventing of different foods [4]. These foods are mainly composed of cereals, fruits, and beans. Vitamin E occurs in all these foodstuffs but its contents depends on the “superfood” composition. For this reason, it is of great importance to determine the appropriate content of the different food components in order to take the recommended daily dose of vitamin without spoiling the taste or the flavor of the foodstuffs.

There are many methods for the determination of vitamin E. Liquid chromatography methods with different detectors are the most common methods used nowadays for the determination of fat soluble vitamins [5-8]. The need for the simultaneous determination of the majority of vitamins led to the use of modern analytical methods with low limits of detection such as LC-MS/MS or LC-MS [9,10]. All methods

require complicate preparation steps in order to decrease the interferences provided by the matrix.

In this article a rapid, precise and accurate method for the determination of Vitamin E in cereal products by GC-FID is presented. The method is based on the method presented by Pyka et al. (2001) for the determination of α -tocopherol in the human plasma and is now modified and expanded for the determination of α -tocopherol in cereal products and biscuits [1]. To the best of our knowledge it is for the first time that a so simple and rapid method is used for the determination of α -tocopherol in cereal products and biscuits with real low limits of detection and high accuracy and precision. The method was applied in a vast number of new nutraceutical and functional cereal products and biscuits that can be characterized as “superfoods” and possible health claims were examined.

2. Materials and methods

2.1 Chemicals

All reagents used were of analytical grade. Vitamin E standard solution and ethanol were purchased from Sigma-Aldrich. Hexane was purchased from VWR and dichloromethane was purchased from Lach-ner.

2.2 Instrumentation

All experiments were performed with a Shimadzu GC 2010 PLUS, GC-FID system, using an Agilent DB-1 30 m x 0.32 mm x 1 μ m. The optimized conditions were: injection volume 2 μ L in splitless mode, pulse time 1.0 min, injector temperature 300 $^{\circ}$ C, carrier gas He at a constant flow 2.0 mL/min, detector temperature 340 $^{\circ}$ C. The initial oven temperature, 120 $^{\circ}$ C was held for 1 min, then programmed at 27 $^{\circ}$ C/min to 320 $^{\circ}$ C, where was held for 15 min.

2.3 Sample preparation

Eighteen different cereal and bakery products, belonging to the class of “superfoods” were selected for vitamin analysis. The ingredients of each product are presented in Table 1. The samples were milled and well homogenized. A sample amount of a well homogenized cereal product (0.5000 g) was diluted in 1 mL of ethanol and left overnight at dark. The solution was then mixed well with an appropriate amount of hexane and dichloromethane (90/10 %v/v), centrifuged at 2500 rpm for 10 min and the hexane:dichloromethane stage was evaporated to dryness at 40 °C. Finally the sample was dissolved in 1 mL of ethanol and 2 µL were injected into the GC system.

Table 1: Content of vitamin E in different cereal and bakery products.

Sample Type	Sample coded as	Ingredients	α -tocopherol (mg/kg)	%Daily value
Sweet bun	S1	Oat and honey	15.0	12.5
Sweet bun	S2	Plum, fig and date	4.00	3.3
Sweet bun	S3	Chocolate, bilberry and raspberry	19.0	15.8
Sweet bun	S4	Nuts, orange and molasses	34.0	28.3
Sweet bun	S5	Nuts and chocolate	31.2	26.0
Sweet bun	S6	Forest fruits	33.2	27.6
Sweet bun	S7	Wholegrain with fruits	34.1	28.4
Sweet bun	S8	Almond	50.2	41.8
Sweet bun	S9	Rye, plum. nuts and raisin	6.12	5.1
Sweet bun	S10	Cereals and nuts	10.0	8.3
Sweet bun	S11	Oat, apple and cinnamon	10.5	8.8
Sweet bun	S12	Plum, nuts and date	60.0	50.0
Biscuits	S13	Zea wheat and vanilla	88.0	73.3
Breadstick	S14	Oat and wheat	18.2	15.2
Breadstick	S15	Zea wheat, tomato and oregano	24.2	20.2
Breadstick	S16	Zea wheat and sesame	20.0	16.7
Breadstick	S17	Zea wheat wholegrain	20.2	16.8
Breadstick	S18	Zea wheat and vegetables	20.5	17.1

2.4 Method validation

Quantification was performed by different dilutions of the stock solution (from 0.5 mg/L to 250 mg/L). Every standard solution was measured in triplicate. LOD and LOQ were calculated by the standard deviation of the intercept. Precision and accuracy experiments were carried out and the relative standard deviation (%RSD) values were calculated from the multiple analysis of the certified reference material (FAPAS T10112QC) (n=6) under repeatability and reproducibility conditions. The uncertainty of the method was also calculated based on the Eurachem/Citac Guidelines [11].

Results and discussion

3.1 *The results of method validation*

For the determination of α -tocopherol, content quantification was performed using by plotting the concentration of different calibration standards (0.5-50 mg/L) versus the peak area of the analyte (PA). The equation of the calibration curve was found equal to $PA = (7.08 \pm 0.16) \times 10^4 C \text{ (mg/L)} (1.5 \pm 3.0) \times 10^3$, $R^2=0.998$. It must be noted that an internal quality control sample was measured every ten samples in order to avoid the use of an internal standard. All samples and standard solution were measured in triplicate.

The instrumental LOD and LOQ were determined by the standard deviation of the intercept of the calibration curve and were equal to 0.17 and 0.51 mg/L. The respective methods LOD and LOQ were found equal to 0.85 and 2.5 mg/kg. The calculated LOD was considered as “fit for purpose” taking into account the reference daily dose of 18 mg/kg as provided in the European Regulation 1169/2011 [3].

Precision experiments were carried out and the relative standard deviation (%RSD) values achieved from three different concentration levels measured six times under repeatability conditions and six times at two different days under reproducibility conditions, were lower than 10% for all different concentration levels. The HORRAT_r and HORRAT_R values achieved from these different concentration levels, ranged from 0.18 to 0.25. These values were lower than the crucial value of two, and the method is ‘fit-for-purpose’. For accuracy estimation the certified reference material FAPAS T10112QC with certified value 49.0 ± 12.5 was analyzed 6 times in two different days by two different analysts (n=12) and the recovery was found equal to 99.5 ± 5.9 (Figure 1). The recovery data are within ± 25 % of the target value, as

provided by the certification of the reference material and for this reason the method was again considered as “fit for purpose”.

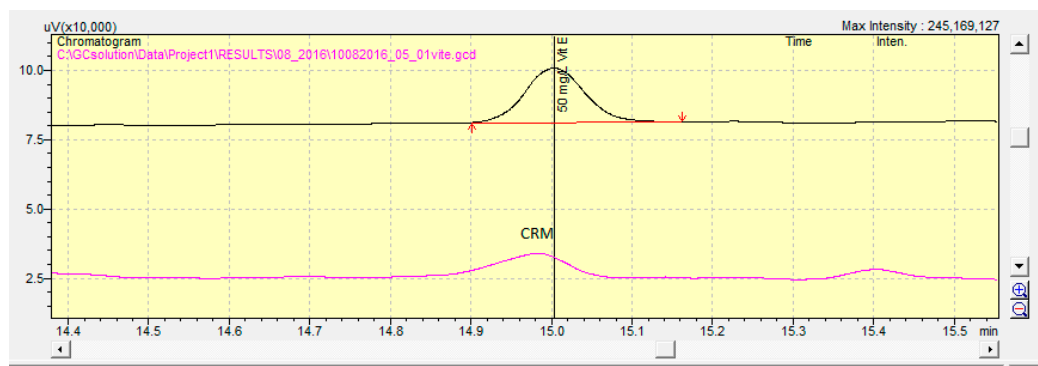


Figure 1: Chromatogram of the certified reference material (CRM) and a standard solution containing 50 mg/L vitamin E.

The uncertainty of the method was also calculated based on the Eurachem/Citac Guidelines [11]. The calculated expanded uncertainties were found equal to 15.0, 12.3 and 8.50% of the content of the analyte in mg/kg for the LOQ, the centroid of the calibration curve, and the upper limit of the linear range, respectively ($k=2$, $CL=95\%$). The main sources of the calculated uncertainty were the calibration uncertainty; the bias uncertainty and the precision uncertainty.

3.2 Determination of α -tocopherol content and diastase activity in real samples characterized as “superfoods”

The method was performed for the determination of vitamin E in eighteen different “superfoods”, produced under ISO 22000 recommendations from Artolife SA, Thiva, Greece. The results proved that the content of α -tocopherol ranged from 4.00 to 88.0 mg/kg. The highest content of vitamin E was found in the sample coded as S13, made from zeaxanthin (Zea mays) and vanilla. Vanilla contains a high amount of

vitamin E, approximately equal to 54.0 mg/kg [12], whereas *triticum dicoomum* wheat also contains a high amount of vitamin E (approximately equal to 10 mg/kg [13,14]). It seems that the temperature of biscuit making did not lead to great loss of vitamin E. Similar results about vitamin E loss during bread making were also reported in Leenhardt et al. work (2006) [13]. The lowest content of vitamin E was found in sweet bun made from plum, fig and date (S4 sample), where the content was approximately equal to the sum of each ingredient content. All superfoods containing nuts and almonds gave high content of vitamin, since nuts are commonly known for their vitamin content.

Having regard to the Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers Vitamin E may be declared on the foodstuffs according to their nutrient reference intake [3]. Significant amount of vitamin can be declared if only a foodstuff contains 15 % of the nutrient reference values specified in the current regulation. For vitamin E the reference intake value is equal to 120 mg/kg or the 15% of the reference intake is equal to 18 mg/kg. According to Commission Regulation (EU) No 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health, the following health claim can be used for a foodstuff containing vitamin E higher than the 15% of the reference daily intake: "Vitamin E contributes to the protection of cells from oxidative stress" [15]. Taking into account as serving size the 100 g only samples coded as S1, S2, S9, S10 and S11 cannot use this specific health claim. Especially, for sweet bun with plum, nuts, and date (S12) and biscuits with zea wheat and vanilla the health claim (S13) can also be used and for the serving size of 35 g (1 biscuit). Summarizing, in

the majority of the samples (over 72%) the health claim: “Vitamin E contributes to the protection of cells from oxidative stress” can be used on the labeling.

4. Conclusion

The method presented in this application can be used for the determination of vitamin E in cereal and biscuit products by GC-FID. The GC-FID method proposed was considered fit for purpose in terms of precision and accuracy. The results proved that the majority of cereal products are rich in vitamin E and can be use the health claim for vitamin E as specified in Commission Regulation (EU) No 432/2012. Vaniila and nuts seems to give an excessive amount of vitamin E in bakery products resulting in whereas, bakery products containing only fruits seem to give lower content.

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