DEVELOPMENT OF ANALYTICAL METHODS FOR DETERMINATION OF WATER SOLUBLE VITAMINS IN FUNCTIONAL FOOD PRODUCTS

RITA ENGEL

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Corvinus University of Budapest
Faculty of Food Science
Department of Applied Chemistry

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PhD. School

Name: PhD. School of Food Science

Field: Food Science

Head: ***Dr. Péter Fodor***,
DSc., university professor
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

Supervisors: *Dr*. ***Éva Stefanovits-Bányai***
DSc., university professor
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

Dr. László Abrankó
PhD, assistant professor
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

…….……. Signature of Had of School

……... Signature of Supervisor

……... Signature of supervisor

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1 INTRODUCTION

Nowadays the different malignant tumours and cardiovascular diseases are widespread throughout the world. In Hungary the three-quarters of the mortality are caused by theses diseases. In Hungary the cancer caused mortality has a growing tendency, and it is the biggest in whole Europe. These facts also point on the seriousness of the circumstance. In several times the improper manner of living and the malnutrition are in the background of these diseases. Inappropriate food choices and economic constrains leading to unbalanced diets and unlikely to provide adequate levels of all micronutrients, which are essential for the normal growth and maintenance of life.

The group of ***vitamins*** belongs to theses essential compounds. The term "vitamin" (vita=life) also indicates their important role in the preservation of health. Vitamins are a diverse group of organic compounds that are, in very small amounts, essential for the normal functioning of several physiological processes. Lack of them can generate several serious diseases. Vitamin malnutrition is a widespread problem throughout the world in both developing and industrialized countries, with serious health and economic implications. The vitamin A deficiency is the most serious global problem. A significant part of the world population is at risk of C-, D-, and B-vitamin deficiency. According to the survey of the National Institute for Food and Nutrition Science among the Hungarian population the most general problem is the suboptimal intakes of "embryo-protective" vitamins (such as folic acid, B_2-, B_6-, B_{12}-vitamins). Their deficiency can lead to developmental abnormality.

The best way of preventing micronutrient malnutrition is to ensure consumption of a balanced diet that is adequate in every nutrient. Thus consumer education programmes to encourage changes in dietary patterns would be the best long-term solution. However, factors affecting food choice are complex (e.g.: bad habits, economic constrains), and therefore programmes to effect changes in dietary patterns may take a relatively long time. For this reason food fortification can be an important tool in helping populations to meet their dietary requirements. In growing numbers of countries fortification of staple foods (salt, flour) with micronutrients becomes an important part of their strategy to improve the nutritional status of the population. Due to the spread of nutrition science knowledge among the population there is a growing segment of the society that strives for health-conscious nutrition. To take such a demand of the people into consideration a growing number of products with health-promoting properties are developed and put into trade circulation by the pharmaceutical and food

industry. Because of this reason an expanding scale of various dietary supplements and fortified foods with micronutritions are commercially available.
Coming out, and fast spreading of the fortified food products require the development of modern, accurate, timesaving, low cost analytical methods for quality control purposes.

2 OBJECTIVES

The growing number of various dietary supplements and fortified food products requires reliable quality control to insure the protection of consumers. However the official analytical methods of water-soluble vitamins in most cases are based on outdated procedures, which are complicated, time-consuming, inaccurate and do not allow the simultaneous determination of the vitamins. For these reasons there is increasing need for the development of well validated, accurate, time-saving, low cost, modern, multicomponent methods.
In the light of the aforementioned the aims of this study were the followings:

- Development of modern analytical methods, which allow the simultaneous, fast, routine determination of those forms of water soluble vitamin (vitamin C and B), which are the most frequently used for food enrichment.
- Development of a sample preparation procedure for the simultaneous extraction of vitamins C and B.
- Application of the developed methods on the most significant, commercially available vitamin enriched food products.
- Development of analytical methods for simultaneous analysis of each B vitamins and vitamers, which allow the determination of total and endogenous B vitamin content of the food samples.
- Estimation of the distribution of the free, bound and bioavailable portion of B vitamin forms in breakfast cereal.

3 MATERIALS AND METHODS

All reagents were of analytical grade. All solutions were prepared in high purity water (Milli-Q 18.2 $M\Omega cm^{-1}$). To prepare different mobile phases KH_2PO_4 and K_2HPO_4 salts, water, formic acid, methanol and as ion-paring reagents triflouroacetic acid and heptafluorobutyric acid were applied. Standard solutions were prepared from the following compounds: L-

ascorbic acid (C), thiamine hydrochloride (B_1), riboflavin (B_2), nicotinic acid, nicotinamide, pyridoxine hydrochloride (PN), pyridoxamine-dihydrochloride (PM), pyridaxal-hydrochloride (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN) and the piridoxal-5'-phosphate-monohydrate (PLP), pantothenic acid (B_5), folic acid (B_9). Hippuric acid was used as internal standard. For the extraction procedures and for the sample solutions preparation the following reagents were applied: HCl, CH_3COOH, $NaCH_3COO$, NH_4OH, NaOH, HPO_3, L-cysteine, taka-diastase, acid phosphatase from potato and b-glycosidase from almond. The examined samples such as vitamin tablet, vitamin enriched breakfast cereal, instant cacao powder and fruit juice were commercially available products. Hydrochloric acid and metaphosphoric acid [EN 14130:2003] were used for the extraction of the free vitamin content of the samples. Sample preparations contain of acid hydrolysis and enzymatic treatment were applied for the determination of the total vitamin B_1, B_2 and B_6 content of food samples [MSZ EN 14122:2006, MSZ EN 14152:2004, MSZ EN 14663:2006].

HPLC-(UV-VIS) and HPLC-(UV-VIS)-ESI-MS/MS systems were used for the measurements. Reversed phase stationary phases were applied for the separation of the vitamins.

4 RESULTS

4.1 Simultaneous determination of water soluble vitamins by RP-HPLC-(UV-VIS) system

The ***selection of the examined compounds*** was focused on those water soluble vitamin forms and vitamers, which are the most frequently applied for enrichment in the food and pharmaceutical industry. Therefore the examined compounds were the following: thiamine, riboflavin, nicotinic acid, nicotinamide, pantothenic acid, pyridoxine, folic acid ascorbic acid.

Since the aim is the ***simultaneous determination*** of the selected eight water soluble vitamins several chromatographic difficulties have to be counted, which are mainly caused by the chemical diversity of these compounds.

The preparation of the ***standard solutions*** already has to be realized carefully, because of the different solubility and high instability of each compound. Diluted acid stock solution of ascorbic acid was prepared to increase its stability. However, despite of the low pH the

degradation of vitamin C was observed. To avoid temporarily the degradation of ascorbic acid the standard solutions measured immediately after preparation.

Reserve phased chromatographic separation of the eight vitamins was performed using pH7 ***phosphate buffer*** and methanol as mobile phases. A four-step gradient elution program was developed for the base-line separation of the vitamins. However the retention of ascorbic acid is quite poor, since as weak acid, under the described pH 7 conditions it is presented in more polar ionized form.

Based on the UV spectra of each vitamin five different wavelengths (***200 nm, 266 nm, 282 nm, 325 nm, 448 nm***) were chosen for their detection in order to improve the selectivity and sensitivity of the method. Pantothenic acid molecule (B_5) does not contain a characteristic chromophore group and hence it exhibits only very weak absorbance at wavelengths around 200 nm. Therefore its UV detection is not advantageous because of the interferences of the applied solvents and the matrix components. Nevertheless the detection of pantothenic acid at 200 nm was feasible under the above described chromatographic conditions by means of the low UV cut-off value (195 nm) of phosphate buffer. The developed method was applied to the measurement of a commercially available vitamin enriched cereal and a dietary supplement. Hydrochloric acid was used for the extraction. The separation of the examined compounds from the matrix components of the cereal sample was not adequate under the described chromatographic conditions. Although the method is not suitable for the examination of complex food samples, it can provide a fast, cheap solution for the routine measurement of ***simpler matrixes*** such as vitamin tablets.

The further aim of my Ph.D. study was to find solutions for the ***analytical problems*** appeared, which are summarised in the following:

1) Problems during the preparation of standard solutions:
    a) Degradation of vitamin C in the stock solution, and in the multicomponent solution.
2) The weak points of the developed chromatography:
    a) Poor retention of vitamin C
    b) Coelution of the examined components with matrix components
    c) The disadvantages of the inorganic pH7 phosphate buffer
        (i) In the present of organic solvent the inorganic salt can be precipitated, which on the one hand can cause the clogging of the system (valves,

capillary tubes), on the other hand settling on the colonna it can modify negatively the separation and the selectivity.

(ii) The pH 7 is disadvantageous in microbiological stability aspect.

3) The applied sample preparation (with hydrochloric acid) does not ensure the simultaneous determination of vitamin C and the B group vitamins:
   a) The degradation of vitamin C
   b) Interferences caused by the matrix components during the separation

4.2 Simultaneous determination of water soluble vitamins using ion-paring reagent by RP-HPLC-(UV-VIS) system

Vitamin C is the critical point of the simultaneous determination of water soluble vitamins. The ***barriers*** of the determination of vitamin C along with B group vitamins derive from the ***high instability*** of vitamin C and from the difficulties of its ***chromatographic separation*** from B vitamins.

The ***stability problems*** were solved by using metaphosporic acid (HPO_3) for the preparation of standard solutions. The metaphosporic acid prevents the oxidation of vitamin C and at the same time provides proper stability conditions for the B group vitamins.

The fast degradation of vitamin C also appears as a serious problem during the ***sample preparation***. Thus, to preserve the vitamin C content of the samples a sample preparation method [EN 14130:2003] originally developed for vitamin C was extended and validated for B group vitamins. This could be accomplished because the added vitamin content of the food stuffs are not strongly embedded in the matrix, thus instead of the complex sample preparation of B vitamins a much general extraction method can be fit for purpose.

The poor retention of vitamin C was the main problem during the simultaneous determination of C and B vitamins applying reserved phase ***chromatographic***. To overcome this problem the phosphate buffer was replaced by trifluoroacetic acid (TFA), which played double role. On one hand as pH stabilizer increased the retention of vitamin C, on other hand as ion-pairing reagent also provided adequate retention for vitamins with basic properties.

The expanded sample preparation procedure and the developed RP-IP-HPLC method were successfully applied to the determination of vitamin C, nikotinamid, vitamin B_2 and B_6 content of different vitamin enriched food matrixes (breakfast cereal, instant cacao powder, fruit juice). However (***i***) the increased UV cut-off value of the mobile phases caused by TFA did not allow the detection of vitamin B_5. Moreover I have to mention that the (***ii***) non-

symmetric peak shape and poor retention of vitamin B_1, (***iii***) the selectivity problem of nicotinic acid and the (***iv***) weak sensitivity of vitamin B_9 remained as disadvantages of the method. To resolve all these problems (retention, selectivity, sensitivity) I decided to transmit the method to mass spectrometry.

4.3 Simultaneous determination of water soluble vitamins by RP-HPLC-(UV-VIS)-ESI-MS/MS coupled system

The development of a method using liquid chromatography coupled with mass spectrometry can offer the most prosperous solution for the aforementioned analytical problems. Therefore I developed and validated a new multicomponent method using ***HPLC-ESI-MS/MS*** coupled system for the simultaneous determination of water soluble vitamins.

The ***source- and compound-dependent parameters*** were optimised as the firs step of the method development in order to rich the best signal-to-noise ratio. The ion source was operated in positive and in negative ion mode and MS data acquisition was performed in the MRM (multiple reaction monitoring) mode by selecting two precursor-to-product ion transitions for each target compound. Of the two selected MRM transitions, that having the highest intensity (quatifier transition) was used to perform quantitative analysis, while the least intense one was used for identification purposes (qualifier transition).

The optimization of the parameters was followed by the development of a new ***MS compatible method*** for the simultaneous determination of water soluble vitamins. In the previously applied TFA suppressed the ionization of the vitamins therefore instead of it formic acid was used to provide the adequate low pH of the mobile phases. The new composition of the mobile phase required the change of the stationary phase. The adequate retention and base line separation were achieved by using a stationary phase with characteristic of high water tolerance. Diode array detector coupled with mass spectrometer was used for the detection of the vitamins. The developed chromatographic method was ***validated*** (limit of detection, linearity, sensitivity, repeatability) and successfully applied to the determination of the vitamin content of several vitamin ***enriched food samples*** (breakfast cereal, instant cacao powder, fruit juice).

Extraction efficiency study was performed as a part of the validation procedure of the before mentioned sample preparation method using metaphosphoric acid solution. The sample extracts gained from a single and a double extraction of the samples were compared by using hippuric acid as internal standard. There was no significant difference between the once and

twice-extracted samples. Above the extraction efficiency the repeatability of the sample preparation was also examined.

In conclusion, the developed ***HPLC-(UV-VIS)-ESI-MS/MS*** method and the extraction using ***metaphosphoric acid*** is suitable for the simultaneous determination of vitamin C and B group vitamins (B_1, B_2, nicotinic acid, nikotinamid, B_5, B_6, B_9)in enriched food products.

4.4 Determination of endogenous vitamin content

For many of the water soluble vitamins, biological activity is attributed to a number of structurally related compounds known as vitamers. Certain vitamers are naturally occurred in ***free*** and chemically ***bound*** forms. Thus, the bound vitamin forms have to be released from the matrix to gain the total vitamin content of the examined food products. In the case of the enriched food products the total vitamin content is consist of the added and the endogenous vitamin content. In the frame of my Ph.D. study I examined that how much percent of the total vitamin content of the enriched foodstuffs can be extracted with the ***extraction procedure using metaphosphoric acid***. For this reason the extraction using metaphosphoric acid was compared with sample preparation procedures of B vitamins offered by European standards and also with extraction using warm water. Standards for the determination of total vitamin B content of the foodstuff by HPLC are available in the case of vitamins B_1, B_2 and B_6. Moreover these standard methods also ensure the conditions required (e.g. acid hydrolysis) to release the total niacin (B_3) content of the foodstuff. The steps of the extraction procedure of vitamin B_1 and B_2 are the same, thus these two sample preparation could be merged. Therefore the extraction using metaphosphoric acid was compared with two different sample preparation made up of ***acid hydrolysis*** and ***enzymatic treatment*** and with extraction using ***warm water*** in the case of ***vitamins B_1, B_2, B_3 and B_6***.

According to the aforementioned the compounds of interest are the followings: thiamine (B_1), riboflavin (B_2), nicotinic acid, nicotinamide, pyridoxine (PN), pyridoxamine (PM), piridaxal (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN) and the piridoxal-5'-phosphate (PLP). The monitoring of the phosphorylated forms is required to control the optimal activity of the dephosphorylating enzymes. The disappearance of the phosphorylated forms from the chromatogram means the optimal activity of the enzymes.

The determination of the compounds was performed by ***RP-HPLC-(UV-VIS)-ESI-MS/MS*** coupled system using ***ion-paring reagent*** (HFBA). The required compound-dependent parameters were also optimised before the development of the separation.

Applying the developed chromatographic method the above mentioned four sample preparation procedures were compared on an enriched breakfast cereal. Based on the results it is proven that the extraction using metaphosphoric acid extracts only the ***free vitamin forms*** of the food samples.

5 THESIS STATEMENTS

1) I developed a group of analytical methods, which provides possibility for the simultaneous determination of the most frequently applied forms of water-soluble vitamins (C, B_1, B_2, B_3, B_5, B_6, B_9) for food enrichment. I offered alternative solutions using different analytical approaches to overcome the chromatographic difficulties caused by the chemical diversity of water soluble vitamins.

   - I developed reserved phase HPLC methods for the determination of the above mentioned vitamins using:
     - UV-VIS detection at one or more wavelengths, and as well as
     - UV-VIS- ESI-MS/MS detection system,
     - With ion-pairing reagent (trifluoroacetic acid),
     - Without ion-pairing reagent, at neutral pH, and also
     - Without ion-pairing reagent under ion suppressed conditions.

   - Regarding the UV-VIS detection of vitamin B_2 I proved that better results can be achieved at 448 nm due to the higher selectivity than at the wavelength of its absorbance maximum (λ_{max}=270 nm).

   - I proved that the B_5 vitamin content of dietary supplements (vitamin tablets) –with the lack of characteristic chromophore- can be determine at 200 nm detection wavelength using 50mM phosphate buffer and methanol as mobile phases.

- I proved that the application of trifluoroacetic acid as ion-paring reagent can serve as one of the solutions for the analytical problems caused by the chemical diversity of water soluble vitamins.

2) I develop an ion-pair, reversed-phase HPLC separation method coupled with ESI-MS/MS detection system, which allows of the simultaneous determination of water-soluble vitamins and some their vitamers. I proved the efficiency of this method for the control of the sample preparation used for the extraction of total vitamin content from foodstuff.

   - I proved that applying 0,05 % (v/v) heptafluorobutyric acid as ion-paring reagent in the mobile phase can ensure the separation of thiamine (B_1), riboflavin (B_2), nicotinic acid, nicotinamide, pyridoxine (PN), pyridoxamine (PM), piridaxal (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN) and the piridoxal-5'-phosphate (PLP).

3) I developed a sample preparation procedure for the determination of water soluble vitamin content of food products and dietary supplements. By applying the method on real food samples I proved the adequacy of the method for quantitative analysis of total vitamin C and free vitamin B_1, B_2, B_3, B_5, B_6 and B_9 content.

   - Performing recovery studies I proved that the quality and quantity of the above mentioned vitamins are preserved during the sample preparation procedure.

   - I developed a new method for the control of extraction efficiency. Using this new procedure I proved that the applied conditions (solution-sample ratio, time of extraction) during the extraction ensure maximal efficiency.

   - I proved that in the case of B vitamins using the optimized sample preparation method with metaphosphoric acid (originally developed for vitamin C) leads to similar results as the use of the common warm water-extraction.

4) I performed a comparison study related to the detection systems (ESI-MS/MS and UV-VIS) applied during the development of the different chromatographic methods.

- I found out that the determination of vitamin B_5 in food samples using UV-VIS detection is not possible applying the developed chromatographic methods, but in the same time under the same chromatographic conditions ESI-MS/MS system provides successful detection of this compound, which is duo to the different working principals of this system.

- I found out that 15 and 17 times lower detection limit can be achieved by ESI-MS/MS system for vitamins B_1 and B_3 respectively, while in the case of vitamin C the UV detection can provide more than one order of magnitude lower detection limit. Detection limits of vitamins B_2 and B_9 achieved by both detection systems do not show significant difference.

- I found out that the ESI-MS/MS detection system responses to the present of the matrix in a more sensitive way than the UV detector. Therefore to prevent the masking effect of the matrix, matrix matched calibration technique is required for the simultaneous determination of the water soluble vitamins when ESI-MS/MS system is used for detection.

- I found out that using the developed chromatographic methods, in the case of the examined compounds better repeatability can be achieved by UV detection then by ESI-MS/MS detection system.

- I proved that if ESI-MS/MS system is used for detection of nicotinic acid and nicotinamide adequate separation of this two molecules with similar chemical structure is required to prevent the interference caused by the appearance of same transitions.

5) I developed an analytical method using HPLC-ESI-MS/MS system for the quantitative determination of the bioavailable B_1, B_2, B_6 vitamin content of cereal based products.

- I proved that the sample preparation procedures serving the single examination of B_1, B_2, B_6 vitamins could be merged. Therefore the bioavailable fraction of the aforementioned vitamins can be simultaneously determined in the samples using this combined extraction procedure followed by the developed HPLC-ESI-MS/MS method.

- I proved the adequacy of the method for the determination of the bioavailable vitamin B_1 (thiamine and its phosphates), bioavailable vitamin B_2 (riboflavin and its phosphates) and the bioavailable vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine, and their phosphates) content of cereal based products.

6 PUBLICATIONS

In journals with impact factor

Hegedűs A., Balogh E., **Engel R**., Sipos B.Z., Papp J., Blázovics A., Stefanovits-Bányai É.: Comparative nutrient element and antioxidant characterization of berry fruit species and cultivars grown in Hungary. HortScience, ISSN: 0018-5345, ***2008***. 43 (6) 1711-1715 (IF:0,79)

Engel R., Abrankó L., Stefanovits-Bányai É., Fodor P.: Simultaneous determination of water-soluble vitamins in fortified food products. Acta Alimentaria, ISSN 1588-2535, ***2010***. (IF: 0,441) in press

Engel R., Abrankó L., Stefanovits-Bányai É., Fodor P.: Simultaneous determination of B group vitamins and vitamin C in various fortified food products. Chromatographia, ***2010***. (IF: 1,48) in press

In journals without impact factor

Engel R., Hegedűs A., Bodor Zs., Heltmann-Tulok M., Stefanovits-Bányai É.: Comparative study of three rosemary (*Rosmarinus officinalis* L.) clones during the growing season. Journal of Horticulture Science, ISSN 1585-0404, ***2005***. 11 (2) 67-72.

Stefanovits-Bányai É., **Engel R**., Hermán R., Blázovics A., Hegedűs A.: Antioxidant characterization of apricot (Prunus armeniaca L.) cultivars and hybrids. International Journal of Horticultural Science, ISSN 1585-0404, ***2005***. 11 (4) 47-51.

Szenthe A., Stefanovits-Bányai É., Blázovits A., Hegedűs A., **Engel R.**, Sipos B.Z., Sárdi É., Papp J.: Influence of strawberry and raspberry consumption on the antioxidant status of human body. International Journal of Horticultural Science, ISSN 1585-0404, ***2006***. 12 (3) 30-36.

Engel R., Hegedűs A., Heltmann-Tulok M., Stefanovits-Bányai É.: Rozmaring-klónokból készült vizes és alkoholos kivonatok antioxidáns kapacitásának és elemösszetételének összefüggései. Olaj, Szappan, Kozmetika, ISSN 0472-8602, ***2005***.54 (1) 26-28.

Stefanovits-Bányai É., Gyúrós J., **Engel R**., Hegedűs A.: Tojásgyümölcs (*Solanum melongena*) fajták szerepe az antioxidáns védelemben. Kertgazdaság, ISSN 1419-2713, ***2005***. 37 (4)10-13.

Stefanovits-Bányai É., Blázovics A., Hegedűs A**.**, **Engel R**., Sipos B.Z., Szenthe A., Papp J.: A szamóca és a málnafogyasztás szerepe a szervezet antioxidáns státuszának kialakításában. Kertgazdaság, ISSN 1419-2713, ***2006***. 38 (1)19-26.

<u>In conference proceedings</u>

<u>Hungarian, full-text</u>

Engel R., Hegedűs A., Heltmanné Tulok M.: A rozmaring (*Rosmarinus officinalis* L.) kivonatok összantioxidáns- kapacitásának valamint összfenol- tartalmának változása a vegetáció során. The 11th symposium on Analytical and Environmental Problems, Szeged, ISBN 963-217-147-0, ***2004***. Összefoglaló 121-125.

Stefanovits-Bányai É., Hegedűs A., Blázovics A., Sztefanov A., **Engel R**., Koczka N.: Az antioxidáns kapacitás szempontjából fontos fenolos vegyületek mennyisége a *Ginkgo biloba* L. hím és nőivarú egyedeiben. The 12th Symposium on Analytical and Environmental Problems, Szeged, ISBN 963-219-675-9, ***2005***. Összefoglaló 410-414.

Engel R., Hegedűs A., Heltmanné Tulok M., Ferenczi A., Stefanovits-Bányai É.: Mintavételi időpontok hatása néhány fontosabb *Lamiaceae* faj drogjából készült kivonat antioxidáns kapacitására. The 12th Symposium on Analytical and Environmental Problems, Szeged, ISBN 963-219-675-9, ***2005***. Összefoglaló 405-409.

<u>Hungarian abstract</u>

Engel R., Hegedűs A., Heltmann Tulok M., Stefanovits-Bányai É.: Rozmaringból készült teakivonatok összehasonlítása az antioxidáns kapacitás szempontjából. Magyar Szabadgyök-kutató Társaság III. Konferenciája, Debrecen, ***2005***. 15.

Engel R., Hegedűs A., Szani Sz., Mózes T., Stefanovits-Bányai É.: Káposztafajták antioxidáns kapacitásának szerepe az élelmiszeriparban. VII. Élelmiszertudományi Konferencia, Szeged, ISBN 963-482-676-8, ***2006***. Előadás és poszter összefoglaló, 94.

Szenthe A., Stefanovits-Bányai É., Stéger-Máté M., Boldoczky D., Balogh E., **Engel R**., Sipos B.Z., Papp J.: Ősszel érő bogyósgyümölcsök friss fogyasztásának szerepe táplálkozásunkban. VII. Élelmiszertudományi Konferencia, Szeged, ISBN 963-482-676-8, ***2006***. Előadás és poszter összefoglaló 115-116.

Szamosi Cs., **Engel R**., Hegedűs A., Némethy-Uzoni H., Tordai E., Sárdi É., Stefanovits Bányai É.: A dinnyefélék táplálkozási jelentősége a téli időszakban. VII. Élelmiszertudományi Konferencia, Szeged, ISBN 963-482-676-8, ***2006***. Előadás és poszter összefoglaló 113-114.

Engel R., Hegedűs A., Blázovics A., Gyúrós J., Stefanovits-Bányai É.: Paprikafajták antioxidáns tulajdonságának összehasonlítása friss és feldolgozott állapotban. Magyar Szabadgyök Kutató Társaság, Munkaértekezlet, ***2006***. 9.

Szamosi Cs., **Engel R**., Tordai E., Sárdi É., Némethy-Uzoni H., Stefanovits-Bányai É.: Milyen szerepe lehet a dinnyefélék fogyasztásának a szervezet antioxidáns státuszának biztosításában? Magyar Szabadgyök Kutató Társaság, Munkaértekezlet, ***2006***. 22.

Engel R., Abrankó L., Fodor P.: Vízben oldódó vitaminok meghatározása vitaminozott élelmiszerekben HPLC-UV technikával. Centenárium Vegyészkonferencia, Sopron, ***2007***.

International, full-text

Engel R., Hegedűs A., Heltmann-Tulok M., Stefanovits-Bányai É.: Microelement ingestion by herb-tea consumption. 6th International Elements in Environment, Medicine and Biology, Temesvár, Románia, Publishing House, ISBN 973-620-035-3, ***2004***. 203-208.

Engel R., Némethy-Uzoni H., Hegedűs A., Stefanovits-Bányai É.: Effect of potassium sulphate fertilization on element content and antioxidant capacity in onion (*Allium cepa* L.). International Symposium on Trace Elements in the Food Chain, Budapest, ISBN 963 7067 132, ***2006***. 299-303.

Engel R., Abrankó L., Stefanovits-Bányai É., Fodor P.: Simultaneous determination of water-soluble vitamins in supplemented cereal by liquid chromatography. Proceedings of 5th International Congress on Food Technalogy, Thessaloniki, Greece, ISBN 978-960-88557-3-1, ***2007***. 619-626.

International abstract

Engel R., Heltmann-Tulok M., Hegedűs A., Ferenczy A., Stefanovits-Bányai É.: Antioxidant capacity of some *Lamiaceae* species. 36th International Symposium on Essential Oils, Budapest, ISBN 963 218 981 7, ***2005.*** September 4-7. Abstract P141.

Máthé Á., Stefanovits-Bányai É., Blázovics A., Sipos B.Z., Szenthe A., Hegedűs A., **Engel R**., Papp J., Sárdi É. Antioxidant activity and element content in elderberry collected from different areas in Hungary. 27th International Horticultural Congress & Exhibition, COEX, Seoul, Korea, ***2006.*** August 13-19. Abstracts 91. SO3-P-47

Máthé Á., Stefanovits-Bányai É., Blázovics A., Sipos B.Z., Szenthe A., Hegedűs A., **Engel R**., Papp J., Sárdi É. Effect of the antioxidant activity and element content in raspberry fruits on human health. 27th International Horticultural Congress & Exhibition. COEX, Seoul, Korea, ***2006.***August 13-19. Abstracts 84. SO3-P-10

Engel R., Stefanovits-Bányai É., Abrankó L.: Simultaneous determination of water-soluble vitamins in supplemented cereal by liquid chromatography. Congressus Pharmaceuticus Hungaricus XIV., Gyógyszerészet Supplementum Budapest, ISSN 0017-6036, ***2009.*** 53, S113