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ORIGINAL PAPER

ANTIOXIDANT DENSITY IN PLANT FOODS TYPICAL IN BULGARIAN DIET AND HEALTHY AGEING

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Abstract

Introduction. The Free Radical Theory initiates a research of antioxidants as preventive factors against degenerative age-related diseases. The importance of antioxidants in diet for strengthening the body antioxidant defense is confirmed by numerous studies, arising the need to determine their content in foods.

The objective of the study was to obtain data about the composition of antioxidant micronutrients and biologically active compounds in foods typical for Bulgarian diet, due to their active role in prevention of age-related diseases, thus providing healthy and available food choices of the elderly for antioxidants density diet.

Materials and methods. The vitamins E, C, and bio-actives β-carotene and flavonoids – quercetin, catechins and flavones in 13 fruits and 13 vegetables were studied. The compounds were analyzed with high performance liquid chromatography methods.

Results. The richest source of vitamin E is apricot (4.04 mg/100 g), of vitamin C – red pepper (142.8 mg/100 g), of β-carotene – carrot (6.3 mg/100 g). Quercetin has the highest value in onion (20.41 mg/100 g); catechins are found only in fruits with maximal content in black grapes (19.53 mg/100 g), while flavones are detected only in

Résumé

La densité antioxydante des aliments végétaux typiques dans l'alimentation bulgare et le vieillissement en bonne santé

Introduction. La théorie des radicaux libres (Denham Harman, 1950) lance une recherche des antioxydants comme facteurs préventifs contre les maladies dégénératives liées au processus de vieillissement. L'importance des antioxydants dans l'alimentation pour fortifier la défense antioxydante est confirmée dans de nombreuses études, d'où la nécessité de présenter leur contenu dans différents aliments.

L'objectif de l'étude est de présenter des données sur la composition des micronutriments antioxydants et des composés biologiquement actifs dans les aliments, typiques du régime bulgare, en raison de leur rôle actif dans la prévention des maladies liées à l'âge, offrant ainsi des choix alimentaires sains et disponibles aux personnes âgées pour un régime de densité des antioxydants.

Matériaux et méthodes. Les vitamines E, C et les bioactifs β-carotène et flavonoïdes – quercétine, les catéchines et les flavones dans 13 fruits et 13 légumes sont étudiées. Les composés sont analysés par les méthodes HPLC.

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the vegetables: carrot, peppers, lettuce, and especially in parsley (79.74 mg/ 100g).
Conclusions. The current study provides information of antioxidants density of fruits and vegetables typical for Bulgarian diet. The present data are a basis for establishment of healthy diet for prevention of age-related diseases and can be used for forming the European database of foods antioxidants, building the variety of diets in the European Union.

Keywords: antioxidants density, fruits, vegetables, anti-ageing.

Abbreviations
Vit C – vitamin C
Vit E – vitamin E
HPLC – high performance liquid chromatography

Résultats. Les résultats montrent que la source la plus riche en vitamine E est l'abricot (4,04 mg/ 100 g), la vitamine C – le poivron rouge (142,8 mg/ 100 g), le β-carotène – la carotte (6,3 mg/ 100 g). La quercétine a la valeur la plus élevée en oignon (20,41 mg/ 100 g); les catéchines ne se trouvent que dans les fruits à teneur maximale en raisins noirs (19,53 mg/ 100 g), tandis que les flavones ne sont détectées que dans les légumes: carotte, poivron, laitue et surtout dans le persil (79,74 mg/ 100g).
Conclusions. L'étude actuelle fournit des informations sur la densité antioxydante des fruits et des légumes typiques de l'alimentation bulgare. Les présentes données constituent une base pour la création d'une alimentation saine pour la prévention des maladies liées à l'âge et peuvent être utilisées dans la formation d'une base de données européenne sur les antioxydants alimentaires, construisant une diversité de régimes alimentaires dans l'Union Européene.

Mots-clés: antioxydants, les fruits, les légumes, anti-âge.

Introduction

There are proofs showing the positive role of food antioxidants for healthy, anti-aging diets. Denham Harman's hypothesis, known as „Free Radical Theory of Aging", published in the 1950s, is considered the start of research in this field[1]. Further discussion with some negative opinions about the theory provoked the author to provide new arguments in 1992 (40 years later) supporting his view. After some analyses, the author succeeded to show that a low-energy diet, containing also free radical inhibitors, increases live expectancy by 5 years or more[2]. The debate continues even today, focusing on the aging process, with a new hypothesis formulated in 2018, „A Free Radical Theory of Frailty", according to which oxidative damage is not entirely related to chronological age, but rather to the health status of the elderly[3]. Antioxidants, as preventive factors against age-related diseases, are confirmed today in many studies[4-7]. Simultaneously with the growing scientific interest in antioxidants, we are witnessing a demographic challenge for the XXIst century – extending live expectancy and corresponding growth of diseases associated with aging. According to World Health Organization (WHO), the proportion of the world's population over 60 years will nearly double, from 12% to 22% between 2015 and 2050, expecting to reach 2 billions. The advancing age is associated with increasing incidence of age-related diseases: neurodegenerative, cardiovascular, cancer, diabetes, eye diseases, and cognitive dysfunctions[8-13].

The role of nutrition in healthy ageing is focused mainly on two dimensions – caloric restriction and increased intake of dietary antioxidants[14,15]. Along with enzymatic antioxidant system, many non-enzymatic antioxidants act to scavenge free radicals[16]. This second defense system against free radicals provides protection against oxidative damage and synergic enhances the function of endogenous antioxidants defense. The most studied representatives of exogenous antioxidants, naturally present in foods, are the micronutrients – vitamin E, vitamin C, and bioactive phytochemicals – carotenoids, and flavonoids.

Antioxidants' intake has shown potential health benefits in the fight against neurodegenerative diseases, associated with oxidative damage to neurons, the primary cause of aging[17]. Higher amounts of vitamin E in the diet are associated with maintenance of an optimal immune response and with enhanced resistance to respiratory infections, particularly harmful in elderly[18]. A large amount of research and epidemiological cohort studies have shown the relationship between the dietary intake of flavonoids and the risk of developing cardiovascular diseases, diabetes, cancer etc, where the advanced age is the basic risk factor[19].

The main food sources of vitamin E, vitamin C, carotenoids and flavonoids are plant foods, namely fruits and vegetables, tee, cocoa.

Our study is focused on antioxidants content in Bulgarian fruits and vegetables, following the general

recommendation for their high consumption. Fruits and vegetables are at the basis of all healthy eating pyramids, and due to their high micro-nutrient density, but low energy, they perfectly match the guidelines of caloric restriction as anti-aging intervention[20]. The initial recommendations for higher intake of fruits and vegetables were justified by the richer fibres content, but the modern identification of their composition explained the positive effect, with the presence of a rich spectrum of bio-active compounds, emphasizing their antioxidant properties and warning about careful use of fibres in diseases of the gastrointestinal system in the elderly[21].

The current data from Global Burden of Diseases, published in Lancet (2019) show that consumption of nearly all healthy food and nutrients was subnormal and more than half of all diet-related deaths and two thirds of diet-related disability-adjusted life-years were attributed to three main dietary factors: high intake of sodium, low intake of whole grains and low intake of fruits[22]. In this aspect, acquiring information for food antioxidants in Bulgarian fruits and vegetables will provide a quantitative base for development of preventive anti-aging diet, founded on national traditional food preferences.

The aim of the study is to present data regarding the composition and content of antioxidant micronutrients and biologically active compounds in foods typical for Bulgarian diet, due to their active role in the prevention of age-related diseases, thus providing healthy and available food choices for the elderly.

Material and methods

The analysis of the data on food consumption of the Bulgarian population gave us a basis for the selection of the respective representatives from the two groups of plant foods, subject of the present study[23]. In the present study, 26 foods were studied – 13 fruits and 13 vegetables, typical for Bulgarian diet. All foods were purchased within their mature ripening stage. The amount of the antioxidants: vitamin E, vitamin C, provitamin A – β-carotene and of the class of flavonoids – flavonol quercetin, catechins – sum of (+)-catechin and (-)-epicatechin, and flavones – sum of luteolin and apigenin were analyzed.

Vitamin E was determined after saponification of the food samples with 50% potassium base in ethanol, by refluxing in water bath for 30 minutes. For prevention of oxidation, 50 mg butylhydroxi toluene was added. After saponification, vitamin E was extracted with three portions of 70 ml diethyl eter. The combined extract was evaporated to dryness using rotary evaporator at 40°C. The dry extract was dissolved in 10 ml methanol. The quantitative analyzes were performed by high performance liquid chromatography (HPLC). Vitamin E was detected as α-tocopherol with fluorescence detector at Ex=358 nm and Em=345 nm. The chromatographic separation was on reverse phase C18 column (250x4.6 mm, 5μm) with mobile phase 94 % methanol: water at 35°C and flow rate of 1 ml/min.

Vitamin C was extracted with 0.5% meta-phosphoric acid and analysed with HPLC and UV detection at λ=254 nm. The chromatographic determination was performed on C18 column with isocratic elution using methanol: buffer (pH=5.5) – 0.1% sodium hydrogen sulfate, containing 0.002 mmol hexadecyl-trimethyl ammonium bromide 35:65 (v/v).

β-carotene was extracted with acetone and analyzed with HPLC and UV detection on C18 column by elution with mobile phase of acetone: water 95:5 (v/v). The flow rate was 0.8 ml/min; the working temperature was 35°C and detection was at λ=450 nm.

The flavonol – quercetin, and the flavones – lutelin and apigenin were measured after acid hydrolysis of the glucosides by refluxing in 1.2 mol HCl in 50% aqueous methanol for 2 h and HPLC analysis with UV detection at λ=360 nm. For stability of flavonoid aglucones, an antioxidant TBHQ (2 mg/ml methanol) was added during refluxing. The chromatographic separation was performed on Alltima C18 column by isocratic elution with 53 % metanol in 2% acetic acid at a flow rate of 0.8 ml/min. A method of internal standard (IS) was applied for quantitative determination and morin (500 μg/ml) was used as IS.

Catechins – (+)-catechin and (-)-epicatechin were extracted with 80% methanol/water solution for 5 minutes in ultrasonic bath. For quantitative determination, a HPLC analysis with fluorescent detection at l_{EX}=280 nm and l_{EM}= 15 nm was applied, by using the method of external standard. The HPLC determination was performed on Alltima (100 ´ 4.6 mm i.d., 3 mm) C18 by elution with 9% acetonitrile in 2% acetic acid at 30°C and 1 ml/min volumetric flow.

All analytical methods were validated, according to ISO 17025 requirements.

Results

The results of analysis of selected Bulgarian fruits and vegetables are presented in Table 1 and Table 2, respectively, expressed in mg/100 g fresh weight. Catechins' content is expressed as sum of (+)-catechin and (-)-epicatechin, and flavones content is expressed as sum of luteolin and apigenin.

The results show that the richest sourse of vitamin E is apricot (4.04 mg/100 g), of vitamin C – red

Table 1. Antioxidant compounds in Bulgarian fruits.

Fruits	Vitamin E, mg/100 g	Vitamin C, mg/100g	β-Carotene, μg/100g	Quercetin, mg/100 g	Catechins, mg/100g
Apples	0.61	5.76	26.5	0.84	4.18
Pear	0.71	4.05	9.6	0.59	4.77
Peach	1.82	7.06	91	-	2.98
Apricot	4.04	10.10	1300	3.41	14.46
Cherry	0.20	5.73	30	2.52	6.36
Sour cherry	0.14	11.10	29	0.32	0.99
Plum	0.55	13.40	180	2.34	3.73
Grapes, white	0.30	15.00	18	1.56	8.67
Grapes, black	0.40	9.70	33	2.32	19.53
Strawberry	0.12	61.95	4.9	1.02	3.29
Raspberry	1.47	26.29	9.3	1.60	5.03
Blueberry	0.57	14.83	49	9.92	6.41
Blackberry	0.61	21.41	100	2.7	5.69

Table 2. Antioxidant compounds in Bulgarian vegetables

Fruits	Vitamin E, mg/100 g	Vitamin C, mg/100g	β-Carotene, μg/100g	Quercetin, mg/100 g	Flavones, mg/100g
Onion	0.43	11.71	160	20.41	-
Carrot	0.45	8.42	6300	-	2.31
Tomato	0.34	17.77	470	1.42	-
Red sweet pepper	2.94	142.8	480	1.49	2.07
Green sweet pepper	0.70	102.1	160	10.27	8.11
Lettuce	0.22	29.35	1500	15.39	8.06
Okra	0.27	23.0	72	20.03	-
Green beans	0.02	16.57	190	2.13	-
Cucumber	0.15	4.73	300	-	-
Eggplant	0.03	1.4	37	-	-
Spinach	2.12	31.8	2900	-	-
Broccoli	0.14	93.2	990	2.94	-
Parsley	2.31	120.0	4000	-	74.79

(-) – non detected

sweet pepper (143 mg/100 g), of β-carotene – carrot (6.3 μg/100 g). Among flavonoids, quercetin has the highest value in onion (20.42 mg/100 g); catechins are found only in fruits, with maximum value in black grapes (19.53 mg/100 g), while flavones are detected only in vegetables – carrot, peppers, lettuce and especially in parsley (79.74 mg/100g).

To identify the richest sources of antioxidants in Bulgarian fruits and vegetables, we have arranged in descending order the analyzed foods considering their antioxidant compounds, presented in Figure 1 and 2, respectively.

Among fruits, strawbery is a leader of antioxidant density, due to the great amount of vitamin C (61.95 mg/100 g). The sum of studied antioxidant varies between 30-35 mg/100 g in raspberry, apricot, black grapes, blueberry and blackberry. Our results show that vitamin C has the highest proportion of the analyzed antioxidant substances in fruits and only in black grapes, pears and apricots the flavonoids prevailed vitamin C amount (Fig. 1).

The leafy condiment – parsley is the reachest source of antioxidants (201.1 mg/100 g as a sum of vitamin C, vitamin E, β-carotene and flavonoids), followed by red and green sweet pepper and broccoli (149.78 mg/100g; 121.34 mg/100g and 97.27 mg/100g correspondingly). For vegetable samples, we have observed that only in onions and lettuce the amount of flavonol – quercetin is higher than the vitamin C.

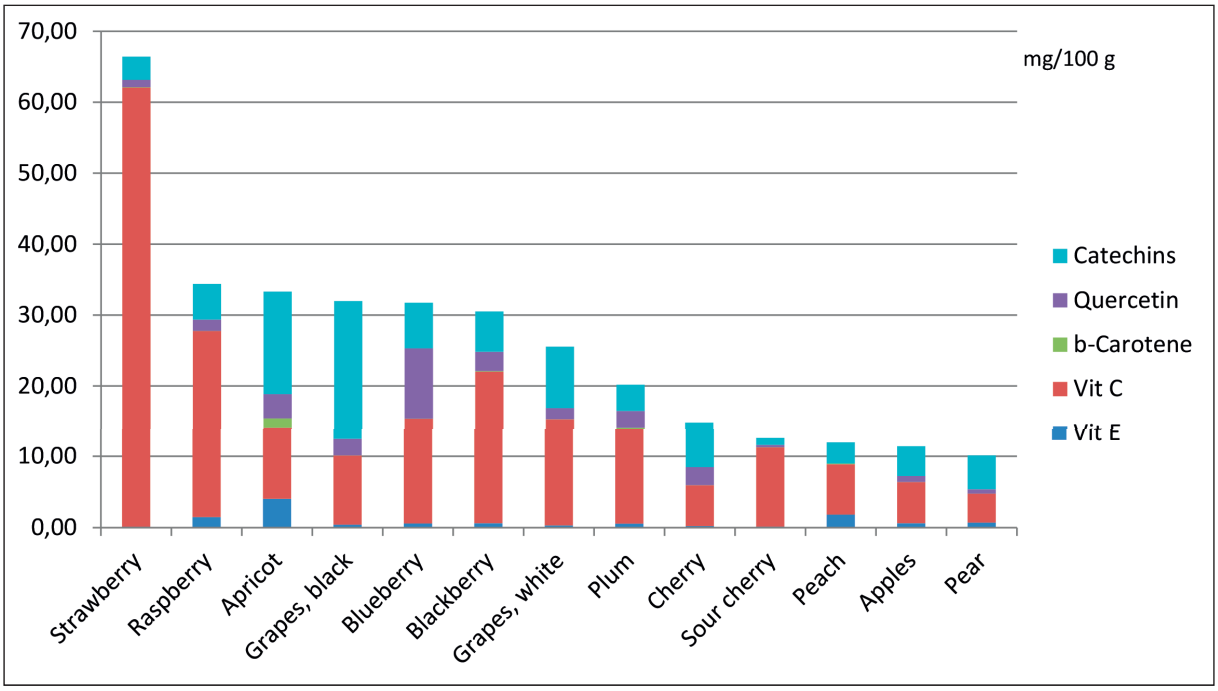

Figure 1. Fruits sorted by antioxidants content.

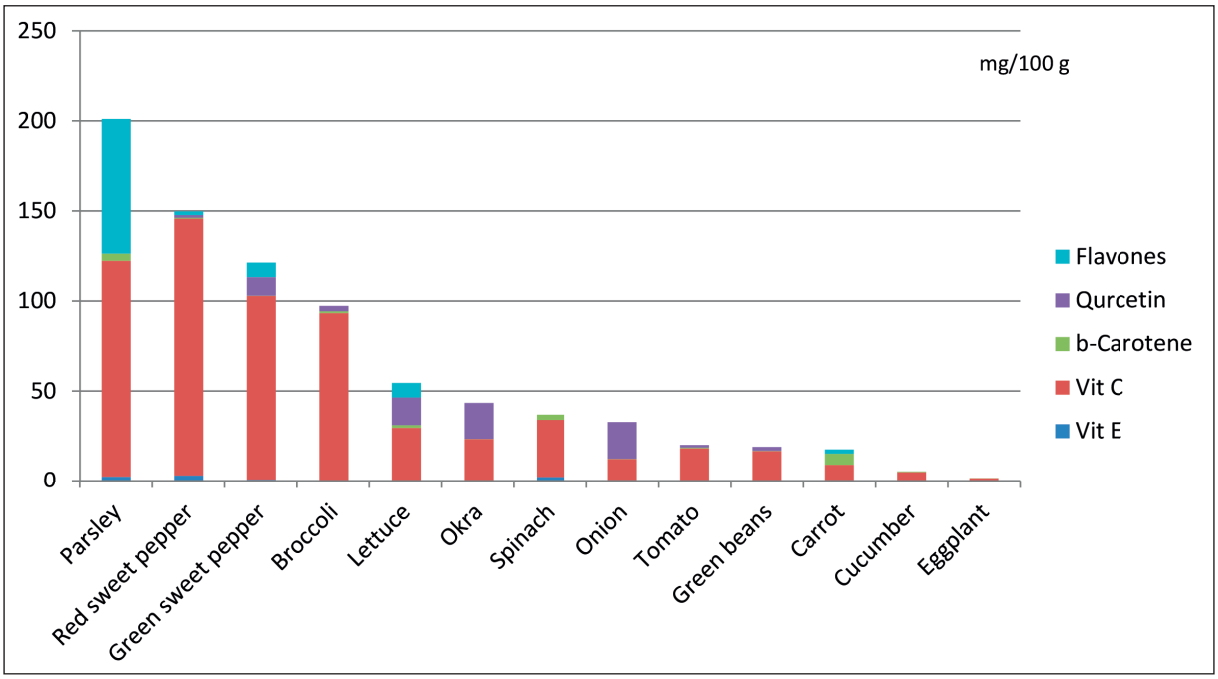

Figure 2. Vegetables sorted by antioxidants content.

Discussion

The discussion on the present results will be presented in two consecutive parts, respectively, concerning the content of antioxidants in fruits and vegetables, considering them in parallel with their consumption and their role in healthy aging.

Antioxidants in studied fruits

According to the data of the National Statistical Institute, apples are the most consumed fruits in Bulgaria – 11.7 kg per year per capita, which comprise 23% of all fruits consumed per year (49.5 kg per capita)[23]. A study conducted by Leontowicz et al has demonstrated that apples have better cholesterol-lowering effects than pears and peaches, due

to their high polyphenol content[24]. Apples have been also proved to be effective in inhibiting LDL oxidation[25] and it has been demonstrated that apple peels can greatly inhibit grow and proliferation of liver and colon cancer cells[26]. Our data show that total antioxidants level in apples is low in comparison to other studied fruits (Fig. 1), suggesting that other fruits can enrich in the larger extend the diet with antioxidants. For instance, such fruit sources are grapes. In Bulgaria, 2.4 kg grapes per year per capita are consumed[23]. Taking into consideration the greatest amount of antioxidants in both white and black grape varieties, we could conclude that grapes are considerable source of antioxidant vitamins and bioactive substances in Bulgarian diet. We must point out that black grapes contain the highest amount of catechins (19.53 mg/100 g).

The consumption of pears is 0.8 kg per capita yearly[23]. Pears appear to provide a low antioxidant intake, due to their lowest level of vitamin C (4.05 mg/100 g) and lowest amount of all other compounds studied and their relatively low consumption.

Bulgarian official statistical information about consumption of other fruits is 6 kg per capita[23]. The review of our results give us a reason to recommend higher proportion of berry fruits in the diet, since strawberries, raspberries and blackberries are abundant source of vitamin C (61.95 mg/100 g, 26.29 mg/100 g, 21.41 mg/100 g). Blueberries have the highest quercetin content, reaching a value of 9.92 mg/100 g.

Other healthy choice of antioxidants is apricot, which is an extremely rich source of β-carotene (1300 μg/100 g), vit E (4.04 mg/100 g) and of catechins (14.46 mg/100 g). It is well-known that apricot also contains high amount of potassium, thus playing a role in managing high blood pressure, since potassium lessens the effect of sodium. The bioactive profile of apricot, with high level of antioxidant vitamins, provitamin A and flavonoids, along with their high potassium density, allow it to be recommended extensively in preventive anti-aging diets.

Antioxidants in studied vegetables

The results of vegetables analysis show that vitamin E has the highest level in red pepper (2.94 mg/100 g), vitamin C – also in red pepper (142.8 mg/100 g), β-carotene – in carrot (6300 μg/100 g), quercetin – in onions (20.41 mg/100 g) and flavones – in parsley (74.79 mg/100 g). Vitamin C in parsley, red and green peppers and broccoli is about two to three time higher in comparison to other vegetables, placing them in the leading position as antioxidant sources.

In Bulgaria, the mean consumption of vegetables per year is 70.7 kg per capita[23]. The information from the National Statistical Institute shows that individual vegetable consumption per capita/per year is 21.0 kg for tomatoes (29.70% of all consumed vegetables), 6.7 kg for peppers (9.47%) and 10.3 kg for onions (14.57%)[23]. Scientific studies have shown that the onion consumption inhibits the blood platelets aggregation. Onion extracts inhibits the enzymes cyclooxygenase and 12-lipoxygenase in thrombocytes, taking part in the arachidonic acid metabolism[27]. Through this biochemistry pathway, the active compounds in onion inhibit the thromboxane synthesis and play a positive role on blood coagulation function. Onions are classified in the third position of vegetables consumed in Bulgaria, after potatoes and tomatoes. However, our data show that in Bulgarian diet peppers are the richest, accessible and sustainable source of antioxidant compounds, providing exceptionally high amounts, not only of both micronutrients, vitamin E and vitamin C, but of β-carotene and flavonoids as well (Fig. 2). The results also demonstrate that leafy condiments, like parsley, should be present extensively in the healthy diet, as parsley is the leader of the total antioxidant substances studied (211.1 mg/ 100 g).

For the public health point of view, it is considered that much of the resources necessary to support the aging population are economic, associated with reorganization of the health system. Nevertheless, an increasing role of preventive, lifestyle approaches, including dietary healthy choices, is eminent to healthy ageing. Summarizing all data provided, we can conclude that the analyzed fruits and vegetables are a good source of antioxidants and their use in various combinations in the diet can provide high efficiency of healthy ageing care.

Conclusions

The current study presents healthy and available food choices of the elderly, by providing information of antioxidants density of fruits and vegetables typical in Bulgarian diet, carrying the potential of reduction the burden of illnesses in the elderly.

The Bulgarian traditional diet has its own specifics and health characteristics, confirmed over time by Bulgarian longevity and the current assessment of high antioxidant density of the studied foods supports the modern recommendations for more fruits and vegetables in the diet, preserving the traditional nutrition.

The aging population needs up-to-date foods information, which intends to raise awareness of the relevant population risk groups and to ensure effective self-management, which largely predetermines the success of health management.

The data provided in this study can be included in the construction of national and regional food

composition databases on antioxidant nutrients and bioactive compounds in foods involved in the rich variety of European diet.

Author Contributions:

Conceptualization, S.T-S. and F.R.; methodology, S.T-S. and F.R.; software, S.T-S.; validation, S.T-S. and F.R.; formal analysis, S.T-S.; investigation, F.R.; resources, F.R. and Z.S.; data curation, S.T-S..; writing–original draft preparation, S.T-S., F.R.; writing–review and editing, S.T-S., Z.S., and F.R.; visualization, S.T-S..; supervision, F.R.; project administration, Z.S. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experimental of this study comply with the ethical standards of the Declaration of Helsinki 1975, revised in 2008, as well as national legislation"

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