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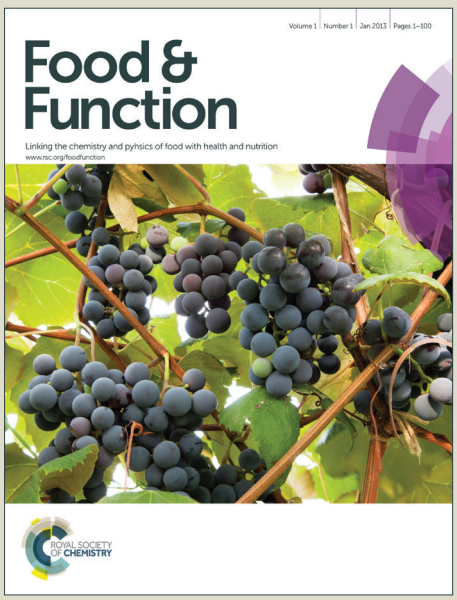

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Targeting specific nutrient deficiencies in protein-restricted diets: some practical facts in PKU dietary management

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Abstract

Among aminoacidopathies, phenylketonuria (PKU) is the most prevailing one. Early diagnosis in the neonatal period with a prompt nutritional therapy (low natural-protein and phenylalanine diet, supplemented with phenylalanine-free amino acid mixtures and special low-protein foods) remains the mainstay of the treatment.

Data considering nutrient contents of cooked dishes is lacking. In this study, fourteen dishes specifically prepared for PKU individuals were analysed, regarding the lipid profile, and iron and zinc contents.

These dishes are poor sources of essential nutrients like Fe, Zn or n-3 fatty acids, reinforcing the need of adequate supplementation to cover individual patients' needs. This study can contribute for a more accurate adjustment of PKU diets and supplementation, in order to prevent eventual nutritional deficiencies.

This study contributes to a better understanding of nutrients intake from PKU patients' meals, showing the need of dietary supplementation.

Keywords:

Phenylketonuria, nutritional deficiencies, fatty acids, vitamin E, iron, zinc.

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Introduction

Protein restricted diets are essential to treat several inherited metabolic disorders, such as aminoacidopathies – phenylketonuria (PKU)/hyperphenylalaninemia, type I tyrosinemia (type I and II), leucinose, type I citrullinemia, argininosuccinic aciduria, hyperargininemia, classical homocystinuria, hypermethioninemia; and organic acidurias – propionic aciduria, methylmalonic aciduria, isovaleric aciduria, type I glutaric aciduria, or 3- methylcrotonylglycinuria.

Among inherited amino acid metabolic disorders, PKU (OMIM 261600) is the most prevailing one.[1] It is estimated that the overall prevalence of PKU in Europe and the United States is about 1 in 10,000 live births.[2]

For more than sixty years, early nutritional intervention and diet therapy have been the basis of the treatment for the great majority of PKU diagnosed individuals.[3,4] The dietary management intends, first of all, to maintain phenylalanine (Phe) blood levels within safe limits, in order to prevent mental retardation, ensure normal growth and a normal and healthy life through adulthood.[4,5] High blood Phe concentrations are neurotoxic, as this is known to inhibit the transport of free L-amino acids, required for protein and neurotransmitters synthesis.[4] Most patients require a natural-protein-restricted diet, which allows the reduction of Phe intake, still ensuring the contribution of some Phe to guarantee normal protein anabolism.[5-7] According to patients' tolerance, protein rich-foods are slightly to strictly restricted. Therefore, meat, fish, eggs, standard bread, dairy products, nuts, seeds and leguminous crops are generally forbidden foods. Protein and essential amino acids intake is basically assured Phe-free amino acids mixtures. Energy needs are met by consuming controlled amounts of low natural protein foods (fruits and vegetables) and special low-protein foods (flour, bread, pasta, biscuits,

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milk substitutes, cheese substitutes, egg substitutes, soups, candies, among others), resembling in a vegan-like food pattern.[7-9]

As a result of restricting high protein animal-derived foods, these patients are likely to ingest small amounts of essential fatty acids and some micronutrients, such as vitamins A, C, D and E, vitamins B_2, B_6 and B_{12}, and folates, as well as selenium, iron, zinc and calcium.[5, 7, 10-13] This is particularly important when planning the diet in a long-term perspective. Most of the existing guidelines in PKU dietary management lay in childhood.[13] The main concerns have been focused in optimizing the growth and nutritional status of PKU individuals, ensure an optimal compliance of the diet and improve their quality of life.[4] Ultimately, the treatment should allow PKU patients to achieve an optimal cognitive and psychosocial development and well-being throughout life. Therefore, it is consensual that treatment and follow up of PKU patients is for life and, based on that, especial attention should be given to specific nutrient supplementation in long-term dietary counseling.[4, 14, 15] As described by Demirkol and colleagues,[14] the dietary management for this disease is somehow complex and time-consuming. It also requires knowledge of foods and recipes, cooking skills and cautious food portions control.

Based on this, and following our previous work,[16,17] we intended to characterize the composition of low protein dishes, selected from a list of recipes specifically proposed for PKU patients, regarding fatty acids profile, and tocopherols, tocotrienols, iron and zinc contents, nutrients for which these individuals may be at risk.

Results and Discussion

Patients with PKU need to follow a low-protein diet, but still healthy balanced.[16] This is a very restrictive vegan-like diet, based in fruits and vegetables, low-protein natural

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foods, Phe-free amino acid mixtures and special low-protein foods, to fulfill their energy needs. The recipes included in this study, described in Table 1, clearly illustrate this feature. As it can be observed, vegetables and fruits, followed by dietetic low-protein products, represent the most significant fraction of the ingredients used to prepare and cook these recipes. The use of significant amounts of different types of fat is also an important aspect to highlight, as this is reflected in the energy contribution and lipid profile of the final product. Spices and aromatic herbs are another feature that stands out in many of the recipes, being used to improve the palatability and final look of the dishes. Typically, this diet provides high intake of carbohydrates rich foods and none or very low intake of animal origin foods, which leads to a low intake of several nutrients such as cholesterol, Fe, Se, Zn, Ca.[4] Moreover, fresh fruits and vegetables do not provide some important nutrients, like vitamin B12, and others (e.g. Ca and Fe) may present low bioavailability.

In addition, cooking methods can affect foods composition, essentially due losses and/or incorporation of water and/or fat. Deep-fat frying is an example of that, in which the oil is incorporated in foods, during the process. Therefore, the final product somehow exhibits the lipidic profile of the frying oil. Moreover, variations in the composition of foods itself may occur, depending on factors like variety, seasonality, soil type, climate or even with procedures related with preparation and cooking. Regarding this latter aspect, precautions were taken in order to accurately replicate home-produced recipes.

The use of seasonings, such as spices and aromatic herb, helps not just to improve organoleptic aspect, such as taste, odor or visual appearance, but may have a particular interest in PKU particular context, as oxidative stress is a concern in these patients.[6] In fact, spices and aromatic herbs are known to be rich in several phytochemical compounds with antioxidant activity.[18]

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Fats are as well an important aspect to highlight in this food pattern context. Besides contributing to accomplish the individual energy needs, they are a source of fatty acids, cholesterol, and liposoluble vitamins (A, D, E and K).

The type of fatty acids consumed plays an important role in the etiology of several diseases. Saturated fatty acids (SFA) intake has been associated with coronary heart disease (CHD). In turn, its prevention has been correlated with monounsaturated fatty acids (MUFA) consumption. Also, long chain *n*-3 polyunsaturated fatty acids (PUFA) have beneficial effects in human health, regarding neurological functions (especially in children), prevention of CHD and metabolic syndrome, and improvement of immune response.[19] Western diets are *n*-6 PUFA rich. These compete with *n*-3 PUFA for several physiological processes, being associated with CHD and pro-inflammatory mechanisms. Recent research suggests that an increased *n*-3 FA intake and/or increased *n*-3/*n*-6 PUFA ratio in the diet has protecting health effects.[20,21] Protein-rich foods, and particularly fat fishes, are good sources of PUFA but, as already mentioned, these foods are not allowed in PKU diet. As a result, patients present lower intakes and lower plasmatic concentrations of PUFA, when compared with healthy controls.[22]

The profile of the ingredients used to prepare and cook the recipes (Table 1) somehow was reflected in the fatty acid profile, which is described in Table 2. Monounsaturated fatty acids (MUFA), essentially oleic acid (C18:1 *n*-9), was the most representative class in the dishes which contained olive oil as the main fat source or olives as ingredient.

Recipes containing vegetal margarine, a well known source of SFA,[23] presented higher relative percentages of these compounds. The main SFA found was palmitic acid (C16:0), followed by stearic acid (C18:0). Recipes containing high quantities of low-protein milk substitute (6, 7, 12, and 14) also presented greater amounts of stearic acid

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(C18:0), followed by myristic acid (C14:0), when compared with the remaining ones. Since low-protein food milk substitute is prepared from cow's milk, which contains a high amount of saturated fat, recipes containing this ingredient, presented higher amounts of stearic (C18:0) and myristic (C14:0) acids.

Linoleic acid (C18:2*n*-6) was the major polyunsaturated fatty acid (PUFA). The highest amount (49%) was found in vegetables patties (recipe 9). Corn oil, a rich source of linoleic acid (C18:2*n*-6), was the major fat contributor in this recipe.

Besides finding low relative percentages of *n*-3 PUFA, ranging from 0.33% in pineapple cake (recipe 11) to 1.50% in carrot soup (recipe 2), we have also found low *n*-3/*n*-6 ratios (<0.26) in all recipes. This strengthens the need of an adequate *n*-3 PUFA supplementation, especially if there is poor compliance of the amino acid mixtures by patients.

Vitamin E includes eight chemically distinct molecules: α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ- tocotrienol, sharing structural homology. α-Tocopherol is the most studied isoform of natural vitamin E, as it appears to have further biological functions than just its antioxidant activity, showing specific interactions with enzymes, structural proteins, lipids and transcription factors. Like tocopherols, tocotrienols also exhibit antioxidant activity. Recently, more attention has been given this second group of compounds, since there is some evidence that they may have superior biological properties, and that their anti-inflammatory and antioxidant activities may prevent cancer, diabetes, cardiovascular and neurodegenerative diseases.[25,26] Vitamin E exists in various extents in most edible oils, the major source of its intake.[25,27]

Total tocopherol and tocotrienols contents were lower than 1 $mg.g^{-1}$ fat for all dishes. Data expressed in $mg.100\ g^{-1}$ of cooked sample are presented in Table 3. α-Tocopherol was present in all recipes. The second most prevailing vitamer was γ-tocopherol,

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followed by γ-tocotrienol. In turn, δ-tocotrienol was the least representative vitamer. Tocopherols and tocotrienols contents were higher in dishes with considerable amounts of fat as ingredient, which is the case of onion pie and biscuits cake (recipes 7 and 10, respectively); in those which were fried, namely, vegetables croquettes and vegetables patties (recipes 8, 9 and 10, in that order); and in sweet vermicelli (recipe 12). In this last case, α-tocopherol was the main vitamer. This may be possibly associated with the low-protein food milk substitute's composition, which is produced from cows' milk cream, an appreciable source of α-tocopherol.[28] Some recipes (7, 10, and 11), containing vegetal margarine, exhibited the 8 vitamers simultaneously, reflecting the vitamin E profile of this ingredient.[25] Dietary Recommended Intakes (DRI) for vitamin E, for adults, is about 15 $mg.day^{-1}$.[29] As PKU patients may be at risk for its deficiency, the selection of good quality fats to prepare and cook foods is important, in order to improve their vitamin E intake and, consequently, promote a better antioxidant protection. Regarding the recipes in the present work, it would be possible to attain this daily total amount of vitamin E, ensuring appropriate combinations of foods.

A well balanced diet ensures an adequate intake of micronutrients, such as minerals and trace elements. However, these undergo significant changes in food, mainly due to processing or manufacturing. For this reason, processed products are, often, fortified with some important nutrients, to prevent nutritional deficiencies even in general population. Decreased reserves of Fe and Zn are a commonly feature associated with vegetarian diets.[30,31] PKU diet is similar to a vegan diet, but taken to the extreme. As early mentioned, micronutrients imbalance in PKU patients is common and a challenging subject, when planning the diet.

Fe is an extremely important trace element, essential for the formation of hemoglobin and myoglobin, which carry oxygen in the blood. Moreover, it is part of many proteins

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and enzymes in the organism.[32] Animal origin foods contain heme Fe, the most bioavailable form of Fe. Plant origin foods, in contrast, contain non-heme Fe, which bioavailability may be affected by absorption inhibitors, including phytates, calcium, and fiber, as well as polyphenols. However, there are ways to significantly improve the absorption of Fe, by combining foods rich in vitamin C and other organic acids, commonly found in fruit and vegetables.[12, 33]

Zinc is one of the most abundant nutritionally essential elements in the human body. It is important for the structure and function of a large number of macromolecules and for over 300 enzimatic reactions, playing also an important role in cell growth, wound healing and proper functioning of the immune and nervous systems.[34] Foods of animal origin are also excellent sources of Zn. There are vegetable sources of Zn as well, namely, legumes, whole grain pasta, wheat germ, fortified cereals and nuts. However, the majority is considered forbidden foods for PKU individuals as they are sources of natural protein.[31]

In the analysed recipes, Fe contents varied between 0.1 mg.100 g^{-1} (sweet vermicelli, 12) and 3.9 mg.100 g^{-1} (onion pie, 7). Concerning Zn contents, all recipes presented lower than 0.5 mg.100 g^{-1} amounts.

As can be observed in Figure 1 dishes with considerable amounts of special low-protein flour were those that presented higher levels of Fe (7 (3.9 mg.100 g^{-1}), 9 (5.5 mg.100 g^{-1}), 11 (1.1 mg.100 g^{-1}) and 13 (2.8 mg.100 g^{-1}). However, it was not possible to relate Zn contents with a particular ingredient.

Figures 2 and 3 represent the differences between estimated contents and the corresponding analytical data obtained. Most of the Food Composition Databases (FCD) overestimated Fe contents in the majority of recipes at about 0.02 to 1.56 mg. However, the opposite situation was observed in 3 recipes, where the three FCD

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underestimated Fe levels at about 0.01 to 1.75 mg. Concerning Zn (Figure 3), the three FCD presented overestimated contents in the majority of recipes at about 0.01 to 0.48 mg. As in Fe case, Zn was also underestimated in some recipes in amounts ranging from 0.01 to 0.18 mg.

For both Fe and Zn, the analytical results were compared to estimated contents based in the three FCD. In what concerns to Fe, FCDs gave generally similar information conducting over or underestimated amounts in the same direction, and in the same order of magnitude, for each recipe. On the other hand, this was not so linear for Zn composition, as the slight variations within some recipes were sometimes overestimated, and in others underestimated. These slight deviations are unquestionably related with several different factors such natural variability of nutritional composition of foods, variability within species, the effect of the cooking methods itself, the methodology of samples analysis, among others.

For healthy individuals these deviations are not relevant. Nevertheless, in clinical situations requiring severe food restrictions, this is of huge significance. Diets based on FCD should be carefully planned (especially regarding micronutrients composition), analyzed and dynamically adjusted whenever required. It is of utmost importance to monitor patients' nutritional status and, if needed, supplementation should always be considered as part of the dietary treatment.

DRI for male and female healthy adults is of 8 and 18 $mg.day^{-1}$ for Fe, and of 11 and 9 $mg.day^{-1}$ for Zn, respectively.[35] Considering the bioavailability of the trace elements in plant origin foodstuffs, a periodical assessment of individuals' nutritional status is important to guarantee balanced intakes.

Experimental

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Standards and reagents

The fatty acid methyl ester standard mixture (FAME 37) was acquired from Supelco (Bellefonte, PA, USA). Boron trifluoride was from (Sigma-Aldrich, Steinheim, Germany). Tocopherols (α, β, γ and δ) and tocotrienols (α, β, γ and δ) were purchased from Calbiochem (La Jolla, California, USA) and tocol was obtained from Matreya Inc. (Pennsylvania, USA). Butylated hydroxytoluene (BHT) was from Aldrich (Madrid, Spain), High Performance Liquid Chromatography (HPLC) grade acetonitrile and 1,4-dioxane were from Fluka (Madrid, Spain) and HPLC grade *n*-hexane was from Merck (Darmstad, Germany). Cesium chloride (CsCl) and nitric acid (HNO3) were p.a. quality from Panreac® (Barcelona, Spain). Ultrapure water from a Simplicity 185 system (resistivity 18.2 MΩ.cm; Millipore, Belford, USA) was used for the preparation of samples and standards. For iron (Fe) and zinc (Zn) determination, stock solutions (1000 $mg \cdot l^{-1}$; Panreac, Barcelona, Spain) were used.

Samples and sample preparation

Recipe books specifically planned for PKU patients[36,37] were used to select 14 recipes, including soups (*n*=2), main courses (*n*=7), desserts (*n*=3) and other daily basic foods (*n*=2). Ingredients of current use were acquired in local supermarkets. Center of Medical Genetics *Doutor Jacinto de Magalhães* (Porto, Portugal) kindly provided dietetic low protein and low-Phe products.

Table 1 presents the list of the recipes, type and main ingredients of low protein and restricted Phe dishes selected and analyzed in this study.

Following the respective instructions, all recipes were prepared and cooked in triplicate, using domestic scale utensils and equipments. Samples were then homogenized

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(Classical A320R1, Moulinex, France) and immediately used for lipid extraction. The remaining was kept at -20 ºC until minerals quantification.

Lipid profile analysis

Lipids were extracted following the Soxhlet procedure with petroleum ether, for 3.5 h.[38] Then, fatty acids (FA) profile and tocopherols and tocotrienols contents were determined.

Fatty acids profile

Fatty acids were determined as methyl esters (FAME), prepared by acid-catalysed transmethylation of total lipids using boron trifluoride methanol according to Shantha and Ackman.[39] These were then analysed by gas chromatography with flame ionization detection in a GC-2010 Shimadzu (Shimadzu Corporation, Tokyo, Japan), according to Fernandes and colleagues.[40] A CPSil 88 fused silica capillary column (Varian, Middelburg, The Netherlands; 50 m x 0.25 mm internal diameter, film thickness 0.19 µm) was used for the FAME separation, using helium as the carrier gas (120 kPa) and a temperature gradient program (120ºC for 5 min, increasing to 220 ºC at 3 ºC.min^{-1}, and hold at 220 ºC for 10 min). Injector and detector temperatures were 250 °C and 270 °C, respectively. A 1.0 µl injection volume and split ratio of 1:50 was used. Triplicate assays were performed, each one injected in duplicate. Data were analyzed using GC Solution software (version 2.30, Shimadzu Corporation, Tokyo, Japan). FAME's were identified by comparison with standard mixtures (FAME 37, Supelco, Bellefonte, PA, USA) and expressed as relative percentage composition (%) of the individual FAMEs in the chromatogram. Analyses were performed in triplicate and results are presented in Table 2.

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Tocopherol and tocotrienol contents

To quantify tocopherols and tocotrienols, fat obtained as previously mentioned was prepared based on the ISO 9936:2006 standard,[41] using tocol as internal standard. Briefly, an accurate solution of oil in *n*-hexane, with tocol, was analyzed by HPLC. The chromatographic analysis was carried out in a HPLC integrated system from Jasco (Japan), equipped with an AS-950 automated injector, a PU-980 pump, an MD-910 multiwavelength diode array detector (DAD) and an FP-920 fluorescence detector, programmed for excitation at 290 and emission at 330 nm. The chromatographic separation of the compounds was achieved on a normal phase Supelcosil^{TM} LC-SI (3 µm; 75 x 3.0 mm; Supelco, Bellefonte, PA, USA) according to Alves *et al.*[42] Chromatographic data were analyzed using a Borwin-PDA Controller Software (JMBS, France). Analyses were performed in triplicate and results were expressed in both mg.100 g^{-1} cooked sample (Table 3).

Iron and Zinc contents

The quantification of Fe and Zn in samples was performed according to Santos *et al.*[43] in a high-resolution continuum source atomic absorption spectrometer (ContrAA 700, from Analytik Jena, Germany), equipped with a xenon short-arc lamp of 300 W (XBO 301, GLE, Berlin, Germany) and an AS52s autosampler (Analytik Jena, Germany), after acid digestion in a microwave (MARS-X 1500W; CEM, Mathews, USA), with pressure and temperature control, using a 100-mL Teflon® HP-500 Plus® vessels (CEM Corporation, Matthews, EUA). Briefly, previously homogenized frozen samples were left to room temperature. The equivalent amount of 0.3 g (dry weight) of each one was weighed into the vessels and dried in the microwave, until constant weight. After

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cooling, 8 ml of 65% nitric acid and 2 ml of 30% peroxide hydrogen were added to the samples and the microwave assisted digestion was carried out, during 30 min at a maximum temperature of 140 ºC. The clear solutions were then atomized in air/acetylene oxidising flame (Linde, Portugal) and quantified at 248.327 (Fe) and 213.857 nm (Zn). Data acquisition and treatment was performed in Aspect CS (1.4.0 version) also from Analytik Jena. Analyses were performed in triplicate and results are expressed as mg.100 g^{-1} of edible sample (Figure 1).

Food Composition Databases (FCD)

Analytical results obtained for Fe and Zn contents were compared with estimated data from different food composition databases (FCD) (Figures 3 and 4). Three FCD, namely, the Portuguese FCD,[28] the United States Department of Agriculture, National Nutrient Database (USDA),[44] and the Danish Food Composition Databank[45] were selected for comparison purposes.

Statistical analysis

Data were reported as mean ± standard deviation. Data treatment was carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

CONCLUSIONS

PKU diet is unquestionably a forcibly very restrictive vegan-like prescribed diet. Patients need to follow a low-protein diet, but still healthy balanced. In this study, it was possible to see the fatty acids and vitamin E profiles strongly depend on the different types of fat used to prepare and cook foods. These dishes are poor sources of

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essential nutrients like Fe, Zn or n-3 fatty acids, reinforcing the need of adequate supplementation to cover individual patients' needs. This study can contribute for a more accurate adjustment of PKU diets and supplementation, in order to prevent eventual nutritional deficiencies.

We believe that these are important information inputs that can help finding alternative strategies to successfully select the best ingredient combinations, facilitate the nutritional and dietetic prescription, a good compliance of the diet, as well as a good metabolic control and nutritional status of the patients.

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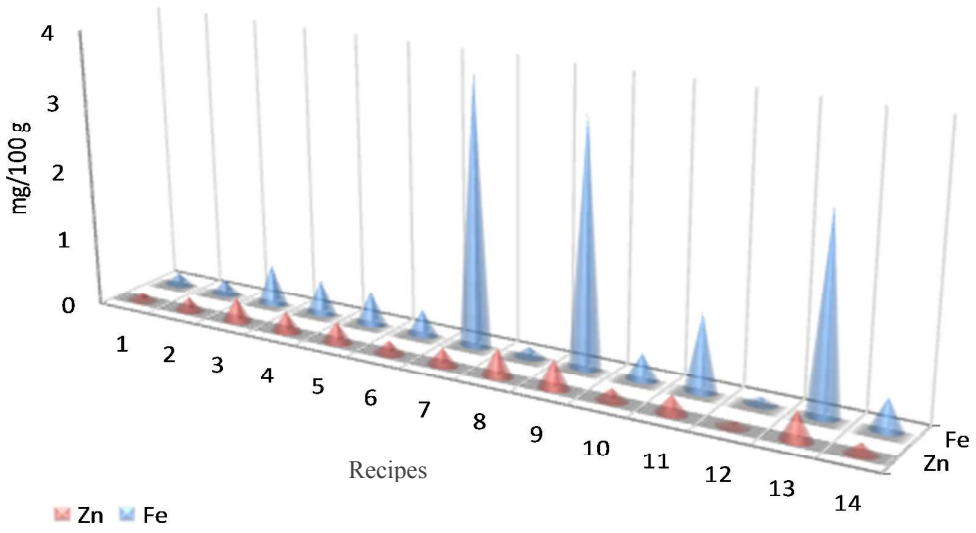

Fig. 1

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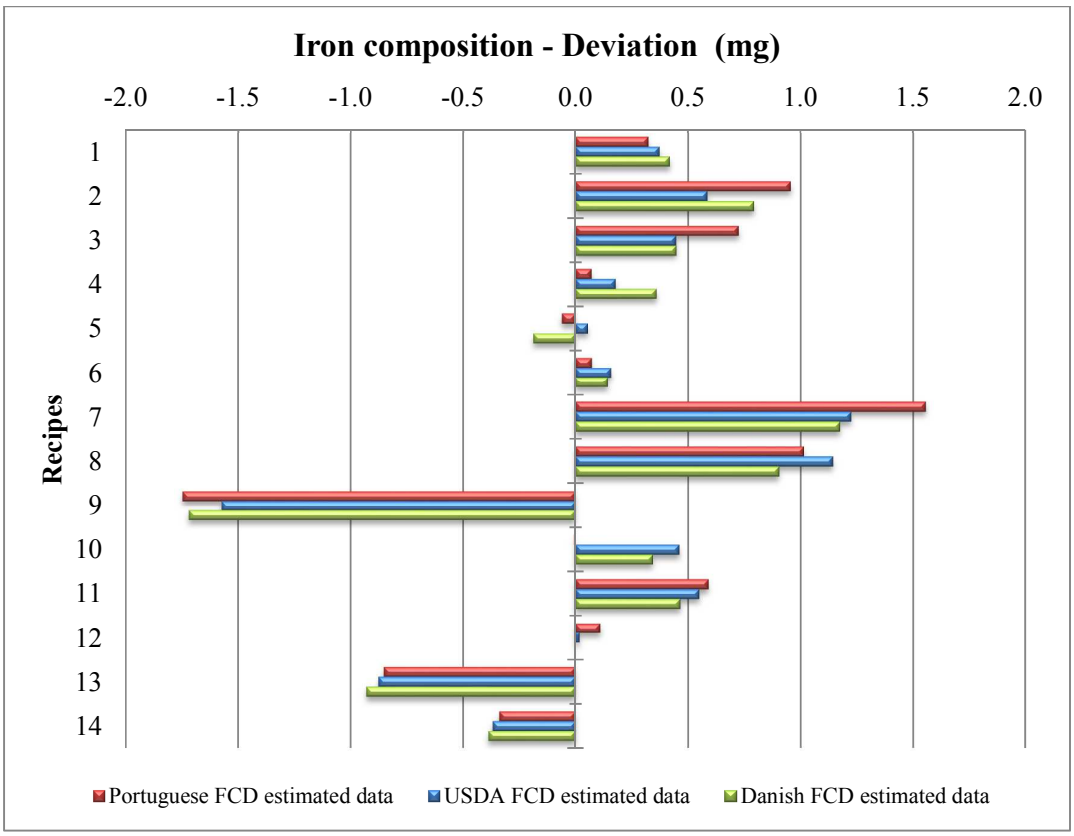

Fig. 2

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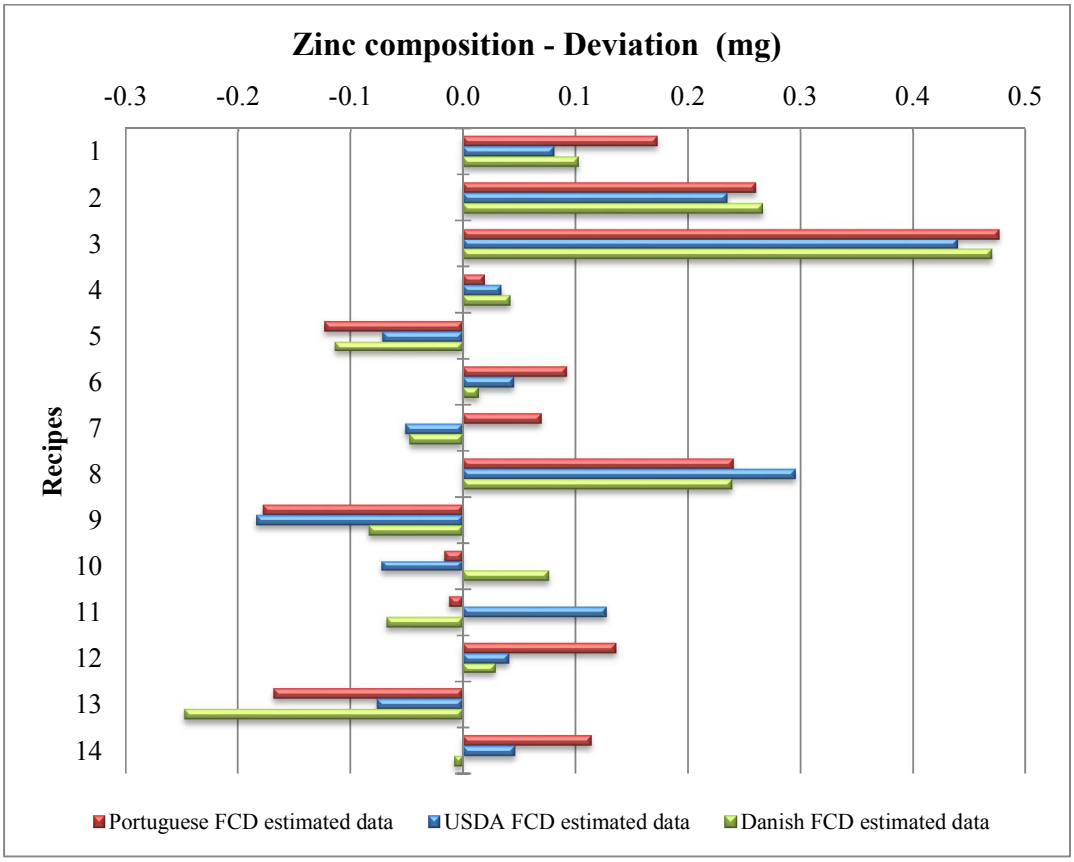

Fig. 3

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Figure Captions

Figure 1. Iron (Fe) and Zinc (Zn) composition of the dishes in mg.100 g^{-1} of edible part. Values are the mean of triplicate analysis.

Legend – Recipes: 1 - Green bean soup; 2 - Carrot soup; 3 - Pasta with vegetables sauce; 4 - Roast potatoes with vegetables; 5 - Stuffed potato; 6 -Vegetables pie; 7 - Onion pie; 8 - Mushrooms croquettes; 9 - Vegetables patties; 10 - Biscuits cake; 11 - Pineapple cake; 12 - Sweet vermicelli; 13- Low protein bread; 14 - Low protein yogurt.

Figure 2. Deviations on iron (Fe) composition of the dishes (mg) based on the comparison of analytical results and estimated data from Portuguese, USA and Denmark Food Composition Databases (FCD).

Legend – Recipes: 1 - Green bean soup; 2 - Carrot soup; 3 - Pasta with vegetables sauce; 4 - Roast potatoes with vegetables; 5 - Stuffed potato; 6 -Vegetables pie; 7 - Onion pie; 8 - Mushrooms croquettes; 9 - Vegetables patties; 10 - Biscuits cake; 11 - Pineapple cake; 12 - Sweet vermicelli; 13- Low protein bread; 14 - Low protein yogurt.

Figure 3. Deviations on zinc (Zn) composition of the dishes (mg) based on the comparison of analytical results and estimated data from Portuguese, USA and Denmark Food Composition Databases (FCD).

Legend – Recipes: 1 - Green bean soup; 2 - Carrot soup; 3 - Pasta with vegetables sauce; 4 - Roast potatoes with vegetables; 5 - Stuffed potato; 6 -Vegetables pie; 7 - Onion pie; 8 - Mushrooms croquettes; 9 - Vegetables patties; 10 - Biscuits cake; 11 - Pineapple cake; 12 - Sweet vermicelli; 13- Low protein bread; 14 - Low protein yogurt.

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Table 1. List of recipes and cooking methods, category and ingredients of low protein and restricted Phe dishes.

	Recipe	Category	List of ingredients (weight in g per 100 g of edible raw foods)
1.	Green bean soup (Boiled)	A	Water (55.0 g), green beans (13.0 g), tomatoes (9.8 g), onions (9.5 g), potatoes (8.8 g), white cabbage (2.4 g), potato starch (0.6 g), carrot (0.4 g), olive oil (0.4 g), salt (0.1 g).
2.	Carrot soup (Boiled)	A	Water (49.8 g), carrots (32.1 g), onions (8.1 g), potatoes (6.1 g), olive oil (0.9 g), potato starch (0.8 g), salt (0.1 g); low protein bread fried crumbs* to serve (2.1 g).
3.	Low protein pasta with spiced vegetables sauce (Stewed)	B	Onions (24.5 g), water (22.7 g), carrots (16.8 g), fresh mushrooms (12.2 g), low protein pasta* (9.3 g), white cabbage (6.3 g), olive oil (4.3 g), margarine (3.4 g), tomato paste (0.3 g), spices (saffron, bay leaf and nutmeg, paprika, garlic powder) (0.2 g).
4.	Roasted potatoes with vegetables (Roasted)	B	Potatoes (35.3 g), onions (20.7 g), carrots (15.9 g), water (11.6 g), white wine (9.2 g), olive oil (3.8 g), garlic (2.7 g), salt (0.3 g), bay leaf (0.3 g), parsley leafs (0.2 g).
5.	Potato stuffed (boiled/ stewed)	B	Potatoes (36.5 g),Water (33.6 g), tomatoes (9.8 g), low protein bread* (6.5 g), mushrooms (5.8 g), onions (5.0 g), olive oil (1.1 g), garlic (0.9 g), parsley (0.4 g), corn starch (0.3 g), salt (0.1 g).
6.	Vegetables pie (Baked)	B	Low protein milk substitute* (28.3 g), water (17.0 g), potatoes (17.0 g), artichokes (12.9 g), carrots (11.9 g), Brussels sprouts (2.3 g), corn starch (7.1 g), margarine (2.5 g), low protein bread dry breadcrumbs* (0.5 g), spices and salt (nutmeg, paprika, dry parsley) (0.5 g).
7.	Onion pie (Baked)	B	Low protein flour* (24.7 g), low protein milk substitute* (19.7 g), onions (15.0 g), margarine (12.2 g), water (10.6 g), carrots (5.1 g), olives (4.7 g), mushrooms (4.3 g), corn starch (1.4 g), salt (0.2 g), garlic powder (0.1 g), pepper (0.1 g), olive oil (0.8 g), corn oil (0.8 g), dry parsley (0.3 g).
8.	Mushroom croquettes (Fried)	B	Mushrooms (31.4 g), low protein milk substitute* (26.3 g), low protein bread crumbs* (15.5 g), olive oil (11.0 g), water (5.5 g), corn starch (5.3 g), margarine (3.9 g), salt (0.2 g), fresh parsley (0.4 g), nutmeg (0.1 g), egg substitute* (0.4 g).
9.	Vegetables patties (Fried)	B	Mushrooms (30.7 g), water (21.1 g), low protein flour* (18.7 g), carrots (9.1 g), corn oil (5.6 g), olives (5.3 g), boiled rice (4.5 g), onions (3.3 g), white wine (1.0 g), garlic (0.5 g), salt (0.2 g).
10.	Biscuits cake (Unprocessed)	C	Low protein biscuits* (37.5 g), sugar (31.7 g), vegetal margarine (20.0 g), instant coffee prepared (7.7 g), low protein chocolate candy* (2.9 g), cinnamon (0.2 g).
11.	Pineapple cake (Baked)	C	Canned pineapple (25 g), low protein flour* (20.4 g), pineapple juice (16.9 g), liquid caramel (14.3 g), water (12.8 g), sugar (5.1 g), margarine (3.3 g), baker's yeast (2.0 g), egg substitute* (0.1 g), vanilla (0.1 g).
12.	Sweet vermicelli (Boiled)	C	Low protein milk substitute* (38.2 g), water (47.8 g), lemon zest (0.6 g), vanilla extract (0.2 g), cinnamon (0.2 g), low protein vermicelli* (9.6 g), sugar (2.9 g), egg substitute* (0.5 g).
13.	Low protein bread (Baked)	D	Water (47.2 g), low protein flour* (46.4 g), baker's yeast (2.6 g), margarine (2.9 g), salt (0.9 g).
14.	Low protein homemade yogurt (Boiled)	D	Low protein milk substitute* (84.4 g), natural yogurt (9.0 g), corn starch (5.4 g), egg substitute* (1.2 g).

*Legend: A- soup; B- main course; C- dessert; D- daily basic foods. * Low protein dietetic products.*

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Table 2. Fatty acids composition (relative percentage).

	Recipes													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fatty acid (%)														
C8:0	-	-	-	-	-	0.24	-	-	-	0.14	-	0.81	-	0.81
C10:0	-	-	-	-	-	0.54	-	-	-	0.12	-	2.02	-	2.27
C12:0	0.12	0.10	-	-	-	0.95	0.43	0.10	-	1.03	0.37	2.50	0.40	2.98
C14:0	0.19	0.21	0.24	0.08	0.09	3.07	0.92	0.32	-	1.05	0.70	8.17	1.15	10.15
C15:0	-	-	-	-	-	0.27	-	-	-	-	-	0.90	-	1.04
C16:0	13.52	14.91	14.25	12.47	13.68	33.66	26.70	15.18	11.03	31.79	28.46	27.75	32.76	29.82
C18:0	3.64	3.78	3.78	3.10	3.27	6.93	4.22	3.24	1.76	4.67	4.36	14.83	5.21	14.36
C16:1	0.71	0.85	0.65	1.14	1.06	0.53	0.21	1.04	-	-	-	1.29	-	1.90
C18:1*n*9 t	-	-	-	0.13	0.17	0.63	-	-	-	-	-	2.17	-	2.47
C18:1*n*9 c	55.68	54.65	64.14	66.43	62.24	31.39	39.20	64.57	32.59	36.36	35.13	21.75	32.70	21.43
C18:1 trans-11	2.21	1.93	1.48	2.32	2.16	0.76	0.91	2.22	0.79	0.73	0.75	0.72	0.74	0.81
C18:2*n*6cc	11.2	17.51	10.91	10.60	9.26	17.78	25.61	9.14	48.55	19.69	27.57	3.81	22.36	3.28
C18:3*n*3	0.39	1.15	0.55	0.70	0.52	0.51	0.43	0.53	0.80	0.41	0.33	0.93	0.40	0.87
Total *n*-3 PUFA	0.39	1.15	0.55	0.70	0.52	0.51	0.43	0.53	0.80	0.41	0.33	0.93	0.40	0.87
Total *n*-6 PUFA	11.2	17.51	10.91	10.60	9.26	17.78	25.61	9.14	48.55	19.69	27.57	3.81	22.36	3.28
n-3/*n*-6	0.03	0.07	0.05	0.07	0.07	0.03	0.02	0.06	0.02	0.02	0.01	0.24	0.03	0.26

Values are the mean relative percentage of triplicate analysis.

Legend: 1 - Green bean soup; 2 - Carrot soup; 3 - Pasta with vegetables sauce; 4 - Roast potatoes with vegetables; 5 - Stuffed potato; 6 -Vegetables pie; 7 - Onion pie; 8 - Mushrooms croquettes; 9 - Vegetables patties; 10 - Biscuits cake; 11 - Pineapple cake; 12 - Sweet vermicelli; 13- Low protein bread; 14 - Low protein yogurt.

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Table 3. Tocopherols and tocotrienols composition (mg.100 g^{-1} cooked sample).

	α-TF	α-TR	β-TF	β-TR	γ-TF	γ-TR	δ-TF	δ-TR
1	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	-	0.04 ± 0.00	0.01 ± 0.00	-	-
2	0.08 ± 0.01	0.08 ± 0.03	-	-	0.09 ± 0.03	-	-	-
3	0.79 ± 0.12	-	-	-	0.35 ± 0.03	0.07 ± 0.00	-	-
4	0.27 ± 0.03	0.37 ± 0.06	0.20 ± 0.03	0.03 ± 0.00	0.41 ± 0.07	-	-	-
5	0.07 ± 0.00	-	-	-	-	-	-	-
6	0.10 ± 0.00	0.14 ± 0.01	0.08 ± 0.00	0.01 ± 0.00	-	0.02 ± 0.00	0.01 ± 0.00	-
7	1.96 ± 0.17	1.08 ± 0.04	0.47 ± 0.01	0.05 ± 0.01	1.40 ± 0.01	2.67 ± 0.45	0.03 ± 0.01	0.21 ± 0.01
8	1.87 ± 0.21	1.19 ± 0.06	-	-	1.16 ± 0.06	0.14 ± 0.03	-	-
9	2.15 ± 0.19	0.71 ± 0.03	0.37 ± 0.01	-	6.26 ± 0.48	0.41 ± 0.01	0.19 ± 0.03	-
10	1.89 ± 0.32	2.19 ± 0.32	0.60 ± 0.05	0.10 ± 0.00	1.22 ± 0.09	5.79 ± 0.69	0.08 ± 0.01	0.42 ± 0.04
11	0.85 ± 0.08	0.46 ± 0.00	0.21 ± 0.01	0.02 ± 0.00	0.41 ± 0.01	0.99 ± 0.12	0.02 ± 0.00	0.03 ± 0.01
12	0.32 ± 0.04	-	-	-	0.10 ± 0.04	0.02 ± 0.00	-	-
13	0.22 ± 0.00	-	0.06 ± 0.00	-	0.03 ± 0.00	0.04 - 0.00	-	-
14	0.11 ± 0.00	0.14 ± 0.00	-	-	-	-	-	-

Values are the mean of triplicate analysis.

Legend: 1 - Green bean soup; 2 - Carrot soup; 3 - Pasta with vegetables sauce; 4 - Roast potatoes with vegetables; 5 - Stuffed potato; 6 -Vegetables pie; 7 - Onion pie; 8 - Mushrooms croquettes; 9 - Vegetables patties; 10 - Biscuits cake; 11 - Pineapple cake; 12 - Sweet vermicelli; 13- Low protein bread; 14 - Low protein yogurt.

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