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Elemental profile of dietary supplements and agricultural byproducts evaluated by neutron activation analysis

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Abstract

Dietary supplements and agricultural byproducts were characterized by neutron activation analysis. The nutritional potential of supplements was evaluated according to alternative and commercial categories, using analysis of variance and cluster analysis, and recommended dietary intake for children. The results indicated statistically significant differences between both categories for the elements Cs, K, Na, and Rb. For the nutritional elements Ca, Co, Fe, K, Na, and Zn, the categories were similar in cluster analysis. The similarity between elemental profiles of alternative supplements and agricultural byproducts was calculated using a dissimilarity matrix, showing that rice and wheat are the predominant ingredients.

Keywords Human diet · Nutrition · Undernourishment · Dissimilarity matrix

Introduction

Hunger and malnutrition are consequences of poverty. Eradicating hunger and poverty requires understanding how these two injustices intertwine. Hunger, and the malnutrition that accompanies it, prevents the poor people from moving out of poverty because it diminishes their ability to learn, work and care for themselves and their families. Current estimates of the number of people suffering from hunger, as measured by the prevalence of malnutrition, point to almost 690 million people or 8.9% of the world's population [1]. In 2021, poverty rates in Brazil rose to 29.5% while the rate of Brazilians in extreme poverty increased to 9.8% of the population (214,326,223). The rate of children up to six years old who lived in households below the poverty line reached 44.7% in the country, the highest level in a decade.

The challenges to achieve SDG2 (Sustainable Development Goal 2: Zero Hunger) by 2030 to end hunger, food insecurity and malnutrition continue to grow steadily. The deleterious effects of malnutrition include stunting and wasting, essential micronutrient deficiencies and overweight in children, as well as maternal anemia and obesity in adults [1]. The COVID-19 pandemic has intensified the world hunger and food insecurity, exposing the fragilities in agrifood systems and inequalities in societies, making it impossible for around 3.1 billion people to afford a healthy diet. The Ukraine conflict has disrupted supply chains affecting prices of grain, fertilizer and energy. Severe climate crises are also hampering food production, especially in low-income countries.

There is a great concern about the global food loss and waste, which amounts to 1.3 billion tons per year and represents 30% of food produced for human consumption, having social impacts associated with nutrient loss and world hunger [2]. This huge amount of lost food could provide a diet of 2100 kcal per day for 2 billion people. Nanotechnological and biotechnological approaches are being used recently to value agricultural food wastes and byproducts and their effects on nutrition, environment, economy and management systems [3].

New food production systems are exploring innovations in food technology that are definitely driving dietary changes, incorporating choices like edible insects, jellyfish, plant-based alternatives and seaweeds, promoted as potential means of achieving environmental sustainability

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and nutritional benefits [4]. The use of alternative foods as sources of nutrients to fight hunger and malnutrition as well as improve food production and economic growth of nations should be encouraged. Boosting underutilized food sources, whose sustainable production and high nutritional quality is a challenging way to ensure food accessibility, has to be popularized and demystified [5].

In Brazil, a social assistance program has started in 1980s with the Pastoral da Criança (Child Pastorate), one of the most important non-governmental organizations (NGOs) in the world devoted to the health, nutrition and education of children from prenatal to 6 years of age. Founded in 1983 by the National Conference of Bishops of Brazil (CNBB), this NGO currently operates in the national territory, serving around 2 million children under 6 years of age and a monthly average of almost 100 thousand pregnant women. To complement the available food, Pastoral da Criança implemented alternative supplementation with a low-cost, quick-to-prepare product that meets regional preferences, based on unconventional ingredients and/or agroindustrial byproducts, such as wheat bran, rice bran, cassava leaves, sweet potato, pumpkin, and seeds, which are rich in diverse nutrients (proteins, vitamins and minerals). Supplement effectiveness may be a combination of nutritional improvement and hygiene education received from volunteers and applied during food preparation. Further studies demonstrated that the bioavailability of Ca, Fe and Zn was not affected by the phytate content in the alternative supplement [6, 7], and that its addition to deficient diets contributed significantly to children's physical development [8]. Furthermore, based on the abundant evidence of improved well-being, in the year 2003, the 12th National Health Conference recommended the use of such supplement as a public policy. This was endorsed by the National Institute of Food and Nutrition (INAN) as an official solution to fight hunger among poor Brazilians [9].

On the other hand, in 2007, the company Nestlé launched in the Brazilian market a new line of products developed exclusively with whole grains, informing that it is preserving all the components of the grains (bran, endosperm and germ), with emphasis on the excellent nutritional qualities of the bran. This fact suggests, at the very least, that the basic principle of the alternative supplement to use agricultural byproducts like rice and wheat bran is appropriate. Obviously, an industrial product of this nature is, because of its high cost, completely beyond the reach of people living below the poverty line. Thus, this product cannot be considered as a viable substitute for the alternative supplement for the target population.

Neutron activation analysis (NAA) has been the analytical technique chosen to investigate nutritional quality of byproducts of the industrial processing of fruits [10], to determine trace and essential elements in baby milk formulas in Algeria [11], to distinguish rice grains of four cultivars from different geographic origins in Brazil [12], to assess mineral composition of beef cattle diets in Brazil [13], to determine trace elements in infant formulas consumed in Egypt [14], to evaluate trace elements to discriminate honey sourced from Montana against honey sourced from North and South Dakota [15], and to establish multi-element profile for identification and traceability of cat food [16].

A comprehensive assessment of the elemental profile of alternative supplements produced in different regions in the country, with distinct proportions of agricultural byproducts, and of commercial supplements most consumed by children was performed by neutron activation analysis in order to compare their nutritional values.

Experimental

The sampling strategy involved the collection of dietary supplements specially designed to meet the nutritional needs and complement the diet of children from six month of age. Commercial supplements (n = 9) from different brands commonly consumed in Brazil (Neston, Farinha Láctea, Mucilon, Sustagen, Sanakids) consisting of combinations of cereals (wheat, oats, barley, rice), milk powder, minerals and vitamins were acquired in local markets. Although nutritious, they are quite expensive and not very accessible to the low-income population. On the other hand, supplements based on unconventional ingredients and/or agricultural byproducts rich in different nutrients (proteins, vitamins and minerals) have been an interesting substitute used in nutrition assistance programs in the country promoted by the charitable institution Pastoral da Criança. Samples of these alternative supplements (n = 9) were collected in the cities of Piracicaba (SP), São Paulo (SP) and Brasília (Federal District). Samples of agricultural byproducts were also collected (n = 23), which are the main ingredients that make up these supplements, including wheat bran, wheat flour, brown rice, polished rice, rice bran (stabilized, whole, parboiled and roasted), rice flour, cassava leaves, and seeds of pumpkin, sesame, sunflower and watermelon.

Samples were dried in oven at 60 ºC until reaching constant weight and ground using a rotor mill Pulverisette 14. Analytical portions of 300 mg of samples were weighed into high density polyethylene vials from Posthumus Plastics, Beverwijk, The Netherlands. Portions of 10 mg of a Ni–Cr metal alloy of known chemical composition and homogeneity [17] were used to monitor the neutron flux. Analytical quality control was performed with the certified reference materials IAEA 336 Trace and Minor Elements in Lichen and IAEA V-10 Hay Powder from International Atomic Energy Agency, Vienna, Austria, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from Institute of Nuclear Chemistry and Technology, Warszawa, Poland.

The irradiation took place in the IEA-R1 nuclear research reactor of the Nuclear and Energy Research Institute, Brazilian Nuclear Energy Commission (IPEN/CNEN) in São Paulo, SP, at a thermal neutron flux of 8×10^{12} cm^{-2} s^{-1} for 4 h. The induced activity was measured by high resolution gamma-ray spectrometry at the Radioisotopes Laboratory (CENA/USP), Piracicaba, SP, after decay times of 4, 7, 15 and 30 days, using coaxial hyperpure germanium detectors, models GEM45190 and GEM50P4-83 from ORTEC, respectively with 45% and 50% relative efficiency at the 1332 keV photopeak of ^{60}Co. Chemical elements mass fractions were obtained by the k_0 method [18], using the Quantu software [19]. En score values were calculated for the elements in the four reference materials.

The mass fractions of chemical elements in both supplement categories (alternative and commercial) were compared to verify univariate differences with the Mann–Whitney U test (NPANOVA) [20]. Following data standardization to z-score (mean = 0 standard deviation = 1) [21], the samples were grouped by unsupervised machine learning using the hierarchical clustering technique by the Ward method [22] to evaluate similarities between the categories for the nutritionally important chemical elements. The similarity between the elemental profile of alternative supplements and their ingredients was assessed by the Euclidean distance matrix for all the elements.

The hierarchical clustering was evaluated by three metrics: Silhouette index [23], Davies-Bouldin index [24] and V-measure [25]. The Silhouette index varies between -1 and 1, values close to 1 indicate high intragroup similarity. The Davies-Bouldin index, in turn, measures the similarity between groups, values close to zero indicate that the groups formed are distinct from each other. The V-measure, a metric that varies between 0 and 1, is used to assess whether the groups formed are homogeneous, that is, their points are mostly from a category (alternative or commercial).

Results and discussion

The mass fractions and respective uncertainties of the chemical elements Br, Ca, Co, Cs, Fe, K, Na, Rb and Zn determined by NAA in samples of alternative and commercial supplements are shown in Table 1.

The En score values obtained for the certified reference materials used for analytical quality control remained between -1 and 1 for all elements, with the exception of Na in the INCT-TL-1 Tea Leaves, as shown in Fig. 1.

Alternative and commercial supplements are not intended to replace meals, therefore, they are not expected to fully meet children's nutritional needs. Assuming the consumption of portion of 30 g per day, the amount of minerals Co, Ca, Zn, Fe, K and Na and respective percentage of the recommended daily intake (RDI) [26, 27] is shown in Table 2.

The Mann–Whitney U test indicated statistically significant differences (p-value < 0.05) between alternative and commercial supplements for the elements Cs, K, Na and Rb. Alternative supplements had higher mass fractions of

Table 1 Mass fractions (mg/kg) of chemical elements determined by NAA in samples of alternative and commercial supplements, with expanded uncertainties (k = 2)

	Br	Ca	Co	Cs	Fe	K	Na	Rb	Zn
Commercial 1	3.23 ± 0.18	2320 ± 200	0.0190 ± 0.0028	0.0455 ± 0.0045	91.3 ± 6.6	4450 ± 200	1070 ± 11	8.70 ± 0.59	27.3 ± 1.3
Commercial 2	3.79 ± 0.21	2330 ± 210	0.0252 ± 0.0026	0.0428 ± 0.0044	89.5 ± 5.0	4650 ± 250	1040 ± 84	10.7 ± 0.64	28.6 ± 1.3
Commercial 3	4.74 ± 0.26	2090 ± 310	0.0255 ± 0.0044	0.0458 ± 0.0048	75.7 ± 5.0	4570 ± 290	1110 ± 110	9.89 ± 0.63	24.4 ± 1.2
Commercial 4	4.66 ± 0.25	2490 ± 280	0.0209 ± 0.0022	0.0426 ± 0.0049	89.5 ± 5.6	4290 ± 290	1170 ± 120	9.50 ± 0.68	24.3 ± 1.1
Commercial 5	3.11 ± 0.18	2350 ± 240	0.0237 ± 0.0023	0.0400 ± 0.0049	103 ± 6.6	4560 ± 23	1100 ± 110	8.96 ± 0.56	27.2 ± 1.3
Commercial 6	4.49 ± 0.28	5610 ± 470	0.0360 ± 0.0032	0.0294 ± 0.0056	145 ± 7.8	10,900 ± 1300	6830 ± 1500	7.17 ± 0.50	40 ± 1.8
Commercial 7	1.83 ± 0.15	2590 ± 500	0.0307 ± 0.0042	0.0142 ± 0.0046	368 ± 18	1140 ± 150	1750 ± 140	1.78 ± 0.26	119 ± 5.1
Commercial 8	38.7 ± 2.32	5970 ± 470	0.0987 ± 0.0063	0.0327 ± 0.0059	235 ± 12	9860 ± 1200	2450 ± 310	10.4 ± 0.64	214 ± 9.9
Commercial 9	6.79 ± 0.34	1980 ± 180	0.148 ± 0.010	0.0302 ± 0.0048	253 ± 14	5510 ± 300	558 ± 46	8.81 ± 0.55	158 ± 7.0
Alternative 1	1.70 ± 0.08	1760 ± 210	0.204 ± 0.013	0.0520 ± 0.0047	91.2 ± 4.6	14,200 ± 650	44.9 ± 2.1	21.8 ± 1.5	45.7 ± 4.0
Alternative 2	2.41 ± 0.12	1140 ± 190	0.151 ± 0.012	0.0457 ± 0.0050	215 ± 13	11,400 ± 500	59.5 ± 2.8	21.7 ± 1.5	73.9 ± 7.7
Alternative 3	1.32 ± 0.06	629 ± 220	0.0093 ± 0.0012	0.156 ± 0.012	83.9 ± 5.4	8720 ± 540	13.5 ± 0.76	56.3 ± 2.8	54.4 ± 2.0
Alternative 4	0.96 ± 0.05	561 ± 180	0.0069 ± 0.0012	0.124 ± 0.0094	83.3 ± 4.8	7310 ± 440	13.5 ± 0.79	45.9 ± 2.2	48.4 ± 1.8
Alternative 5	6.91 ± 0.33	2734 ± 300	0.0434 ± 0.0075	0.259 ± 0.020	101 ± 5.9	9430 ± 400	644 ± 35	32 ± 1.8	48 ± 2.2
Alternative 6	4.04 ± 0.19	1120 ± 210	0.0540 ± 0.0046	0.268 ± 0.030	101 ± 10	8160 ± 830	28.5 ± 2.6	31.6 ± 2.1	51.9 ± 3.2
Alternative 7	4.50 ± 0.23	7740 ± 680	0.133 ± 0.0099	0.314 ± 0.028	123 ± 12	13,000 ± 1900	81.7 ± 9.2	40.9 ± 2.9	80.4 ± 4.9
Alternative 8	2.54 ± 0.12	3270 ± 430	0.0692 ± 0.0053	0.101 ± 0.0095	54.8 ± 5.0	4670 ± 450	27.2 ± 1.7	34.3 ± 2.3	26.9 ± 1.7
Alternative 9	5.88 ± 0.28	728 ± 230	0.394 ± 0.044	0.606 ± 0.084	141 ± 11.3	16,000 ± 2100	45.8 ± 5.7	204 ± 14	73.2 ± 4.5

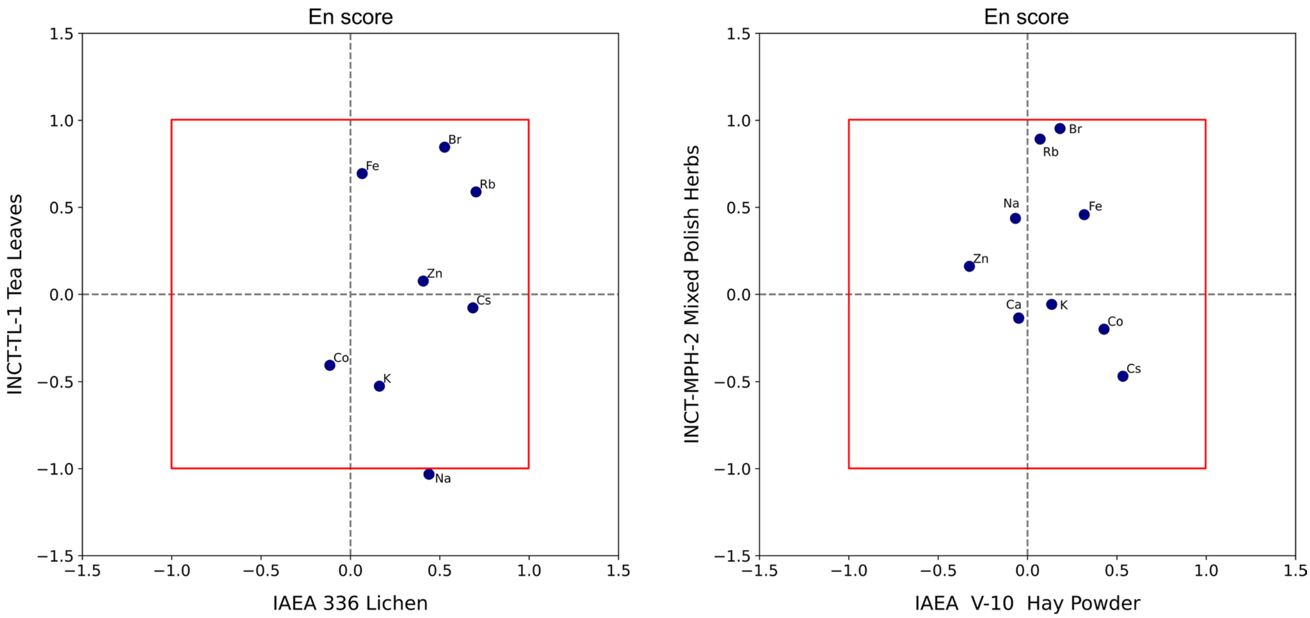

Fig. 1 En score for IAEA 336 Trace and Minor Elements in Lichen, INCT-TL-1 Tea Leaves, IAEA V-10 Hay Powder and INCT-MPH-2 Mixed Polish Herbs

Table 2 Mean mass of minerals (mg) and RDI (%) in 30 g of alternative and commercial supplements

	RDI 4–8 years	Alternative		Commercial	
Minerals	mg/day	mg/30 g	% RDI	mg/30 g	% RDI
Ca	1000	65.6	6.6	92.4	9.2
Co	0.005	0.0036	71.0	0.0014	28.5
Fe	10	3.32	33.2	4.83	48.3
K	2300	310	13.5	167	7.2
Na	1500	3.20	0.2	57.0	3.8
Zn	5	1.68	33.5	2.21	44.2

Cs, K and Rb, while Na content was much higher in commercial supplements.

The statistically significant differences between categories for Cs, K and Rb can be attributed to the intrinsic characteristics of agricultural byproducts used in manufacturing alternative supplements, since such ingredients are commonly derived from food processing, especially leaves and seeds rich in K, a fundamental macronutrient for plant development. K uptake in plants is positively correlated with the uptake of Cs and Rb [28]. Higher amounts of Na in commercial supplements are expected, as this element is an important preservative used in the food industry [29].

Using the mass fractions of the nutritionally important chemical elements Ca, Co, Fe, K, Na and Zn, a hierarchical cluster was made by the Ward method, using Euclidean distances. The dendrogram of cluster can be seen in Fig. 2.

In the cluster, considering 4 groups, the Silhouette index was equal to 0.48, indicating that the points of the groups are close to each other and that there is no overlap of groups. The Davies-Bouldin index was equal to 0.60, indicating that the groups formed are different.

The first group was formed by 10 samples, being 5 alternatives and 5 commercials, the second was formed by 4 samples of alternative supplement, the third by 3 samples of commercial supplement. A commercial supplement sample was not grouped with any others. The V-measure index of this cluster was 0.33. Thus, 55% of alternative and commercial supplements are nutritionally closer to supplements in the other category than to others in the same category. These results indicate that the alternative and commercial categories cannot be considered different.

With the elemental profile of 23 agricultural by products used to produce alternative supplements, a matrix of Euclidean distances was calculated to verify which agricultural byproduct is chemically similar to each alternative supplement. Wheat bran and wheat flour appeared among the 6 chemically similar ingredients for 7 of the 9 alternative supplement samples. The seed mix powder (pumpkin, watermelon and sunflower) was another ingredient that appeared among the 6 most similar ingredients for 6 of the 9 samples, followed by the sesame seed that appeared as similar to 2 samples. These seeds are normally added to enrich the alternative supplements. Rice-derived ingredients were also among the 6 chemically similar for 8 samples. Therefore, rice or wheat appeared among the 6 most

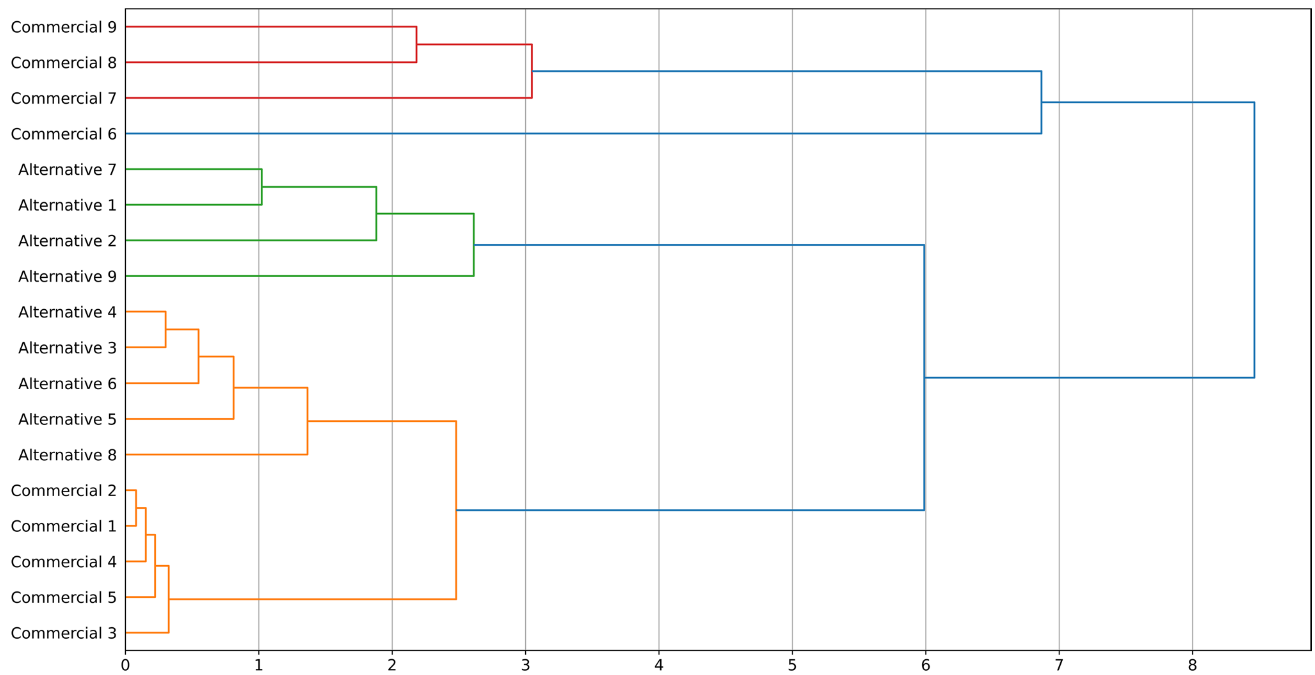

Fig. 2 Dendrogram of hierarchical cluster with alternative and commercial supplements

similar ingredients to all alternative supplements, confirming that they are made from one of these two grains, or even a mixture of both.

Conclusions

It was possible to demonstrate that alternative supplements prepared with agricultural byproducts have nutritional potential similar to those commercially available. In view of the increase in hunger and malnutrition in the post-Covid19 world, dietary supplements made from agricultural byproducts, especially those resulting from industrial food processing that would be discarded, can play a relevant socioeconomic role contributing to improved nutrition of children for the reduced cost and ample accessibility.

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Declarations

Competing interests All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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