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Antioxidant-rich foods and nutritional value in daily kindergarten menu: A randomized controlled evaluation executed in Slovenia

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ABSTRACT

Little is known about Total Antioxidant Capacity (TAC) of daily kindergarten menus. The objective of the present study was to determine whether, antioxidant-enriched kindergarten menu had, compared to a standard one, more optimal proximate composition, energy value and higher antioxidant capacity of free and bound dietary antioxidants. Antioxidant-enriched kindergarten meals, on average, contained significantly more vegetables, nuts, and whole grain foods ($p < 0.05$) and the average proximate composition and mineral content were more consistent with Dietary Reference Intake (DRIs). Additionally, in antioxidant-enriched vs standard daily meals, average TAC was 1369 mg vs 586 mg vitamin C equivalent (determined by 2′-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid] radical scavenging capacity (ABTS) assay) and 1734 mg vs 810 mg vitamin C equivalent determined by ferric reducing antioxidant power (FRAP) assay. Our study shed light on free and bound antioxidants in daily kindergarten meals and highlighted that supplementing kindergarten meals with foods rich in antioxidants can significantly improve dietary intakes.

1. Introduction

Multiple studies suggest that a high intake of dietary antioxidants has many beneficial effects on children's health related to obesity (Rendo-Urteaga et al., 2014), allergic diseases (Gref et al., 2017) and lung function (Sdona et al., 2020). Due to the high prevalence of overweight and obese children, even among preschool children (Garrido-Miguel et al., 2019), there has been a growing tendency in recent years to improve kindergarten nutrition (Kovacs et al., 2020).

Children in the European Union (EU) spend up to 45 h per week in kindergartens (European Commission/EACEA/Eurydice, 2019), consuming up to 75 % of their energy and nutrient requirements in full-day programmes. When it comes to children aged 4–6 years, Europe is characterised by almost 95 % kindergarten enrolment (European Commission/EACEA/Eurydice, 2019), which gives us an exceptional opportunity to foster good eating habits in children by providing them with proper kindergarten nutrition, and thus contribute to better health later in life (Kovacs et al., 2020). For all these reasons, it is very important to pay more attention also to antioxidants when planning for meal times in kindergarten programmes.

As antioxidant compounds can be chemically very diverse, their separation and individual quantitation represents a big challenge; therefore, the concept of Total Antioxidant Capacity (TAC) was introduced, primarily to explore whether a protective role of a certain plant food correlates with their antioxidant content (Pellegrini et al., 2020).

The methods used to evaluate antioxidant activity are based on different mechanisms and may lead to non-comparable results (Apak et al., 2013; Cömert & Gökmen, 2017, Pellegrini et al., 2020). It is necessary to realise that the complex structure of food, together with the place where the antioxidants are located and their solubility, affects the efficiency of extraction. Proper assessment of antioxidant capacity requires adequate hydrolysis to release insoluble antioxidant compounds

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and multi-step extraction to allow them to exert their antioxidant activity *in vitro* (Cömert & Gökmen, 2017). Since there is no universal test, the use of at least two different methods is recommended (Apak et al., 2013).

Often used assays are the 2′-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) assay, which is based on the ability of the antioxidants to capture and decolorize $ABTS^{+\bullet}$ and the ferric reducing antioxidant power (FRAP) assay, which is based on the ability of the antioxidants to reduce the ferric complex in a ferrous complex at low pH (Apak et al., 2013). Other commonly used assays comprise measurement of oxygen radical antioxidant capacity using different fluorescent probes (ORAC), measurement of total radical-trapping antioxidant parameter (TRAP), inhibition of lipid peroxidation, measurement of 2,2-diphenyl-1-picrylhydrazyl (DPPH), measurement of Folin–Ciocalteu's phenol reagent reducing capacity, and the cupric reducing antioxidant capacity (CUPRAC) assay (Pellegrini et al., 2020). To elucidate the relationship between antioxidant activity and chemical content multivariate techniques such as principal component analysis (PCA) may be applied (Fernandes et al., 2021).

Antioxidants in food microstructure may be present in two different forms: 1) free or soluble antioxidants (e.g. vitamins C and E, carotenoids, low-molecular-weight polyphenols) that are completely or partially absorbed either in the stomach or in the small intestine and 2) bound or insoluble antioxidants that are present in complex foods and are mostly embedded in the matrix and linked to various macromolecules such as polysaccharides, proteins and lipids, and in particular dietary fibre (high-molecular-weight polyphenols). Bound antioxidants constitute an important part of the dietary antioxidants as they reach the colon almost unchanged where they are released from the matrices and absorbed. Cömert and Gökmen revealed that free antioxidant compounds ingested with food prevent oxidative stress, but their beneficial effects are rather short-lived, while bound antioxidant compounds exert their antioxidant effects much longer, thus playing an important role in reducing local oxidative stress and protecting the gastrointestinal tract from disease (Cömert & Gökmen, 2017).

Nutritional studies as well as studies on the chemical properties of foods highlight the importance of the antioxidant properties of food that were, however, in the past often underestimated. In the tissues of fruits, vegetables, legumes and seeds, bound antioxidants represent 20–60 % of all phenolic compounds whose impact on human health should not be underestimated (Çelik & Gökmen, 2022).

The TAC data of individual raw foods are available from many studies (Carlsen et al., 2010; Pérez-Jiménez et al., 2015), while studies evaluating the antioxidant properties of complex and thermally processed foods are scarce (Durazzo et al., 2017; Saura-Calixto et al., 2007). To the best of our knowledge, there are no studies available that would provide reliable data on the antioxidant capacity of free and bound antioxidants of any kindergarten or other institutional whole diet. We found a similar study (Medoua et al., 2009), determining only the content of free antioxidants while failing to address bound antioxidants.

In this study we, according to Guidelines for Healthy Eating in Educational Institutions (Guidelines) (Gabrijelčič Blenkuš et al., 2005), created a prototype kindergarten 5-day menu (breakfast, lunch and two snack), in which we deliberately included foods high in antioxidants (selected vegetables, fruits, nuts, whole grains and oils) (Carlsen et al., 2010). The ease of transfer to other kindergarten environments was also taken into consideration when designing the menu. Prototype meals were compared with standard kindergarten meals, in addition to the content of free, bound and total antioxidants, also from the perspective of energy, nutrients and minerals content. Our hypothesis was that with the prototype menu, we can significantly increase the TAC of daily kindergarten meals, as well as improve the energy content and nutritional value of meals.

Studies dealing with TAC, proximate and mineral composition of kindergarten nutrition are rare, especially studies that include data obtained through chemical analyses. Therefore, the main objective of our study was to obtain and compare these data in differently designed kindergarten meals, within the same country and with the same nutritional guidelines, to assess whether there is a significant difference. The advantage of the study is that the whole daily kindergarten menus, composed of four meals, were taken into account and not just a single meal, which is the main research objective in most other published studies. Also, the antioxidant-enriched prototype menu can be easily transferred to other kindergarten environments. We believe that the greatest value of the study will be the first-time revealed data on the content of free and bound antioxidants in the all-day kindergarten diet, which will facilitate for kindergartens to plan and assess the quality of meals. In addition, results could contribute to more specific recommendations in national Guidelines.

The weaknesses of this research are the relatively small number of participating control kindergartens, meaning that the results may not reflect the reliable data on differences between prototype meals and standard meals on national level, and some limitations with antioxidant assays. Limitations at antioxidants assay relate to concerns that the concentrations of antioxidants tested in antioxidant assays are usually higher than *in vivo*, the chemistry is unclear and assays are not standardized (Schaich, et al., 2015).

2. Materials and methods

2.1. Study design

The research is part of a larger study that was registered at ClinicalTrials.gov (NCT04252105) and examines the effect of antioxidant-rich kindergarten diet on oxidative stress in healthy children. Due to the amount of data obtained in the study, in this manuscript we present just comprehensive data on the nutritional quality of organized kindergarten nutrition, including data on the content of free and bound antioxidants, which in our opinion importantly contribute to filling a gap on this field.

Ethical approval was obtained from the Commission of the Republic of Slovenia for Medical Ethics (No. 0120-66/2019/8).

We invited 6 kindergartens with own kitchens to participate in the study, 3 from the Zasavje Region and 3 from the Central Slovenia Region, to avoid biases resulting from possible differences in meal preparation. We informed the management team of each kindergarten about the details and obligations required by the study protocol. The inclusion criteria included written consent to voluntary participate in the research and a commitment to fulfil all obligations without a request for material compensation.

After obtaining approval, we randomized the invited kindergartens in each region into two groups. Randomization was made in Microsoft Excel (2013) with the RANDBETWEN function to select a control kindergarten in the region. The VLOOKUP function found the name of the randomized kindergarten. The first group included two kindergartens from each of the two regions ($n = 4$) and was given a plan of a 5-day (Thursday to Wednesday, without weekend) antioxidant-enriched prototype menu (the prototype group). The second group included one kindergarten from each region ($n = 2$) preparing food according to the standard menu (the standard group) and in accordance with the Guidelines (Gabrijelčič Blenkuš et al., 2005).

We did not specifically consider the costs of food and ingredient purchases, since in Slovenia the municipalities that establish kindergartens determine the maximum acceptable cost of purchasing food per month/child. For meals prepared in this study, the approved cost for food purchase was not exceeded in any of the participating kindergartens.

Daily kindergarten meals samples were collected in spring of 2020.

In this study, we use two terms – daily menu and daily meals. The daily menu is a list of foods and dishes that cooks must prepare for each meal each day. Daily meals are a composite of all individual foods and dishes from the menu in the mass that were actually offered to one child in a single day spent in kindergarten.

2.2. Prototype menu

Nutrition in Slovenian kindergartens is systemically regulated. Children do not bring food from home. All meals are cooked and prepared in kindergarten kitchens. When planning for meal times, Slovenian kindergartens are obliged to follow the Guidelines (Gabrijelčič Blenkuš et al., 2005), which stipulate that kindergartens must offer 4 meals per day (breakfast, lunch and two snacks), which are served within the 11-hour opening of the kindergarten (mostly from 8 am to 3 pm). The 5-day antioxidant-enriched prototype menu was designed by considering the recommendations regarding the inclusion of main food groups and recommendations concerning the inclusion of less appropriate foods (foods high in sugar and fat) (Gabrijelčič Blenkuš et al., 2005). Prior to its use, the prototype menu was also evaluated using the online dietary assessment tool OPEN (https://opkp.si/), which serves as the National Food Composition Database (Korošec et al., 2013). The menu included some of the foods that are most common and popular among children (spreads, pasta, stews), which were combined with foods with high antioxidant capacity (e.g. berries, dried fruits and nuts, broccoli, kale, tomatoes, red cabbage, whole grains, olive oil, lyophilized herbs and spices) (Carlsen et al., 2010). Although the 5-day menu was the same for all 4 "prototype" kindergartens, individual dishes were prepared according to their own recipes, using mostly staple foods. Foodstuffs were purchased through established routes, namely from the existing contractual suppliers of each kindergarten. The entire prototype menu, containing all dishes and recommended serving sizes, in line with the required percentage of energy needs, is listed in Table 1. The 5-day menu started on Thursday and ended on Wednesday of the following week. The prototype antioxidant-rich menu was designed with the aim of being easily transferred to other kindergarten environments. Some selected dishes or food ingredients used in this work are characteristic of Slovenian cuisine, but they could easily be replaced by other types of fruit, vegetables, whole grains, and nuts, which are recognised as foods with high antioxidant activity (Carlsen et al., 2010). In this context, it is important to bear in mind that diversity is a key determinant of dietary intake throughout the course of life, as it is mostly associated with better nutritional value and food quality (Embling et al., 2021).

The antioxidant-rich prototype menu was well accepted by the children, which was expected, as it does not include foods or dishes they are not familiar with.

2.3. The two standard menus

To reduce the possible risk of bias arising from the fact that kindergartens were aware of their participation in the study, the kindergartens with a standard diet were asked to use last year's menu for the duration of the study. Both standard menus – from Zasavje and Central Slovenia regions – are reported in Table S1 and Table S2.

2.4. Recipes and food groups

All food ingredients were cleaned, peeled, and weighed in grams on a calibrated professional kitchen scale before use. For each raw food or dish served to the children (a dish consisting of several different ingredients), the kindergartens provided a precise recipe, specifying exact quantities of all ingredients (including water) and the information on the number of meals prepared ($n = 129$). Brand names of all foods and beverages used to prepare daily meals were also provided. Two cooking techniques most commonly used in preparing dishes were cooking and baking. Breakfast spreads were prepared by mixing different ingredients in a cutter. On the basis of these data and by means of the OPEN dietary assessment tool (Korošec et al., 2013), the dishes were classified into five main food groups (meat and its substitutes, milk and dairy products, vegetables, fruits, and starchy foods). For our study, starchy foods were separated into whole grain foods and refined foods. For the time being, the OPEN tool does not provide the possibility of separating starchy foods into whole grain and refined starchy foods, so we classified starchy foods manually. Grain foods were classified as whole-grain products only if they contained at least 30 % whole grain ingredients in overall product. Potatoes were classified as refined foods due to their low dietary fibre content ($\leq$6 g) (Jones et al., 2020). Legumes were not dealt with separately, they were classified as vegetables.

2.5. Meal sampling

For 5 days, we took duplicate samples of daily meals prepared in each individual kindergarten; therefore, the final number of collected samples was 60 (40 prototype meals and 20 standard meals). Each daily composite sample (in two replicates) was placed into a separate container, and comprised all edible raw foods as well as prepared/heat-treated dishes that were on the menu that day, in the amounts corresponding to the suggested portion size for one child (Table 1). The samples of heat-treated dishes were taken directly from the boiling pan

Table 1
Prototype of 5-day kindergarten anti-oxidant enriched menu, including the recommended serving portions (g/child) and approximate energy intakes by individual meals.

	Breakfast 20 %[a]	Morning snack 5 %[a]	Lunch 30–35 %[a]	Afternoon snack 15 %[a]
1st DAY Thursday	hot cocoa (180 g), wheat germ bread (60 g), cottage cheese spread with chives (30 g), slice of apple (20 g)	cherry tomatoes (50 g), radish (50 g)	beef soup (170 g) with noodles (50 g), beef (60 g), sauté potatoes (130 g), steamed broccoli (25 g) and cauliflower (25 g), red cabbage salad (50 g)	blueberries (100 g), buckwheat bread with walnuts (40 g)
2nd DAY Friday	herbal tea with honey and lemon (150 g), spelt bread (70 g), egg spread with vegetable and chicken breast salami (30 g), orange pepper (50 g), slice of apple (20 g)	kiwi (50 g), raspberry (50 g)	kale stew with chicken and quinoa (260 g), rye bread (30 g), yogurt panna cotta (80 g) with blueberry sauce (50 g), 100 % orange juice (120 g)	spelt bread roll (50 g), red pepper (50 g), black olives (6 g), cheese (20 g)
3rd DAY Monday	millet porridge with milk (280 g), cinnamon and ground chocolate topping (3 g), slice of apple (20 g)	dried cranberries (15 g) and plums (25 g), walnuts (15 g)	broccoli soup (188 g), whole grain spaghetti (130 g), tuna in tomato sauce with pine nuts and basil (147 g), green salad (50 g) with pomegranate (5 g)	apple sauce with cinnamon and cloves (130 g), whole grain breadsticks (grissini) (20 g)
4th DAY Tuesday	peppermint tea with honey (150 g), sesame bread roll (70 g), chicken breast salami (15 g), edam cheese (15 g), tomato (50 g), slice of apple (20 g)	strawberries (100 g)	barley stew with turkey (260 g), rye bread (20 g), buckwheat cubes with dark chocolate and apples (80 g), lemonade (140 g)	yogurt (100 g) with raspberry puree (50 g) and ground hazelnuts (20 g), multigrain bread (20 g)
5th DAY Wednesday	cereal coffee with milk (160 g), whole grain bread roll (60 g), dried cranberries (30 g), walnuts (30 g), slice of apple (20 g)	blueberries (100 g)	tomato soup (199 g) with noodles (49 g), baked brown rice with chicken and vegetables (203 g), parmesan cheese (5 g), beetroot salad (59 g)	nuts bar (42 g), 100 % pomegranate juice (150 g)

[a] Distribution of recommended energy intake throughout the day by individual meals (Gabrijelčič Blenkuš et al., 2005).

in which they were prepared (in each kindergarten for a minimum of 150 meals). Other dishes such as fruit, raw vegetables, spreads, bread, and beverages were also placed in a container, in a form suitable for consumption (without peels, seeds, packaging, etc.). The composite samples were stored in a cooling bag at the maximum temperature of 8 °C and taken to an analytical laboratory in the same day. There they were weighed and homogenized in a blender. The average wet mass of a daily prototype meal was 1051 g ± 145 g (mean ± SD), and 836 g ± 125 g (mean ± SD) of a standard daily meal. Each homogenized composite sample was divided into aliquots. Aliquots used for determining TAC were lyophilized and stored at −20 °C until analysis. Aliquots used for determining total dietary fibre were stored intact at −20 °C. Aliquots used for determining protein, fat and selected elements were dried at 50–60 °C to approx. 6 % water content, then milled and also stored at −20 °C until analysis.

2.6. Measurement of proximate composition and total energy values

The proximate composition of each daily kindergarten meal, in the amount for one child, was performed through triplicate determinations of proximate and minerals compositions, no later than two months after collection. All analyses were carried out according to the AOAC methods (2002). Protein was determined by the Kjeldahl method using 6.25 factor for nitrogen to protein conversion (AOAC 981.10). For total fat evaluation, the Weibull-Stoldt method was employed (AOAC 963.15). Dietary fibre was determined according to the enzymatic–gravimetric AOAC method 991.43. For the determination of selected elements, samples were ashed at 550 °C for five hours. The ash was dissolved in 3 ml HCl (25 % v/v), filtered through filter paper (Whatman Grade 589/1, Darmstadt, Germany) and diluted to 50 ml with deionized water (Milli-Q® IQ 7003, Merck, Darmstadt, Germany). The content of Ca, Mg, Na, K, Fe, Cu, Mn and Zn was measured by atomic absorption/emission spectroscopy (AOAC 999.11). A flame atomic absorption spectrometer (Aanalyst200, PerkinElmer, Waltham, Massachutes, USA) was used to perform the measurements. The experimental conditions for each mineral analysed (wavelength, gas mixtures, and flow rates) were set according to the manufacturer's specifications (PerkinElmer Aanalyst200 User Manual). The instrument was calibrated daily using individual external standard solutions (Inorganic Ventures, Christiansburg, Virginia, USA). If the measurement results were outside the range of the calibration curve, the test solutions were diluted accordingly and the measurement was repeated.

Available carbohydrates were calculated by difference. Energy values are expressed in kilocalories (kcal) and have been calculated using energy conversion factors. We collected data on the average nutritional value and mineral composition for each individual daily kindergarten meal from all 6 kindergartens and classified them as either data of the prototype or the standard group. The average results of both groups were compared with the DRI for children aged 4–6 years (NIJZ, 2020).

2.7. Estimation of total antioxidant capacity (TAC)

The total antioxidant capacity of the daily meal samples was determined according to the methods described by Durazzo et al. (Durazzo et al., 2017), with some modifications. The antioxidant capacity of the free and bound antioxidants of the sample extracts was evaluated using the FRAP (Ferric Reducing Antioxidant Power) and ABTS (2′-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]) radical scavenging assays. All analyses were carried out in triplicate. All procedures are described in detail in the Supplementary material. Antioxidant capacity was expressed as vitamin C equivalent (VCE) per daily meal to enable an easier comparison between both assays and between free and bound antioxidants. Vitamin C equivalent represents the amount of vitamin C in mg that gives the same antioxidant assay response as a particular sample of a daily kindergarten meal when the assay is performed under the same experimental conditions.

2.8. Statistical analysis

Variables are expressed as mean with standard deviation (SD). The Shapiro-Wilk test of normality was used to check the normal distributions of the data. Levene's test for homogeneity of variances was used to assess the equality of variances. Since the parametric analysis was possible, the independent samples Student test was performed to compare parameters between the experimental and control group. Otherwise, where the parametric analysis was not possible, the independent samples Mann–Whitney *U* test was applied to evaluate significant differences between groups. All two-tailed p values were presumed statistically significant when the values were below 0.05. All statistical analyses performed were done using R version 4.0.5 programming language.

3. Results and discussion

3.1. Food groups, proximate composition and mineral content in daily kindergarten meals from prototype and standard menus

In this study, we analysed daily kindergarten nutrition from 6 kindergartens in Slovenia in the period from Thursday to Wednesday (5 days). Kindergartens were randomized in two groups – prototype ($n = 4$) and standard ($n = 2$). All results are expressed as weight per daily fresh kindergarten meal offered per child and compared with the official Slovenian DRI (NIJZ, 2020) for a moderately active child aged 4–6 years. Because in our case it is important what the differences between meals are in relation to the daily kindergarten offering, presenting the results in g/100 g seemed less meaningful.

Table 2 provides insight into what average part of selected food groups represented daily meals of prototype and standard menus per day and also in 5-day average. At first glance, on average there were no major differences between menus, which was expected, as all meals have to be planned according to the Guidelines. The total supply of fruit and vegetables was 331 g in the prototype group vs 300 g in the standard group, but the prototype menu placed more emphasis on a sufficient amount of vegetables, including raw vegetables, while the standard menu gave prominence to fruit intake.

By comparing the 10 most commonly consumed vegetables and fruits in the United States, it has been established that vegetables are a better source of minerals such as potassium, copper, magnesium, iron and manganese, dietary fibre and numerous vitamins, while fruits contain a high antioxidant content (Offringa et al., 2019). Both groups also offered a similar amount of starchy foods (211 g in prototype vs 223 g in standard), with the prototype menu including significantly more whole grain foods (wholemeal bread, quinoa, barley, brown rice, etc.), while the standard menu offered significantly more refined foods on average (polished rice, refined pasta, white bread, white buns) and potatoes. In order to attain optimal health, it is recommended that at least half of the starchy foods be of whole grain origin (Jones et al., 2020), which was achieved within the prototype menu. In addition, children in the prototype group were also provided with nuts, whereas this was not the case for the standard group.

In the prototype menu, the greatest progress compared to the standard menu was expressed in a significantly higher offer of vegetables, whole grain foods and nuts, which are frequently referenced in the literature as food with higher biological value and greater beneficial health effects (Jones et al., 2020; Ros, 2017; Wang et al, 2012). The average portion size offered to children in kindergartens with the prototype menu was 982 g/day, compared with 814 g/day in kindergartens with the standard menu ($p < 0.05$). The difference is mainly due to daily supply of vegetable soups and the occasional supply of 100 % fruit juice, which were basically not offered in the standard group. The differences in the planning of kindergarten menus in terms of including foods with a

Table 2

An average daily and 5-day amount of food groups offered by kindergartens and their share (%) to total amount in a daily kindergarten prototype (n = 4) and standard (n = 2) meal.

		Food group[a]							Other food g (%)[c]	Total amount g (100 %)
		Meat and meat product g (%)[c]	Milk and dairy product[d] g (%)[c]	Fruit g (%)[c]	Vegetable g (%)[c]	Whole grain food g (%)[c]	Refined grain food and potatoes g (%)[c]	Nuts[e] g (%)[c]		
	RI[b] 4–6 y. (g)	115	215–400	200	300	150	150	8		
DAY 1	Prototype	75 (7.5)	194 (19.5)	139 (14)	175 (18)	110 (11)	141 (14)	4 (0.5)	154 (15.5)	990 (100)
	Standard	58.8 (7)	125.8 (14)	264.0 (30)	96.4 (11)	72.5 (8)	156.5 (18)	0	100.0 (12)	874
DAY 2	Prototype	82 (8)	88 (9)	142 (14)	233 (23)	122 (12)	55 (5)	0	294 (29)	1.014
	Standard	79 (9)	44 (5)	209 (24)	77 (9)	92 (11)	194 (23)	0	162 (19)	857
DAY 3	Prototype	63 (8)	165 (21)	57 (7)	151 (19)	195 (25)	12 (2)	13 (2)	131 (16)	786
	Standard	89 (12)	70 (9)	140 (19)	67 (9)	16 (2)	305 (40)	0	68 (9)	754
DAY 4	Prototype	116 (10)	167 (14)	206 (18)	128 (11)	133 (12)	149 (13)	21 (2)	237 (20)	1.156
	Standard	40 (6)	189 (27)	176 (25)	169 (24)	20 (3)	106 (15)	0	11 (1)	711
DAY 5	Prototype	57 (6)	164 (17)	173 (18)	251 (26)	127 (13)	13 (2)	31 (3)	148 (15)	963
	Standard	79 (9)	122 (14)	200 (23)	104 (12)	70 (8)	83 (10)	0	217 (25)	876
5-day average	Prototype[g]	78 ± 36 (8)	155 ± 60 (16)	143 ± 61 (15)	188*±66 (19)	138*±62 (14)	74 ± 71 (7.5)	14*±13 (1.5)	199 (19)	982*±162
	Standard[g]	69 ± 28 (8.5)	110 ± 91 (13.5)	198 ± 64 (24)	103 ± 58 (13)	54 ± 45 (6.5)	169*±108 (20.5)	0	112 (14)	814 ± 107
	p-value	0.482	0.114	0.233	0.001	0.001	0.016	0.003		0.001

[f]Other foods included beverages such as herbal tea, lemonade, juice and water used to make a vegetable soup (vegetable soup contains 35–50% of vegetables, the rest is water).

[*] Values in the same column of 5-day average amounts are significantly different with regard to different variables ($p < 0.05$).

[a] The food groups represent the groups used in dietary assessment tool OPEN and separated starchy foods into whole grain and refined grain – mean.

[b] RI – Recommended Intake per day for 4–6 years old children according to the Guidelines.

[c] % of each food group contributing to the total amount.

[d] The range is given because this group includes, in addition to milk, dairy products (e.g. cottage cheese), which are consumed in small quantities.

[e] Nuts – The recommended amount of daily nuts portion was calculated based on the doses recommended for adults, adjusted on children body weight (on average 0.43 g/kg of body weight).

[g] Mean ± SD of daily amounts of food regarding the group and type of menu.

more appropriate nutritional profile (whole grains, nuts, better fruit-vegetable supply ratio) were in the prototype group reflected in significantly more adequate proximate composition and mineral content, which was the case for all parameters except for carbohydrates and sodium (Table 3 and Table 4).

However, it should be emphasized that the antioxidant-enriched prototype menu is suitable only for the diet of healthy people, without hypersensitivity to individual allergens, such as nuts, eggs, milk, gluten, wheat, etc. Special care should be taken when providing nuts to children in kindergartens, as tree nut allergies are among the eight most common food allergies that may cause severe reactions (Weinberger & Sicherer, 2018). In addition, there is also a risk of suffocation, which should be considered in the mode of supply. In recent years, the prevalence of nut allergy in children under the age of 18 has increased from 0.2 % in 1997 to 1.1 % in 2008 (Weinberger & Sicherer, 2018). Nevertheless, it is highly recommended that healthy children, without allergies, consume nuts regularly, as there is strong evidence of their numerous health benefits (Ros, 2017).

We highlight the average amount of dietary fibre, which was 17.7 g in prototype meals and 12.4 g in standard meals. Providing a sufficient amount of dietary fibre is very important, as a recent study states that a higher fibre intake is associated with better glucose tolerance levels and lower cardiovascular risk (Larrosa et al., 2021). Larrosa et al. (2021) also state that children aged 4–6 years from Germany, Poland, Italy, Belgium and Spain consume on average 7.1 to 7.3 g of dietary fibre per 1000 kcal, while Finnish 5–6-year-old children consume on average 16.7 g of dietary fibre per day (Korkalo et al., 2019).

Furthermore, the content of all of the analysed minerals, except calcium, in the prototype menu is high, which seems encouraging, given that as many as 80 % of European children do not regularly consume sufficient amounts of iron, zinc and calcium (Zaragoza-Jordana et al., 2018). Excessive sodium content in all kindergarten meals has been found, which is also the result of excessive salt use in food preparation processes, as evidenced by the recipes of each kindergarten in our study.

3.2. Comparison of offered kindergarten meals with the DRI for 4–6-year-old children

The average daily contribution of energy, nutrients and minerals in prototype and standard kindergarten meals and their comparison with the minimum value in the range of DRIs for children aged 4–6 years is shown in Fig. 1. The results show that prototype daily kindergarten meals offer more adequate nutrition compared to standard daily meals, as they covered more than 66 % of DRIs for energy and macronutrients and standard meals roughly 44 % of DRIs. In the prototype meals, the average energy content was slightly below guidelines (68 %), which coincides with being low in fat (72 % of the DRI for the minimum recommendation) and low in carbohydrates (66 % of the DRI). Other macronutrients were in line with DRIs. In control meals, the content of most macronutrients (except carbohydrates) was significantly lower than in prototype meals (Table 3) and was less compliant with DRIs.

From Fig. 1 we can assume that the amount of protein in the prototype meals is higher than 75 % of the DRI, but in Slovenia the DRIs for some nutrients are set in a range. In Fig. 1, the black line presents the recommendation for 75 % of DRIs, taking into account the minimum value, although the highest recommended value, presented in Table 3, is also considered as an adequate intake. The energy and nutrient content of the prototype meals does not present a risk for developing obesity in children, as it only roughly corresponds to DRIs for intakes in kindergartens.

The content of manganese, magnesium, copper and zinc corresponded to more than 100 % of the DRI in all kindergartens' meals, and their content, with the exception of zinc, was significantly higher in prototype meals ($p < 0.05$). The content of all those minerals in kindergarten meals is not concerning, although it is higher than the maximum DRI, as either the content is much lower than tolerable upper intake levels, or the upper intake level cannot be set (European Food Safety Authority, 2006). The significantly higher amounts of manganese, magnesium, iron and copper in the prototype group correlate with the significantly higher amounts of whole grains, nuts and vegetables in prototype meals (Table 2), which are the main sources of those minerals. The content of zinc is adequate in kindergarten meals from both groups, and does not differ significantly, which is probably caused by the similar amount of meat in the meals (Table 2). The iron content in the prototype meals complies with the DRI, while the iron content in the standard meals is insufficient. The results correlate with the results of Table 2, since, in addition to meat, the main source of iron is whole grain foods, the amount of which is significantly higher in prototype meals. Due to the common occurrence of insufficient supply, special attention should be paid to the rich sources of iron in preschool menus (Myszkowska-Ryciak & Harton, 2018).

In contrast, the average calcium content was below the DRI. On average, antioxidant-enriched prototype meals, compared to standard meals in our study and kindergarten meals from some other studies (Đermanović et al., 2017; Myszkowska-Ryciak & Harton, 2018), contained more calcium, but still insufficient. As a sufficient amount of calcium is necessary for normal and healthy growth and maintenance of the skeleton, especially in childhood (Abrams, 2021), kindergarten menus would need to be corrected by more frequent inclusion of calcium-rich foods (parmesan, hard cheese, sardines, etc.) or calcium-fortified non-dairy products.

The sodium content in all kindergarten meals significantly exceeded the recommended minimum DRI; moreover, it exceeded the amount that represents a safe daily intake (1.3 g/day). The American Heart Association reports that sodium intake of >2.0 g/day is associated with 6.3 % of all CVD deaths in the United States, 9.5 % of all CVD deaths globally, and 13.1 % of CVD deaths in the United States before the age of 70 (Mozaffarian et al., 2016). Furthermore, although kindergarten meals in prototype and standard groups contained more potassium than recommended, the sodium/potassium ratio is too high, as many studies show that the ratio of potassium to sodium intake is a greater risk factor for cardiovascular disease than sodium alone (Levings & Gunn, 2014). While sodium and potassium are naturally present in a variety of foods, more than 75 % of sodium intake is from sodium added to processed foods (Levings & Gunn, 2014). The obtained results indicate the urgent need to implement an effective policy to control these risk factors.

3.3. Total antioxidant capacity (TAC)

In order to obtain representative and reliable TAC data for the studied daily meals and minimize bias, two different analytical tests based on different antioxidant mechanisms were used, namely reduction of Fe^{3+} to Fe^{2+} (FRAP) and free radical scavenging (ABTS). Very often the radical scavenging activity is preferably being determined by means of a DPPH assay, but acid mixtures were not suitable for the DPPH assay here, since DPPH reagent showed optimal stability between pH 4 and 8. These results clearly show that the use of DPPH in the presence of acid reaction mixtures may lead to false positive results. The acid present in the solvent may in fact influence the ionisation equilibrium as pointed out by Ferri et al. (Ferri et al., 2013). A direct comparison of TAC values between bound and free antioxidants can only be made when these values are obtained by using the same method and under the same analytical conditions, using the same solvents. Hence, precedence was given to the ABTS assay, which is insensitive to pH fluctuations.

We determined the antioxidant activity of free antioxidants from the aqueous-organic extracts of whole daily meals. The solid residue from these extractions was used for determining the antioxidant activity of bound antioxidants after hydrolysis. It is these solid residues that make a considerable and, in some cases, even a major contribution to TAC, even though they might not always get the attention they deserve (Saura-Calixto et al., 2007).

Table 5 presents FRAP and ABTS values of free, bound and total

Table 3
The average daily and 5-day energy content and proximate composition of meals (breakfast, lunch and 2 snacks) prepared by kindergartens within the prototype (n = 4) and standard (n = 2) menu.

	Menu	E [c] kcal	Fresh meal weight g	Moisture g/DM [d] (%)	Ash g	Total fat g	% Fat/DM	Protein g	% Protein/DM	Dietary fibre g	CH[e] g	% CH/DM
	DRI[a] 4–6 y. (75 % DRI)[b]	1550 (1162)				52–60 (39–45)	30–35 % of E	39–58 (29–44)	10–15 % of E	> 15 (> 11)	>194 >146	> 50 % of E
DAY 1	Prototype	827	988	797(80)	9.2	26.6	28	42.4	21	18.23	94.7	46
	Standard	950	826	614(74)	7.7	30.1	28	35.5	15	9.44	128.4	55
DAY 2	Prototype	960	1036	818(79)	9.4	30.8	28	40.7	17	16.46	120.5	51
	Standard	816	869	685(79)	7.0	24.5	26	32.3	16	9.55	110.6	55
DAY 3	Prototype	1000	937	719(77)	8.0	37.0	32	33.1	14	14.32	125.8	51
	Standard	754	833	656(79)	7.7	20.7	25	32.2	17	14.53	101.5	54
DAY 4	Prototype	1137	1184	929(78)	9.7	38.28	29	44.9	16	19.92	142.2	51
	Standard	810	736	546(74)	7.6	20.19	22	31.9	16	13.45	117.1	58
DAY 5	Prototype	1330	1112	828(74)	11.5	55.22	36	41.7	13	19.60	156.1	48
	Standard	803	918	728(79)	8.3	20.21	22	30.3	15	15.22	116.1	59
5-day average	Prototype[f]	1051*±229	982*±162	818*±113	9.6*±1.8	37.6*±14.6	31	40.6*±6.6	16	17.7*±4.2	128 ± 29	50
	Standard[f]	827 ± 108	814 ± 107	646 ± 1178	7.7 ± 1.2	23.2 ± 6.3	25	32.5 ± 2.9	16	12.4 ± 3.2	115 ± 19	56
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001		<0.001	0.077	

[*] Values in the same column of 5-day average amounts are significantly different with regard to different variables ($p < 0.05$).
[a] DRI – Dietary Reference Intake per day for 4–6 years old children with PAL (Physical Activity Level) 1.6 (Moderately Active).
[b] 75% – DRI for time spent in kindergarten.
[c] E – energy.
[d] DM – daily meal.
[e] CH – available carbohydrate.
[f] Mean ± SD: Mean of 5-day daily kindergarten samples. Two daily menus were sampled every day in each kindergarten and analysed in triplicate.

Table 4
The average daily and 5-day mineral content of meals (breakfast, lunch and 2 snacks) prepared by kindergartens within the prototype ($n = 4$) and standard ($n = 2$) menu.

	Menu	Ca (mg/DM[c])	Mg (mg/DM[c])	Mn (mg/DM[c])	Fe (mg/DM[c])	Cu (mg/DM[c])	K (mg/DM[c])	Na (mg/DM[c])	Zn (mg/DM[c])
	[a]DRI 4–6 y.	750	120	1.5–2.0	8.0	0.5–1.0	1300	500 -max 1180	4.0
	[b]75 % DRI 4–6 y.	563	90	1.1–1.5	6.0	0.4–0,8	975	(880)	3.0
DAY 1	Prototype	333.5 ± 25	166.4 ± 15	2.62 ± 0.3	7.56 ± 2.1	0.91 ± 0.1	2058 ± 138	1729 ± 292	7.71 ± 1.6
	Standard	270.6 ± 149	147.2 ± 19	1.79 ± 0.04	5.77 ± 1.8	0.64 ± 0.1	1838 ± 161	1495 ± 266	4.75 ± 1.6
DAY 2	Prototype	392.1 ± 50	178.0 ± 29	3.44 ± 1.1	6.69 ± 1.9	0.81 ± 0.2	1829 ± 240	1988 ± 471	4.74 ± 1.3
	Standard	171.4 ± 36	130.5 ± 5	1.97 ± 0.3	4.96 ± 0.6	0.57 ± 0.1	1351 ± 105	1567 ± 123	4.98 ± 0.4
DAY 3	Prototype	375.5 ± 73	203.2 ± 22	3.06 ± 0.6	7.72 ± 1.4	1.01 ± 0.2	1598 ± 197	1703 ± 258	4.94 ± 1.1
	Standard	201.7 ± 72	138.5 ± 28	1.82 ± 0.5	4.39 ± 1.4	0.65 ± 0.1	1834 ± 276	1485 ± 505	5.58 ± 2.2
DAY 4	Prototype	466.5 ± 37	224.4 ± 23	3.45 ± 0.3	8.16 ± 1.5	1.10 ± 0.2	1982 ± 111	2111 ± 372	5.56 ± 1.1
	Standard	358.7 ± 53	163.6 ± 38	2.01 ± 1.1	6.84 ± 2.1	0.74 ± 0.2	1771 ± 363	1497 ± 711	5.51 ± 0.2
DAY 5	Prototype	449.4 ± 88	248.6 ± 43	4.83 ± 0.9	6.48 ± 0.6	1.18 ± 0.2	2282 ± 240	2456 ± 730	5.08 ± 1.3
	Standard	305.6 ± 14	156.8 ± 9	1.80 ± 0.5	4.55 ± 0.4	0.69 ± 0.1	1812 ± 117	1704 ± 25	4.04 ± 0.9
5-day average	Prototype[d]	403.4*±74.7	204.1*±40.3	3.48*±0.99	7.32*±1.62	1.00*±0.20	1949*±295	1998*±516	5.61 ± 1.63
	Standard[d]	261.6 ± 99.5	138.8 ± 40.1	1.88 ± 0.54	5.30 ± 1.57	0.66 ± 0.11	1721 ± 278	1550 ± 376	4.97 ± 1.28
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.001	0.133

[*] Values in the same column of 5-day average amounts are significantly different with regard to different variables ($p < 0.05$).
[a] DRI – Dietary Reference Intake per day for 4–6 years old children.
[b] 75% – DRI for time spent in kindergarten.
[c] DM – daily meal.
[d] Mean ± SD: Mean of 5-day daily kindergarten samples. Two daily menus were sampled every day in each kindergarten and analysed in triplicate.

dietary antioxidants, separately for each day and on average in 5 days. Prototype meals, according to the ABTS assay, contained 4.3, 1.1 and 2.3 times higher TAC values. FRAP values were even higher, namely 5, 1.3 and 2.4 times. We assume that the higher TAC values in prototype meals reflected higher TAC of selected fruit, vegetables, grains and nuts used as staple food in recipes or as part of meals (dried fruit, blueberries, red beets, tomato soup, walnuts, nuts bar, pomegranate juice, etc.). At the same time, antioxidant properties of spices and herbs such as cinnamon, basil, cloves, pepper, thyme and extra virgin oil, which were used to prepare the meals in kindergarten with the prototype menu, are also well documented (Carlsen et al., 2010).

In the prototype meals, on average free antioxidants contributed significantly to higher TAC values of kindergarten meals (71 % and 51 %, determined by ABTS and FRAP assay, respectively), which can be explained by Table 2. The average daily contribution of fruit, vegetables and beverages (other food) to daily kindergarten diet is almost 60 % and antioxidants from these foods mostly come in free form (Cömert & Gökmen, 2017; Pellegrini et al., 2006). On the other hand, in the standard meals, it was bound antioxidants that made a major contribution to TAC levels (61 % and 79 % determined by ABTS and FRAP assay, respectively). Also, in the standard menu, the supply of total amount of fruit, vegetables and beverages was high at 51 %, but the choice of these foods did not place emphasis on foods rich in antioxidants. The higher contribution of bound vs free antioxidants in standard meals is in line with the study by Perez-Jimenez & Saura-Calixto (2015), which reports that fruit such as apples, bananas and pears contain more bound than free antioxidants (Pérez-Jiménez & Saura-Calixto, 2015), and these types of fruit were the most often offered in standard menus. It is interesting to notice the wide range of TAC values in the same meal, measured with different assays; in fact, bound ABTS vs FRAP value differed from 393 to 855 mg VCE/DM in prototype meals and from 361 to 638 mg VCE/DM in standard meals. This significantly bigger amount of bound FRAP values in prototype meals is likely due to the different phenolic compounds, which are high in nuts, spice extracts, wholegrain cereals and pulses (Pellegrini et al., 2006). Furthermore, the content of bound antioxidants may also be related to the average dietary fibre content, which was significantly higher in the prototype meals (Table 3). A recent study (Çelik & Gökmen, 2022) suggests that dietary fibres are the main carriers of insoluble antioxidants, indicating that approximately 95 % of known phenolic compounds found in cereals are linked to cell wall polysaccharides. The bio-accessibility of bound antioxidants is linked to the colon where antioxidants are slowly released from the fibrous matrix under the influence of the intestinal microbiota, contributing to the formation of a stable antioxidant environment that acts against continuously emerging radical species and prooxidants (Çelik & Gökmen, 2022). Studies show that free antioxidants cause a significant increase in plasma antioxidants immediately after they are consumed; however, the antioxidant levels also decline rapidly, whereas bound antioxidants help maintain plasma concentration stable over a longer period of time (Cömert & Gökmen, 2017).

High average TAC in the prototype 5-day menu, 1369 mg VCE/DM (ABTS assay) and 1734 mg VCE/DM (FRAP assay), suggests that using prototype kindergarten menus can have a protective effect on children's health. Wang states that higher dietary TAC (453–2428 mg VCE/day) is a good indicator of diet quality, as well as a predictor of plasma antioxidant status in healthy young adults (Wang et al., 2012). Several studies have also confirmed that a diet rich in antioxidants is associated with lower markers of oxidative stress and obesity, and reducing the risk of cancer and lung disease (Franzini et al., 2012; Rendo-Urteaga et al., 2014; Sdona et al., 2020).

The prototype menus were tested in 4 different randomly allocated kindergartens situated in two different statistical regions, while just one standard menu was analysed from each region. A possible disadvantage of the study was a somewhat limited number of kindergartens that participated in the research, so the results may not fully reflect the differences between prototype and standard meals. In the future, a larger pool of samples might provide more reliable data on the differences between prototype meals and standard meals with regard to their TAC. The TAC data obtained here from assays based on different mechanisms of antioxidant action (ABTS and FRAP) in general led to the

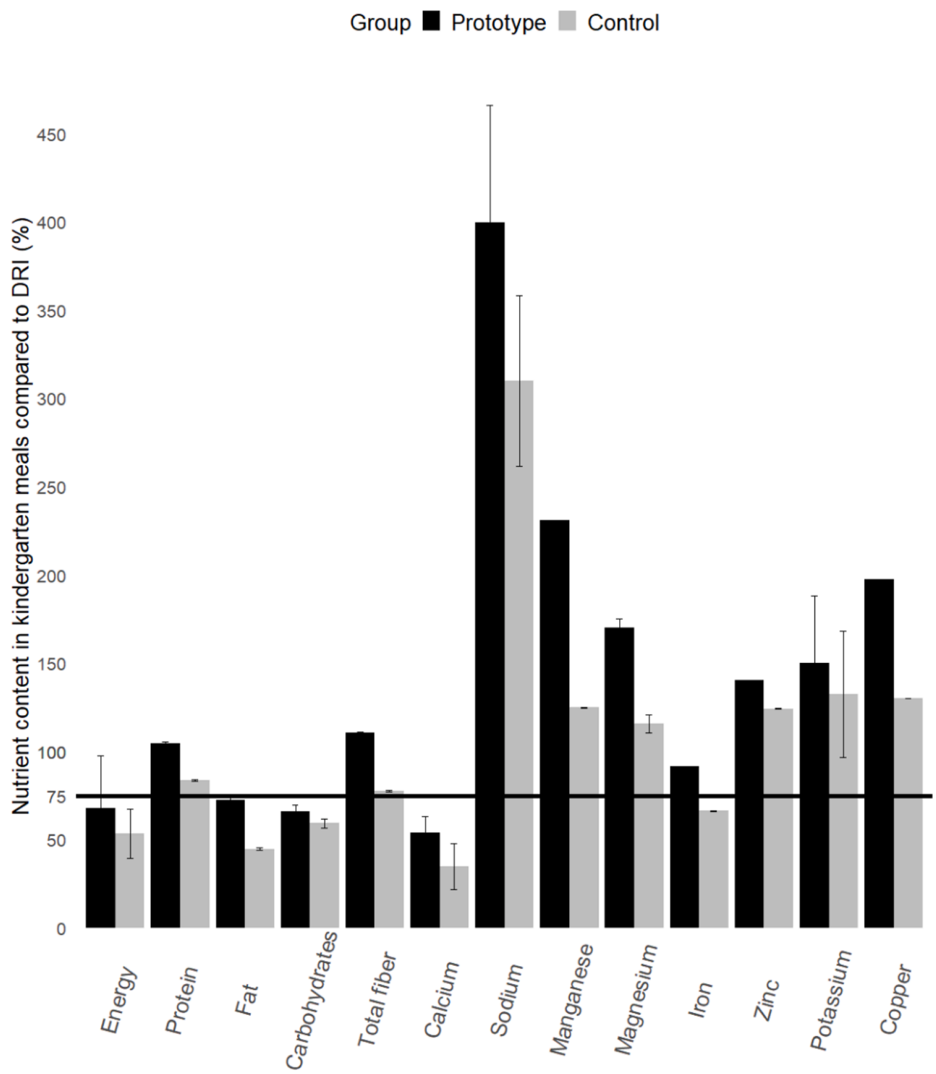

Fig. 1. Average energy and nutrient values for prototype and standard daily meals. Data source: chemical analysis of daily kindergarten meals (prototype group $n = 40$, control group $n = 20$). The black line represents 75 % of the recommended daily intake for each parameter. Error bars indicate 95 % CIs.

same conclusions. This indicates that both approaches could be considered fit-for-purpose.

The effects of cooking were taken into account, as samples of daily meals also included cooked dishes. However, the uptake of antioxidants from the gastrointestinal tract was not considered (neither *in vivo* nor *in vitro*). Nonetheless, as the extraction of bound antioxidants was performed at strongly acidic conditions, the effects of digestion could arguably be estimated as well, but a more suitable study design would be required to confirm such claims (e.g. uptake of major food antioxidants by Caco-2 cell lines, etc.).

Nonetheless, in some cases statistical differences were observed (e.g. in the case of bound antioxidants), which highlights the advantage of using a combination of two complementary methodologies for a reliable TAC evaluation of complex samples. On these occasions, further in-depth research is warranted to identify the source of observed discrepancies that could facilitate the avoidance of bias in future studies. The colorimetric assays used here are non-specific and since many foods are often characterized by heavy matrices, certain chemical interferences may positively or negatively contribute to the measurement output. Due to this reason, whole daily meals were analysed in this study for the first time to account for potential enhancement/suppression effects or other interfering effects from individual food components or chemicals that could affect the measurements and the interpretation of data. This also demonstrates a more relevant real-life scenario in contrast to TAC evaluation of individual foods or ingredients for which the data are in many cases already available in the literature or online databases (Sdona et al., 2020). For a more comprehensive and systematic determination of antioxidant activity of the studied meals (outside of the scope of this study), detailed information on the individual food antioxidants is needed, which requires additional time and high-resolution, high-throughput, and high-performance analytical techniques and methodologies, such as LC-MS.

The evidence from this study clearly shows that, although kindergarten menus are planned according to the Guidelines, without the inclusion of high-TAC foods the meals can be deprived of free antioxidants and to a lesser extent also bound antioxidants. At the same time, standard kindergarten meals in our study offered children a less adequate proportion of energy, nutrients and also some minerals.

The principal advantage of our study is that the 5-day prototype antioxidant-rich menu can be easily transferred to other kindergarten environments. Every dish or food ingredient could be replaced by other types of fruit, vegetables, whole grains, and nuts, which are recognised as foods with high antioxidant capacity and are characteristic of other national cuisines.

To the best of our knowledge, our study is the first one analysing free and bound antioxidants in daily kindergarten meals. The availability of this new and expanded information on TAC of a daily kindergarten diet is bound to facilitate further nutrition-related studies in all institutional

Table 5
TAC of prototype (n = 4) and standard (n = 2) daily fresh kindergarten meals, determined by ABTS and FRAP assays.

	Menu	ABTS mg VCE/DM[c]			FRAP mg VCE/DM[c]		
		Free[a]	Bound[b]	Total[a,b]	Free[a]	Bound[b]	Total[a,b]
DAY 1	Prototype	676 ± 219	404 ± 65	1080 ± 227	622 ± 138	785 ± 169	1407 ± 146
	Standard	200 ± 42	493 ± 133	693 ± 176	197 ± 56	832 ± 255	1029 ± 310
DAY 2	Prototype	511 ± 148	254 ± 70	765 ± 101	500 ± 94	633 ± 38	1133 ± 88
	Standard	426 ± 467	315 ± 97	740 ± 437	190 ± 40	610 ± 185	800 ± 149
DAY 3	Prototype	793 ± 349	279 ± 73	1071 ± 405	699 ± 292	645 ± 123	1344 ± 395
	Standard	159 ± 22	329 ± 190	488 ± 208	138 ± 22	511 ± 297	648 ± 316
DAY 4	Prototype	1029 ± 436	475 ± 101	1504 ± 502	886 ± 346	1113 ± 353	1999 ± 671
	Standard	160 ± 39	341 ± 159	500 ± 148	157 ± 41	620 ± 206	777 ± 186
DAY 5	Prototype	1878 ± 465	549 ± 115	2427 ± 53	1687 ± 339	1098 ± 116	2784 ± 449
	Standard	184 ± 21	328 ± 114	512 ± 134	182 ± 34	615 ± 218	798 ± 245
5-day average	Prototype[d]	977*±581	392 ± 139	1369*±69	879*±493	855*±276	1734*±74
	Standard[d]	226 ± 214	361 ± 144	587 ± 246	173 ± 42	638 ± 235	810 ± 255
	p-value	<0.001	0.491	<0.001	<0.001	0.011	<0.001

[*] Values in the same column of 5-day average amounts are significantly different with regard to different variables ($p < 0.05$).
[a] Antioxidant capacity of acidic methanol/water and aceton/water extracts.
[b] Antioxidant capacity of hydrolyzates (methanol/H_2SO_4) of the residues of the aqueous-organic extracts.
[c] Daily meal.
[d] Mean ± SD: Mean of 5-day daily kindergarten samples. Two daily menus were sampled every day in each kindergarten and analysed in triplicate.

organizations.

The results of our research show that standard meals are low for energy, low for total fat, carbohydrates, calcium and iron, which correlated with insufficient supply of food. On the other hand, proximate composition and mineral content of prototype meals are very close to the DRIs. Shortages in the supply of calcium were observed in both groups; furthermore, the content of sodium in kindergarten meals is too high, also the sodium/potassium ratio. All these findings indicate the urgent need to implement an effective policy to control these risk factors and to formulate practical recommendations for improvement.

Our study also provides exclusive data on TAC of free and bound antioxidants in daily kindergarten meals. The content of free antioxidants was significantly higher in the prototype meals, measured by both methods (FRAP and ABTS). Interestingly, an average TAC of bound antioxidants (measured with ABTS) was, in the standard meals, only slightly lower than the TAC in the prototype meals, but FRAP assay did not confirm that. Anyway, the results are very interesting and can confirm that bound antioxidants are often underestimated.

The new data will facilitate kindergartens to plan and assess the quality of nutrition easier. They can also contribute to further measures to improve nutrition in kindergartens, especially by supplementing healthy eating guidelines with more specific recommendations for the inclusion of foods rich in antioxidants.

4. Conclusion

Very few studies have provided detailed analyses of the composition of daily kindergarten meals. The design of an antioxidant-enriched prototype kindergarten menu (breakfast, lunch and two snacks) and the comparison of these meals with standard kindergarten meals in proximate analysis, mineral content, and free as well as bound antioxidants content fills an important gap in the evaluation of kindergarten menus. It has been established that it is necessary to evaluate TAC from the perspective of free and bound antioxidants, since bound antioxidants also contribute significantly to the total TAC and should not be overlooked.

Antioxidant-enriched prototype kindergarten menus differ from standard menus in terms of their energy content (67.8 vs 53.3 % DRI), nutritional composition (on average, > 66 % DRI vs > 45 % DRI) and high TAC value (1369 vs 587 mg VCE/DM by ABTS assay and 1734 vs 810 mg VCE/DM by FRAP assay).

Even though one should be aware of the relatively small sample size used here (i.e. the number of participating kindergartens), this study clearly demonstrates, that when antioxidant rich foods are combined together to devise menus that conform to the national nutritional guidelines, the TAC value of daily kindergarten meals, as well as their energy value and nutritional quality, can be significantly improved. The uptake of meal antioxidants in the gastrointestinal tract (*in vitro* or *in vivo*) should provide an even better understanding of their physiological pathways and their relevance in kindergarten nutrition, so this is something to aspire to in the future studies.

Due to the above reasons, the prototype menu will be strongly encouraged in all kindergarten environments and beyond, and antioxidant-rich foods specifically highlighted in the Guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134566.

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