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Bioaccessibility of minerals in school meals: Comparison between dialysis and solubility methods

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Abstract

Determinations have been made of content and bioaccessibility of Ca, Fe, Zn and Cu in 13 dishes collected from a catering service delivering to a school. Bioaccessibility was estimated by measuring the soluble or dialyzable mineral fraction resulting from in vitro gastrointestinal digestion of the meal. The analyzed dishes had mineral contents (μg/g) in the following ranges: Ca (74.1–913), Fe (2.8–17.9), Zn (2.8–13.1), Cu (0.28–1.90). Mineral solubility and dialysis percentages were as follows: Ca (1.7–96.2; 0.75–61.3), Fe (16.0–97.8; 0.23–19.0), Zn (22.6–93; 5.78–31.45), Cu (35.7–92.3; 0.66–25.0). The highest bioaccessible Ca content corresponded to fish-based dishes, while vegetables were poor sources of Ca. The lowest Fe and Zn bioaccessible percentages corresponded to vegetable-based dishes, particularly spinach omelet, whereas dishes having meat as the main ingredient exhibited the highest bioaccessible percentages. In the case of Cu, vegetable-based dishes could be considered the best sources.

Keywords: Bioaccessibility; Calcium; Iron; Zinc; Copper; School meals

1. Introduction

It is widely accepted that, in childhood and adolescence, diet influences, not only the immediate health of children, but may also have an important impact on adult health. The childhood diet must be adequate to support normal growth and development, and appropriate amounts of minerals are required since a deficient intake of certain minerals can produce diseases and lead to abnormal development. Mineral deficiency is usually caused by a low mineral content in the diet when rapid body growth is occurring and/or when there is poor absorption of minerals from the diet (Favier, 1993). However, even in the "affluent" diet found in western societies, intakes of some minerals, such as Ca, Fe and Zn, are often marginal in certain population groups, e.g., toddlers or female adolescents (Räsänen & Ylönen, 1992; Southon, Bailey, Wright, Belsten, & Finglas, 1993).

Calcium is an essential element. A good Ca intake during childhood is associated with increased peak bone mass during adolescence, reduced development of precancerous cells in colonic mucosa, breast cancer, bone fragility and hip facture, with a possible delay in the development of high blood pressure and a reduction in the prevalence of osteoporosis (Barrer-Lux & Heany, 1994; Hallfrisch et al., 2000).

Iron serves metabolic and enzymatic functions (e.g., Fe for the synthesis of hemoglobin or myoglobin, and Fe-containing enzymes which participate in electron transfer and redox reactions) (Yip, 2001). According to WHO/UNICEF (1998), iron deficiency is the most prevalent single nutritional deficiency in the world and

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is the main cause of anemia in infants, children, adolescents, and women of childbearing age. Iron deficiency anemia is related to delayed cognitive development and intellectual impairment in children (McGregor & Ani, 2001), reduced work capacity (Haas & Brownlie, 2001), an increased risk of maternal and neonatal mortality (Allen, 2002), and altered immune function (Cook & Lynch, 1986).

Zinc, in turn, is essential for normal growth and development, because it plays an important role in gene expression, regulation of cellular growth and differentiation (Hambidge, 2000), and development of the immune response. It has a recognized action on more than 300 enzymes implied in the metabolism of nucleic acids, carbohydrates and proteins, participating as a cofactor (Salgueiro et al., 2002). Marginal Zn deficiency is a common nutritional problem in developing and developed countries (Michelson, Samuelson, Graham, & Lonnerdal, 1994). Among children, it produces serious consequences for health, such as retarded growth, an increase in infectious diseases, and impaired cognitive function (Rosado, 1998).

Copper play a pivotal role in cell physiology as a catalytic cofactor in the redox chemistry of enzymes for proteins that carry out fundamental biological functions required for growth and development (Linder, 1991). It is needed for mitochondrial respiration, Fe absorption, free radical scavenging and elastin cross-linking (Tapiero, Townsend, & Tew, 2003). Copper deficiency is less frequent than other element deficiencies and has been described mainly in premature infants and children recovering from malnutrition (Cordano, 1998), or who change to a poor intake of this mineral in the diet.

Most school age children and adolescents have their main meal of the day in the school lunchroom, and this meal often constitutes the principal mineral contribution to the daily recommended intake.

To estimate the quality of a dietetic source of a given mineral, it is necessary to precisely define the amount of mineral available for absorption and utilization, i.e., its bioavailability (Barberá & Farré, 1992). The bioavailability of minerals and trace elements has generated increasing interest in the field of nutrition.

Bioavailability should be determined by in vivo measurements (Van Dyck, Tas, Robberecht, & Deelstra, 1996). Ideally, this kind of research should have been performed in humans; however, such studies are difficult, expensive, and provide limited data with each experiment (Hansen, Sandstrom, & Lonnerdal, 1996). While animal assays are less expensive, they are somewhat limited by uncertainties with regard to differences in metabolism between animals and humans. As an alternative to human and animal in vivo studies, the availability of minerals or trace elements has also been estimated, based on simple, rapid and inexpensive in vitro methods (Miller, Schricker, Rasmussen, & Van Campen, 1981).

In vitro estimation of the bioavailability of minerals and trace elements from foods involves the simulation of gastrointestinal digestion and measurement of the mineral soluble fraction or the mineral fraction that dialyses across a semipermeable membrane of a certain pore size (Wienk, Marx, & Beynen, 1999). In fact, these methods measure only the amount of mineral available in the gastrointestinal tract for absorption, i.e., its so-called bioaccessibility (Salovaara, Sandberg, & Andlid, 2002). However, these methods are widely used because of their good correlation with in vivo studies (Roig, Alegría, Barberá, Farré, & Lagarda, 1999; Wienk et al., 1999). Bioaccessibility values must be taken as relative indices of bioavailability, which means that the method used provides a good basis for establishing tendencies, comparisons and the determination of effects caused by different factors (Azenha & Vascondecelos, 2000).

In recent years, the mentioned in vitro methods have been used to estimate the bioaccessability of minerals from different foods (Bosscher, Van Cauwenbergh, Van der Auwera, Robberecht, & Deelstra, 2002; Jovaní et al., 2000; Roig et al., 1999; Sahuquillo, Barberá, & Farré, 2003; Sebastiá, Barberá, Farré, & Lagarda, 2001) and also from dishes and composite diets (Kennefick & Cashman, 2000; Luccarini, Canali, Cappelloni, Di Lullo, & Lombardi-Boccia, 1999; Pushpanjali & Khokhar, 1996; Van Dyck et al., 1996).

The present study uses two in vitro methods (solubility and dialysis) to assess the bioaccessible amounts of Ca, Fe, Zn and Cu provided by different dishes usually distributed to a Spanish school lunchroom with the purpose to compare mineral bioaccessibility of the dishes as a function of the ingredients used for their elaboration.

2. Materials and methods

2.1. Materials

Digestive enzymes and bile salts were supplied by Sigma Chemical Co. (St. Louis, MO, USA). The working solutions of these enzymes were prepared immediately before use.

Pepsin solution was obtained by dissolving 1.6 g of pepsin (P-7000 from porcine stomach) in 10 ml of HCl (0.1 M). The solution of pancreatin and bile salts was prepared by dissolving 0.4 g of pancreatin (P-170 from porcine pancreas) and 2.5 g of bile salt (B-8631 of porcine origin) in 100 ml of 0.1 M $NaHCO_3$.

The dialysis membranes, with a pore size (MMCO) of >12,000 Å (Dia. Inf. 36/32″–28.6 mm, 30 m, Bestl no. 1063F09, Medicell Int. LTD, England), were rinsed several times with distilled deionized water before use.

Standard Ca, Fe, Zn and Cu solutions were prepared immediately before use by dilution (with distilled deionized water) of a 1000 mg/l standard solution (Titrisol, Merck, Darmstadt, Germany). The lanthanum solution (5 g/100 ml) was prepared with La_2O_3 (Merck).

All reagents used were of analytical grade, and Millipore-MilliQ distilled deionized water was used throughout. Glass and polyethylene material was soaked in HNO_3 (sp. gr. 1.38) for 15 min and then rinsed three times with distilled deionized water.

2.2. Samples

Thirteen dishes habitually included in the monthly programme of the school menu with different formulations and forms of preparation were supplied by a catering establishment (Table 1).

Aliquots of dishes were frozen in a still-air freezer at −18 °C until required for analyses and processing.

2.3. Analytical procedure

2.3.1. In vitro digestion

2.3.1.1. Dialysis method. The method described by Jovaní et al. (2000), with slight modifications, was applied to estimate dialyzable minerals. Forty grammes of each dish were homogenized with 60 g of deionized-distilled water, and the pH was adjusted to 2.0 with 6 N HCl. To carry out pepsin–HCl digestion, 0.5 g of pepsin solution per 100 g of sample were added. The mixture was then incubated for 2 h at 37 °C in a shaking water bath. A dialysis bag (molecular mass cut-off value 10–12,000 Da) containing 25 ml of water and an amount of $NaHCO_3$ equivalent to the titrable acidity (previously measured) was placed in the flasks, together with 30 g aliquots of the pepsin digest. Incubation was continued for 45 min, the pancreatic-bile salt mixture (7.5 ml) was added, and incubation was continued up to 2 h.

After incubation, the segments of dialysis tubes were removed from the flasks, washed and weighed.

The titratable acidity was defined as the number of equivalents of NaOH required to titrate the combined pepsin digest pancreatin–bile salts mixture to pH 7.5.

The mineral contents of the dialysis tubes were analyzed by flame atomic absorption spectrophotometry (FAAS) (Perkin–Elmer. Model 2380) for Ca, Fe and Zn elements and by graphite furnace-atomic absorption spectrophotometry (GFAAS) with Zeeman effect (Perkin–Elmer Analyst 600) in the case of Cu.

2.3.1.2. Solubility method. The method described by Sahuquillo et al. (2003) with slight modifications was applied. Thirty grammes of each dish were homogenized with 70 ml of deionized distilled water, and the pH was adjusted to 2.0 with 6 N HCl. Pepsin–HCl digestion was carried out as mentioned in the dialysis method.

Prior to the intestinal digestion step, the pH of the gastric digests was raised to 5 by drop-wise addition of 1 M $NaHCO_3$. Then 18.8 ml of the pancreatin–bile salt mixture were added and incubation was continued up to 2 h. To stop intestinal digestion, the sample was maintained for 10 min in an ice bath. The pH was adjusted to 7.2 by drop-wise addition of 0.5 M NaOH. Aliquots of 20 g of the digested sample were transferred to polypropylene

Table 1
Description of the dishes of the school menu

Type	Dish	Formulation/preparation
With cereal base	Cuban style rice	Rice*, fried egg*, chicken broth, fried tomato*, sausage*, sunflower oil, garlic and salt
	Rice with lean meat	Rice*, lean meat*, mushrooms, peppers, green peas, tomato, onion*, sunflower oil, garlic and salt
	Spaghetti with sausage	Spaghetti*, sausage, tomato, sunflower oil and salt
	Macaroni with tuna	Macaroni*, tuna, tomato, sunflower oil and salt
With leguminous base	Lentils with sausage (chorizo)	Lentils*, sunflower oil, mashed tomato, salt, coloring, laurel, chicken broth, carrot, sausage*, onion, pepper, garlic, potatoes*
	Stew	Chickpeas*, green beans, carrot, pork bone, chicken, potato*, veal ragout*, salt pork, chicken broth and salt
With tuber base	Potato stew	Potatoes*, veal ragout*, white wine, mashed tomato, carrot, chicken broth, onion, pepper, sunflower oil, laurel, coloring and salt
With meat base	Chicken in breadcrumbs with vegetable stew	Chicken in breadcrumbs*, vegetables and sunflower oil
	Chicken in sauce	Chicken breast*, chicken broth, flour, onion, almonds, sunflower oil, potatoes, coloring matter and salt
Fish	Fried hake	Hake filet and sunflower oil
	Precooked hake filet	Hake in breadcrumbs and sunflower oil
With egg	Spanish/potato omelet	Potatoes, egg, sunflower oil and salt
	Spinach omelet	Spinach, eggs, sunflower oil and salt

* Main ingredients.

centrifuge tubes (50 ml, Costar Corning Europe, Badhoevedorp, The Netherlands) and centrifuged (3500*g*) for 1 h at 4 °C. Then, the supernatant (soluble fraction) was collected, its organic matter destroyed by dry ashing, and the mineral content measured by FAAS.

2.4. Mineral content determination

Ca, Fe, Zn and Cu total contents and Ca, Fe and Zn in soluble and dialyzed fractions were measured by FAAS. The Cu content of the dialysates was determined by (GFAAS) with Zeeman effect. Previously reported instrumental conditions were applied (Garcıa, Alegria, Barberá, Farré, & Lagarda, 1998; Roig et al., 1999).

Prior to the atomic absorption spectrophotometry (AAS), determination of Ca, Fe, Zn and Cu from dishes and mineral soluble fractions, the organic matter was destroyed by ashing in a temperature-programmed furnace (Heraeus M1100/3, Hanau, Germany) at 450 °C for 48 h (the temperature being slowly increased at a rate of 50 °C/h). To the black ashes, 3 ml of HNO_3 were added; the sample was then heated to dryness and placed in a muffle furnace at 450 °C for 24 h. The process was repeated as many times as necessary to obtain a white residue. After cooling, the residue was dissolved with 1 ml of HCl (sp. gr. 1.19) and 10 ml of distilled deionized water.

Dialysis mineral percentages were calculated as follows: Dialysis (%) = $100 \times D/C$, where D = dialyzed mineral content (μg/g sample) and C = total mineral content (μg/g sample). Soluble mineral percentages were calculated as follows: Solubility (%) = $100 \times S/C$, where S = soluble mineral content (μg/g sample) and C = total mineral content of the sample (μg/g sample).

2.5. Statistical analysis

A simple regression analysis (Statgraphics Plus 4.0 for Microsoft Windows) was applied between Ca, Fe, Zn and Cu contents and their solubility and dialysis percentages.

3. Results and discussion

Total Ca, Fe, Zn and Cu contents in the analyzed dishes are listed in Table 2. Values found for the four measured minerals were in the ranges given by other authors for composite dishes (Table 3). In five of the analyzed dishes (rice with lean meat, chicken with vegetables, potato omelet, macaroni and potato stew), two different mean contents are mentioned, because two samplings in different seasons (spring and autumn) were carried out. The differences found could be due to the ingredients or food origin (Gibson, 1994), and, even in the case of the same ingredients and recipes, to preparation on different days and by different people (Torelm, Danielsson, Appelqvist, & Bruce, 1997).

The best models obtained in the correlation of each total mineral content (Ca, Fe, Zn and Cu) to the corresponding soluble and dialyzable fractions are shown in Table 4.

Table 2
Total mineral content (μg/g) (mean ± standard deviation, *n* = 3)

	Ca	Fe	Zn	Cu
Lentils with sausage (chorizo)	184 ± 15.8	10.7 ± 1.15	7.81 ± 0.76	1.48 ± 0.25
Stew	140 ± 13.4	9.79 ± 2.18	7.11 ± 0.70	1.90 ± 0.18
Cuban style rice	85.60 ± 3.86	7.56 ± 0.30	4.35 ± 0.13	1.05 ± 0.03
Rice with lean meat*	89.9 ± 1.44	15.9 ± 0.17	7.60 ± 0.16	1.14 ± 0.02
	102 ± 4.61	2.92 ± 0.07	7.03 ± 0.13	1.46 ± 0.03
Chicken with vegetable stew*	114 ± 9.26	8.15 ± 1.60	6.59 ± 0.83	0.69 ± 0.02
	323 ± 4.82	7.14 ± 1.96	5.13 ± 0.09	0.75 ± 0.05
Chicken in sauce	106 ± 3.22	10.5 ± 0.86	13.1 ± 0.66	0.76 ± 0.04
Potato* omelet	315 ± 6.58	13.7 ± 0.20	7.01 ± 0.17	0.63 ± 0.01
	294 ± 7.67	8.92 ± 0.93	6.53 ± 0.19	1.01 ± 0.02
Spinach omelet	913 ± 31.1	17.9 ± 0.51	9.00 ± 0.27	0.79 ± 0.01
Macaroni with tuna*	109 ± 3.83	6.48 ± 0.56	3.27 ± 0.08	0.83 ± 0.04
	137 ± 8.50	6.28 ± 0.65	4.68 ± 0.05	1.16 ± 0.01
Spaghetti with sausage	145 ± 1.53	7.03 ± 0.31	6.29 ± 0.46	0.99 ± 0.02
Pre-cooked hake filet	190 ± 18.0	4.40 ± 0.51	3.70 ± 0.19	0.61 ± 0.01
Fried hake	357 ± 52.0	3.95 ± 0.08	3.88 ± 0.07	0.28 ± 0.01
Potato stew*	74.1 ± 1.85	2.82 ± 0.30	3.12 ± 0.07	0.76 ± 0.01
	99.0 ± 1.84	4.85 ± 0.09	2.82 ± 0.17	0.80 ± 0.02

* Dishes samples in two different time periods (spring–autumn).

Table 3
Calcium, Fe, Zn and Cu contents (μg/g) of different types of diet

Sample	Ca	Fe	Zn	Cu	References
Kuwaiti composite dishes	1230–99.7	51.2–1.7	41.6–1.0	12.5–0.3	Dashti et al. (2004)
French dishes	1594–85	20.6–1.28	26.6–0.160	1.22–0.046	Noël et al. (2003)
Oman dishes	345–41	16.0–0.5	22.5–0.5	2.9–0.2	Musaiger et al. (1998)
Western dishes	2684–40.1	–	79.9–0.17	7.39–0.12	Ekmekcioglu et al. (1999)
Polish fast food	2481–95	27–5.9	–	–	Grajeta et al. (2002)

Table 4
Solubility mineral (SM) and dialyzed mineral (DM) percentage (%) (mean ± standard deviation of $n = 6$)

	Ca		Fe		Zn		Cu	
	SM	DM	SM	DM	SM	DM	SM	DM
Lentils with sausage	35.3 ± 3.15	10.3 ± 1.32	16.0 ± 2.89	3.43 ± 0.35	69.9 ± 3.61	25.3 ± 1.38	70.9 ± 4.12	25.0 ± 3.42
Stew	91.7 ± 9.83	30.5 ± 4.18	49.7 ± 0.91	2.14 ± 0.31	37.1 ± 0.52	26.0 ± 0.99	35.7 ± 4.76	13.9 ± 4.03
Cuban style rice	67.6 ± 7.47	23.8 ± 3.94	81.7 ± 4.88	8.17 ± 0.79	58.9 ± 5.92	20.9 ± 2.79	71.0 ± 4.70	19.1 ± 2.63
Rice with lean meat	32.8 ± 5.70	29.3 ± 1.70	97.1 ± 0.98	1.62 ± 0.08	45.5 ± 2.63	7.45 ± 0.83	42.5 ± 3.47	3.53 ± 0.97
Chicken vegetable stew	38.0 ± 3.01	24.8 ± 2.76	53.8 ± 6.03	8.29 ± 0.99	67.0 ± 4.21	19.1 ± 1.68	60.1 ± 6.07	1.58 ± 0.17
Chicken in sauce	26.5 ± 1.89	61.3 ± 2.06	55.1 ± 2.98	5.12 ± 0.41	39.2 ± 6.98	12.0 ± 1.72	57.2 ± 4.57	0.66 ± 0.10
Potato omelet	35.9 ± 4.77	11.3 ± 2.68	73.7 ± 1.48	1.95 ± 0.19	66.9 ± 12.5	8.53 ± 1.16	92.3 ± 4.38	22.0 ± 0.51
Spinach omelet	1.73 ± 0.29	0.75 ± 0.14	45.4 ± 2.44	0.23 ± 0.01	46.7 ± 1.64	5.78 ± 1.11	88.3 ± 5.70	7.64 ± 1.11
Macaroni with tuna	40.8 ± 3.84	12.0 ± 0.47	80.0 ± 11.0	2.31 ± 1.01	61.6 ± 3.18	7.64 ± 1.00	64.4 ± 2.14	14.8 ± 0.92
Spaghetti with sausage	45.0 ± 2.59	17.5 ± 1.46	59.9 ± 4.95	3.39 ± 0.25	22.6 ± 2.55	9.57 ± 0.55	92.12 ± 7.47	3.70 ± 0.41
Pre-cooked hake filet	47.7 ± 3.82	21.1 ± 4.23	79.1 ± 4.83	15.5 ± 2.97	58.7 ± 3.97	8.98 ± 0.93	87.5 ± 3.71	N.D.
Fried hake	46.8 ± 7.41	17.4 ± 0.83	97.9 ± 3.65	3.39 ± 0.84	29.8 ± 9.20	7.67 ± 1.45	87.0 ± 6.09	N.D.
Potato stew	96.2 ± 7.95	28.1 ± 2.98	96.5 ± 9.20	19.0 ± 0.93	93.0 ± 5.52	31.5 ± 1.61	66.5 ± 8.04	15.7 ± 0.99

N.D., non detectable.

In the multiple regression analysis applied to evaluate the relationship between Ca, Fe, Zn and Cu total contents, and mineral solubility and dialysis, no statistically significant correlations were found.

3.1. Calcium

The total Ca content in the analyzed dishes ranged from 74.1 to 913 μg/g, the lowest and highest contents corresponding to potato stew and spinach omelet, respectively (Table 2). Solubility and dialysis percentages ranged from 1.7% to 96.2%, and from 0.75% to 61.3%, respectively (Table 4)

Solubility and dialysability, expressed as μg/g, showed a significant ($p < 0.05$) correlation with Ca content – soluble Ca ($r = 0.76$) and dialyzable Ca ($r = 0.72$) – the values being greater with increasing total Ca content (Table 4). Values corresponding to

Table 5
Regression analysis: Models and correlation coefficients

Element	Factor	Model	p	r
Total Ca, μg/ g	Soluble Ca, μg/g	$y = 24.24 + 36x$	0.0042	0.76
	Soluble Ca, %	$y = \exp(4.52 - 3.93 \times 10^{-3}x)$	0.0001	−0.88
	Dialyzed Ca, μg/ g	$y = 1/(2.16 \times 10^{-2} + 1.11 \times 10^{-4}x)$	0.0053	0.72
	Dialyzed Ca, %	$y = \exp(3.70 - 4.18 \times 10^{-3}x)$	0.0000	−0.91
Total Fe, μg/g	Soluble Fe, μg/ g	$y = 2.49 + 0.32x$	0.0002	0.87
	Soluble Fe, %	$y = 101.16 - 3.77x$	0.0024	−0.79
	Dialyzed Fe, μg/g	$y = 1/(-1.40 + 0.71x)$	0.0618*	0.53
	Dialyzed Fe, %	$y = \exp(3.09 - 0.20x)$	0.0009	−0.80
Total Zn, μg/g	Soluble Zn, μg/g	$y = 1.06 + 0.34x$	0.0077	0.70
	Soluble Zn, %	$y = 30.73 + 122.28/x$	0.0907*	0.48
	Dialyzed Zn, μg/ g	$y = 0.16X^{0.88}$	0.0496	0.55
	Dialyzed Zn, %	$y = 17.44 - 0.44x$	0.6466*	−0.14
Total Cu, μg/g	Soluble Cu, μg/g	$y = 0.66X^{0.64}$	0.0016	0.78
	Soluble Cu, %	$y = 1/(6.61 \times 10^{-3} + 8.78 \times 10^{-3}x)$	0.0060	0.72
	Dialyzed Cu, μg/ g	$y = -8.46 \times 10^{-2} + 0.21x$	0.0032	0.75
	Dialyzed Cu, %	$y = 0.96 + 9.66x$	0.1263*	0.45

r, Correlation coefficient.
* $p > 0.05$.

spinach omelet were not included. However, when Ca solubility and dialysability were expressed as percentages, negative correlations were found ($r = -0.88$ and $r = -0.91$) for solubility and dialysability, respectively (Table 4).

Spinach omelet, the dish with the highest Ca content, showed the lowest Ca solubility and dialysability percentages (Table 5). The high oxalic acid content in spinach (6.62 g/kg; Chawla, Saxena, & Seshadri, 1988) favors Ca precipitation and thus decreases its bioaccessibility. Kennefick & Cashman (2000) observed that oxalate addition to semisynthetic diets decreases Ca dialysability in a dose-dependent manner.

Vegetable components, such as fibre and phytic acid, negatively affect Ca dialysability (Kennefick & Cashman, 2000; Periago et al., 1997). The presence of these inhibitors could explain the relatively low dialysis percentages obtained for pasta dishes (12.0% and 17.5% for macaroni and spaghetti, respectively) and lentils (10.3%) – these values being similar to those reported by Luccarini et al. (1999) for macaroni with broccoli (18.07%), and for macaroni with cauliflower (19.0%), where the Ca dialysis percentages were lower than those corresponding to broccoli (22.9%) and cauliflower (23.4%).

Calcium dialysabilities (μg/g) of pre-cooked (40.0) and fried hake (62.2) were in the lowest end of the range of dialysability percentages (63.6–83.1%) reported by Martínez, Santaella, Ros, & Periago (1998) in fish-based infant food. It has been reported that different fish species are a good dietetic source of Ca (Larsen, Thilsted, Kongsbak, & Hansen, 2000; Losso, Munene, & Moody, 2003), and the data obtained in the present study indicate both fish Ca content and bioaccessibility to be good.

3.2. Iron

Iron contents in the analyzed dishes ranged from 2.8 to 17.9 μg/g, corresponding to potato stew and spinach omelet, respectively. Solubility and dialysability percentages ranged from 16.0% to 97.8%, and from 0.23% to 19.0%, respectively (Table 5).

A linear positive correlation ($p < 0.05$) was found between Fe content and Fe soluble fraction content (μg/g) ($r = 0.87$; Table 4) when values corresponding to lentils were excluded from the model, since the high Fe content of lentils was associated with a low soluble Fe content. Lentils yielded lower soluble Fe contents than chick peas or white beans (Sahuquillo et al., 2003), despite the higher Fe content of lentils. This could be explained by the higher phytate content of lentils (Máléz, Alegría, Farré, & Frigola, 2002).

Negative correlations between Fe content and Fe solubility percentage ($r = -0.79$) or Fe dialysis percentage ($r = -0.80$) (Table 4) were found. A similar correlation coefficient ($r = -0.72$) between total Fe content and dialysis Fe percentage was reported in a dialysis assay of Fe from weaning dishes containing different vegetable ingredients (Bosscher et al., 2002).

The highest Fe contents corresponded to spinach omelet, potato omelet, rice with lean meat, chicken in sauce, lentils and stew (Table 2) and - with the exception of chicken in sauce – these dishes yielded the lowest dialyzable Fe percentages (Table 4).

Spinach has a high Fe content (Chiplonkar et al., 1999), but also contains oxalic acid (Yadav & Sehgal, 2003) that affects Fe bioaccessibility. This could explain why spinach omelet presented one of the lowest percentages of soluble Fe (45.4%), and the lowest percentage of dialyzable Fe (0.23%) – despite having the highest Fe content (17.9 μg/g) among the dishes analyzed. Similar results were reported by Chawla et al. (1988), who indicated that, among the analyzed foods, spinach presented the lowest solubility (2.8%) percentage and the highest oxalic acid content (6.62 g/kg).

The low solubility and dialysability of Fe from spinach omelet could also be related to the egg used as additional ingredient. The low dialysability percentage of Fe (1.95%) from potato omelet, whose main ingredient is egg, would support this assertion. The negative effect of egg proteins upon Fe bioavailability has been reported elsewhere (Beard, Dawson, & Pinero, 1996; Fairweather-Tait, 1989; Kapsokefalou & Miller, 1995). Some egg proteins (ovotransferrin from egg white and phosphovitin from yolk) form complexes with Fe. Phosphovitin binds more than 50% of Fe (III) in yolk, thus reducing Fe bioavailability (Thapon, Audiot, Proteis, & Sauveur, 1994).

Iron dialysis percentages of pasta dishes are low (2–3%) (Table 4), in agreement with the low Fe bioavailability (3%) obtained by Galán, Cherouvier, Fernández-Ballart, Marti-Henneberg, & Hercberg (1990) in a study among volunteers fed Spanish dishes labelled with Fe radioisotopes.

The highest Fe dialysis percentages corresponded to dishes having meat as the main ingredient, such as breadcrumb chicken with vegetables (15.5%) or potato stew (19.0%). Similar dialysis percentages have been reported for meat-based beikost (4.9–8.6%) (Santaella, Martínez, Ros, & Periago, 1997) and fish-based beikost (9.3–11.6%) (Martínez et al., 1998).

Meat protein favors Fe bioavailability (Engelmann et al., 1998) – this effect having been observed in in vitro (Kapsokefalou & Miller, 1991; Mulvihill & Morrisey, 1998) and in vivo (Baech et al., 2003; Kapsokefalou & Miller, 1995) – and this could be related to the Fe (III) to Fe (II) reducing ability of sulfhydryl groups in sulfur-containing amino acids from meat proteins (Mulvihill, Kirwan, Morrisey, & Flynn, 1998).

3.3. Zinc

In the analyzed dishes, zinc contents ranged from 2.8 to 13.1 μg/g (Table 2), these values corresponding to potato stew and chicken in sauce, respectively. Solubility and dialysability percentages (Table 4) ranged from 22.6% to 93.0%, and from 5.78% to 31.4%, respectively.

Significant correlations ($p < 0.05$) between Zn content and soluble Zn ($r = 0.70$) and dialyzable Zn fractions ($r = 0.55$) were found, showing soluble and dialyzable Zn to increase with the Zn content of the meal.

The highest Zn dialysis percentages corresponded to dishes having tubers or legumes as the main ingredient, but also containing meat – such as potato stew, lentils and stew, followed by dishes having meat as the main ingredient (chicken with vegetables, chicken in sauce). The latter yielded Zn dialysis percentages similar to those reported for meat-based weaning foods (13.7–16.1%) (Santaella et al., 1997). Several studies have shown Zn absorption to increase with the animal protein intake (Sandström, 1992).

Zinc dialysis percentages in cereal-based dishes (rice with lean meat, macaroni and spaghetti) were low, with the exception of Cuban style rice (20.9%), in which the presence of egg as a main ingredient could explain the higher Zn bioaccessibility.

3.4. Copper

Copper contents were in a narrow range in the analyzed dishes, from 0.28 to 1.90 μg/g, corresponding to fried hake and stew, respectively (Table 2). Copper solubility and dialysis percentages ranged from 35.9% to 92.3%, and from non-detectable to 25.0%, respectively (Table 4). The lowest dialysis percentages correspond to dishes having meat as the main ingredient, dialyzable Cu being non-detectable in hake. On the other hand, the latter yielded Cu solubility percentages of over 80%, thus showing Cu to be bound to compounds larger than the pore of the dialysis membrane used.

Significant correlations between Cu content and soluble Cu ($r = 0.78$) and dialyzable Cu fractions ($r = 0.75$) were found (Table 4).

Available information on the solubility and dialysability of Cu from foods is scarce, particularly in reference to Cu from dishes and menus. According to the results obtained, meat and fish were poorer Cu dietetic sources than vegetable-based foods, since low dialysis percentages must be added to the low Cu contents (Tables 2 and 5).

4. Conclusions

The mineral contents of the analyzed dishes, usually found in Spanish school lunchrooms, can be ranked as follows: calcium > iron = zinc > copper. An increase in solubility (μg/g) with increasing element content was observed for all four minerals in the analyzed dishes. When Ca and Fe contents increased, the corresponding dialysis amount and percentage decreased. In the case of Zn and Cu, increased mineral content implied a larger dialyzed amount.

In the analyzed dishes, no significant correlations were found between the Ca, Fe, Zn and Cu solubilities and dialysis percentages. High mineral solubility was not always related to a high dialysis percentage, because the mineral may be bound to compounds of molecular sizes in excess of the pore size of the dialysis membrane. Only for Ca in chicken in sauce was the dialysis percentage seen to be greater than the solubility percentage.

The main dietetic Ca sources, on considering Ca content and dialysability, were fish dishes. The negative effect of oxalates on Ca bioaccessibility was evident in spinach omelet, where a high Ca content was nevertheless associated with the lowest soluble and dialyzable Ca percentages.

The highest Fe contents corresponded to cereal, legumes and spinach omelet dishes. However, the dialysability percentages were low and unrelated to the Fe contents. The low solubility percentage of Fe from lentils, and the low dialysis percentage of Fe from spinach omelet were salient findings – particularly considering that the corresponding Fe contents were high.

The highest Zn dialysis percentage corresponded to legume-based dishes, despite their high phytic acid contents; however, heat treatment was applied to legumes, thus reducing their phytic acid contents (Mañez et al., 2002).

Finally, Cu showed opposite behaviour to Ca, Fe, and Zn in the sense that vegetable-based foods had higher mineral bioaccessibility (solubility and dialysability) percentages than meat-based dishes – even dialyzable Fe being non-detectable in fish. This implies that phytic acid and other well known inhibitors of Ca, Fe and Zn absorption would not affect Cu bioaccessibility.

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