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# Dialysability of trace elements in infant foods containing liver

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## Abstract

Response surface methodology, based on a Box–Behnken experimental design, was used to study the effect of liver and other ingredients (carrot, rice, pea and potato), on the dialysability of copper, zinc and iron in both chicken with rice (CR) and veal with carrot (VC) beikosts.

Although modern nutrition programme recommend an increased intake of dietary fibre, this may be particularly problematic in infants. Fibre-increasing ingredients, especially carrot, also simultaneously increased tannin and phytic acid content and reduced the dialysability of Cu and Zn; carrot content must therefore be minimized in infant food formulas. Rice showed a strong negative effect on Cu dialysability whereas, in the formulations used, neither pea nor potato prompted any significant inhibition of trace-element dialysability.

Inclusion of liver in infant food formulas significantly reduced Fe dialysability in the CR beikost (negative quadratic effect) and Cu dialysability in the VC beikost (negative linear effect); both of these findings have been linked to the presence of phytic and gallic acid in liver. The reduction in Cu dialysability was attributed to the tannin content of some of the raw materials used in the VC beikost, while reduced Fe dialysability in the CR beikost was ascribed to the presence of phytic acid or its metabolites.

*Keywords:* Infant foods; Trace-element dialysability; Liver; Phytic acid; Tannins

## 1. Introduction

The weaning period, also known as the food-accustoming period, is the most critical in the life of infants and preschool children. Between 4 and 6 months and 3 years of age, the child's increasing nutritional requirements are no longer met by breast milk alone. Accordingly, pediatric guidelines advocate the gradual replacement of exclusive milk feeding by complementary foods from the fifth month of life onwards (Kersting, Kaiser, & Schoch, 1995). At this age, therefore, most infants begin to eat supplementary semi-solid foods, and weaning foods or beikosts therefore play a major role in their nutrition. The term "beikost" has been defined as "any additional food used in infant nutrition other than human milk and formulas" (Fomon, 1974).

Several factors have been reported to inhibit trace-element absorption. Phytic acid, for example, can reduce the bioavailability of certain trace elements such as zinc (−62%), iron (−48%) or copper (−31%) (Lopez et al., 2000); tannins have been found to depress non-heme iron absorption (Cook, Reddy, & Hurrell, 1995); studies addressing the influence of fibre on trace-element absorption have yielded conflicting results, but there is evidence to support in vitro inhibition (Carnovale & Lintas, 1995). Although certain dietary factors appear to affect trace-element (e.g., iron) absorption when examined individually, their influence largely disappears in multiple regression analysis (Reddy, Hurrell, & Cook, 2000); these factors should therefore be studied in ready-to-eat foods rather than in single ingredients in isolation.

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Because the in vivo system is much more complex, in vitro studies of mineral binding may serve to pinpoint the mechanisms involved; they show reasonably good correlation with in vivo availability and can be used to obtain relative predictions of mineral bioavailability (Wolters, Schreuder, van den Heuvel, van Lonkhuijsen, & Voragen, 1993). It has been reported, for example, that dialyzable $Fe^{2+}$ is a reasonable predictor of non-heme iron bioavailability (Kapsokefalou & Miller, 1991). Studies of meat systems, including liver, have reported significant enhancement of non-heme iron dialysability through reduction of ferric iron to ferrous iron (Mulvihill & Morrissey, 1998). However, although it has been concluded that Fe absorption studies in adults may be used to estimate Fe absorption in infant formulas, further information is required with regard to weaning foods and complete meals (Hurrell, Davidsson, Reddy, Kastenmayer, & Cook, 1998).

Response surface methodology (RSM) is a very useful statistical tool, comprising a set of techniques used in the empirical study of relationships between one or more responses and a group of input variables, in order to locate the region of lowest response values, where the lowest is considered to be the best (Myers & Montgomery, 1995).

The aim of the present study was to determine, using RSM, the extent to which inclusion of liver and other ingredients in meat-based homogenized infant foods modifies the dialysability of certain trace elements.

## 2. Material and methods

### 2.1. Infant food samples

Two types of beikost, chicken with rice (CR) and veal with carrot (VC), were manufactured using different formulations of several ingredients in accordance with the experimental design described below. Beikost samples (250 g bottles) were manufactured by Hero España SA, using a process described previously (Ros, Abellán, Rincón, & Periago, 1994).

### 2.2. Experimental design and statistical analysis

Each beikost contained constant and variable ingredients. Constant ingredients were: chicken or veal meat for the CR and VC beikosts, respectively, onion, celery, tomato, sunflower oil and water; variable ingredients were carrot, rice, pea or potato, and either chicken liver or veal liver, according to the experimental design shown in Table 1. Variable ingredients were selected for reasons detailed in earlier papers (Abellán, Rincón, Ros, & López, 1994; Rincón, Ros, Periago, & Martínez, 1996). In summary, ingredients and experimental ranges were selected according to commercial formula and, on this basis, the effect of liver addition was assayed while the carrot, rice and pea (CR) or carrot, rice and potato (VC) contents in commercial formula were included in the experimental design in order to balance the liver inclusion.

Table 1
Levels of key ingredients as % considered in the experimental design for both chicken with rice (CR) and veal with carrot (VC) beikosts

| Factors (%) | Symbol | | Levels | | |
|---|---|---|---|---|---|
| | Coded | Uncoded | −1 | 0 | +1 |
| *CR beikost* | | | | | |
| Carrot | $X_1$ | $\Phi_1$ | 5.70 | 6.67 | 7.64 |
| Rice | $X_2$ | $\Phi_2$ | 7.20 | 8.00 | 8.80 |
| Pea | $X_3$ | $\Phi_3$ | 1.40 | 2.00 | 2.60 |
| Chicken liver | $X_4$ | $\Phi_4$ | 0.15 | 1.00 | 1.85 |
| *VC beikost* | | | | | |
| Carrot | $X_1$ | $\Phi_1$ | 10.83 | 13.33 | 15.83 |
| Rice | $X_2$ | $\Phi_2$ | 2.95 | 4.20 | 5.45 |
| Potato | $X_3$ | $\Phi_3$ | 2.20 | 3.40 | 4.60 |
| Veal liver | $X_4$ | $\Phi_4$ | 0.15 | 1.00 | 1.85 |

The weaning food formulation process can be considered as a system including four *input* factors (ingredients included in the experimental design as shown in Table 1) and seven *output* factors (responses). The general design was a second-order full factorial design including three levels for each factor, following a previously-described model (Box & Behnken, 1960). When each factor is measured at three or more levels, a quadratic response surface can be estimated by least-squares regression.

Each response-variable was assumed to be influenced by four independent variables or factors (carrot, rice, pea and chicken liver for CR and carrot, rice, potato and veal liver for VC), $\Phi_i$ ($i$ = from 1 to 4), so that $\xi_m = f(\Phi_1, \Phi_2, \Phi_3, \Phi_4)$, where $\xi$ is each response ($m$ = from 1 to 7), $\Phi_1$ is the percentage of carrot formulated, $\Phi_2$ is the percentage of rice formulated, $\Phi_3$ is the percentage of pea or potato formulated (for CR and VC, respectively), and $\Phi_4$ is the percentage of liver formulated (chicken liver for CR, veal liver for VC).

The basic analysis for a response surface experiment consists in fitting a quadratic model of the form,

$$\xi = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \varepsilon,$$

where $\xi$ is each response, $X_i$ are factors or key ingredients considered for each beikost type as coded independent variables, since it is advisable to transform natural variables into coded variables, which are usually defined as dimensionless with mean zero and the same spread or standard deviation (Myers & Montgomery, 1995), $X_iX_j$ are the two factor interactions, $b_0$ is the intercept, $b_i$, $b_j$ $b_{ij}$ are linear, quadratic and cross product regression terms, respectively, and $\varepsilon$ is the model error. In order to describe each response–factor relationship by a polynomial equation with squared terms, trials at three levels of variable formulation were required; the levels considered are shown in Table 1.

The seven responses measured were: phytic acid, tannin, total dietary fibre, pH and dialyzed Cu, Zn and Fe.

Both Statistica (StatSoft, Inc., Tulsa) and Design-Expert (Stat-Ease, Inc., Minneapolis) software were used to generate designs, fit the response surface model to the experimental data and draw response surface figures. Differences were considered significant at $p < 0.05$.

### 2.3. Methods

Phytic acid was extracted following Plaami and Kumpulainen (1991); phytic phosphorus was then determined using Standard Method 970.39 (AOAC, 1990). Total dietary fibre (TDF) was determined using the method described by Prosky, Aspm, Schweizer, Devries, and Furda (1988). A specific method covering a wide range of ingredients of vegetable origin has been described for determination of tannins in infant foods (Martínez, Rincón, & Ibánez, 2000).

Dialyzable Cu, Zn and Fe were determined using the technique described by Miller, Schricker, Rasmussen, and Van Campen (1981), as subsequently modified by Vaquero et al. (1992). Fresh beikost samples were thawed and mixed with distilled water in a 1:2 ratio. The pH of the homogenates was adjusted to 2 with 6 M HCl. Pepsin was added in a 0.5% ratio to the homogenized sample and incubated in a shaking water-bath for 2 h at 37 °C. After the incubation, 40 g of the digest were placed in a 250 ml plastic bottle. First, the titratable acidity of the digest was determined as meq of $NaHCO_3$. After that, segments of dialysis tubing containing 25 ml deionized water and an amount of $NaHCO_3$ equivalent to the titratable acidity were put into each flask. The flasks were incubated in the shaking water bath for about 30 min or until the pH was 5. Then 5 mL of the pancreatin–bile extract mixture (1/6 ratio in 0.1 M $NaHCO_3$) were added, and the incubation was continued for further 2 h. The diffusates were subsequently analyzed for mineral content following a previously defined technique (Martínez, Rincón, Ibáñez, & Abellán, 2004).

## 3. Results and discussion

### 3.1. General

Table 2 shows the results obtained for each trial, and Table 3 the significant standardized effects ($p = 0.05$) of each ingredient (as coded independent variable $X_1$–$X_4$) on each response ($Y_1$–$Y_7$) for both CR and VC beikosts.

Table 2
Results obtained in weaning foods for different responses[a]

| Run | Trial | Chicken with rice beikost (CR) | | | | | | | Veal with carrot beikost (VC) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ | $Y_6$ | $Y_7$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ | $Y_6$ | $Y_7$ |
| 1 | 25 | 3.68 | 5.89 | 3.73 | 6.11 | 7.99 | 8.08 | 12.69 | 7.15 | 7.08 | 2.30 | 6.03 | 5.52 | 9.55 | 9.19 |
| 2 | 23 | 3.00 | 7.39 | 4.27 | 6.11 | 7.03 | 7.97 | 13.83 | 6.52 | 7.50 | 2.40 | 6.04 | 5.00 | 9.41 | 10.15 |
| 3 | 11 | 3.11 | 5.76 | 5.38 | 6.11 | 6.27 | 9.42 | 13.88 | 6.41 | 8.28 | 1.37 | 6.03 | 5.91 | 8.45 | 8.95 |
| 4 | 24 | 3.11 | 6.40 | 4.88 | 6.08 | 5.17 | 8.64 | 13.30 | 6.57 | 6.75 | 1.80 | 6.04 | 3.97 | 9.41 | 9.36 |
| 5 | 3 | 5.86 | 5.99 | 5.05 | 6.05 | 4.94 | 7.89 | 13.33 | 7.50 | 8.13 | 2.43 | 6.00 | 5.20 | 8.88 | 8.74 |
| 6 | 1 | 1.67 | 6.96 | 4.44 | 6.03 | 6.89 | 11.26 | 14.63 | 7.38 | 7.58 | 1.91 | 6.03 | 5.47 | 7.67 | 10.00 |
| 7 | 9 | 4.04 | 7.50 | 8.29 | 6.02 | 7.39 | 11.38 | 11.53 | 8.69 | 7.86 | 1.91 | 6.01 | 6.49 | 8.94 | 8.73 |
| 8 | 6 | 4.85 | 6.54 | 3.27 | 5.99 | 6.77 | 9.36 | 12.86 | 6.07 | 7.12 | 2.85 | 5.98 | 5.29 | 11.64 | 9.34 |
| 9 | 13 | 3.65 | 7.62 | 2.77 | 6.00 | 7.35 | 11.44 | 12.08 | 5.75 | 7.37 | 2.83 | 5.90 | 5.40 | 7.98 | 9.74 |
| 10 | 18 | 4.77 | 6.81 | 7.52 | 6.00 | 7.83 | 13.26 | 13.71 | 6.18 | 6.42 | 3.63 | 5.91 | 6.19 | 7.74 | 9.34 |
| 11 | 10 | 6.22 | 8.52 | 10.45 | 6.13 | 8.57 | 13.21 | 10.83 | 6.57 | 5.69 | 2.45 | 5.93 | 6.44 | 6.15 | 8.96 |
| 12 | 12 | 6.35 | 7.53 | 6.62 | 6.03 | 6.06 | 9.30 | 11.39 | 5.52 | 8.26 | 2.59 | 5.86 | 3.82 | 11.68 | 3.81 |
| 13 | 16 | 5.33 | 7.19 | 7.68 | 6.03 | 4.41 | 5.09 | 12.03 | 6.54 | 7.04 | 5.65 | 5.89 | 2.70 | 7.32 | 8.55 |
| 14 | 2 | 4.73 | 5.79 | 6.69 | 5.98 | 6.13 | 9.66 | 9.30 | 6.65 | 6.45 | 5.34 | 5.88 | 3.91 | 11.88 | 8.72 |
| 15 | 7 | 5.34 | 8.32 | 3.94 | 6.01 | 6.49 | 11.55 | 10.98 | 7.51 | 7.17 | 4.39 | 5.87 | 2.90 | 10.87 | 9.62 |
| 16 | 15 | 5.93 | 8.56 | 5.19 | 6.08 | 5.47 | 7.28 | 9.96 | 6.81 | 6.31 | 2.72 | 5.93 | 3.08 | 10.19 | 9.54 |
| 17 | 8 | 4.11 | 8.85 | 7.52 | 6.04 | 6.69 | 13.55 | 12.51 | 6.37 | 6.31 | 1.67 | 5.94 | 4.45 | 11.79 | 10.43 |
| 18 | 21 | 3.64 | 10.82 | 5.20 | 6.04 | 5.71 | 13.68 | 10.88 | 6.86 | 5.88 | 2.13 | 5.95 | 3.24 | 8.08 | 9.41 |
| 19 | 4 | 5.29 | 7.00 | 9.84 | 6.03 | 6.57 | 11.79 | 12.26 | 5.25 | 7.53 | 2.33 | 5.90 | 2.91 | 10.39 | 10.98 |
| 20 | 19 | 5.97 | 7.18 | 7.98 | 6.03 | 5.63 | 11.80 | 11.10 | 11.64 | 6.75 | 1.45 | 5.88 | 3.20 | 9.13 | 11.43 |
| 21 | 22 | 5.21 | 7.55 | 3.65 | 6.05 | 6.92 | 11.78 | 10.50 | 9.42 | 6.66 | 4.86 | 5.92 | 3.07 | 9.74 | 10.22 |
| 22 | 14 | 4.51 | 8.94 | 8.59 | 6.05 | 6.00 | 11.83 | 11.60 | 5.96 | 6.17 | 3.13 | 5.96 | 4.69 | 9.80 | 9.32 |
| 23 | 17 | 4.41 | 8.13 | 7.24 | 5.98 | 5.75 | 8.42 | 12.10 | 7.84 | 6.40 | 2.14 | 5.93 | 3.51 | 8.77 | 11.99 |
| 24 | 5 | 4.17 | 8.11 | 9.60 | 5.97 | 7.32 | 11.68 | 11.26 | 7.81 | 5.87 | 2.70 | 5.93 | 3.49 | 10.01 | 11.88 |
| 25 | 20 | 3.47 | 8.06 | 6.38 | 5.98 | 6.14 | 9.30 | 12.57 | 6.60 | 6.15 | 1.42 | 5.91 | 2.59 | 9.67 | 10.30 |

[a] $Y_1$, total dietary fibre TDF (%); $Y_2$, phytic acid (mg/g); $Y_3$, tannin (catechin units/g); $Y_4$, pH; $Y_5$, dialyzed copper; $Y_6$, dialyzed iron; $Y_7$, dialyzed zinc (%). From $Y_1$ to $Y_6$, results are expressed on a dry weight basis, while from $Y_9$ to $Y_{14}$ results are expressed on a wet weight ready-to-eat basis.

Table 3
Significant ($p =$ 0.05) standardized effects obtained for each ingredient considered as a factor in the responses evaluated, for both chicken with rice (CR) and veal with carrot (VC)

| Response[a] | $X_1$ | | $X_2$ | | $X_3$ | | $X_4$ | |
|---|---|---|---|---|---|---|---|---|
| | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic |
| $Y_{1\text{-CR}}$ | 1.98 | | | | | | | |
| $Y_{2\text{-CR}}$ | 3.35 | | 3.05 | | | | | |
| $Y_{3\text{-CR}}$ | 1.98 | | | | | | | |
| $Y_{4\text{-CR}}$ | −3.62 | | | | −3.20 | | | |
| $Y_{5\text{-CR}}$ | | | −1.81 | −2.51 | | −2.58 | | |
| $Y_{6\text{-CR}}$ | | | | −2.90 | | −2.32 | | −2.67 |
| $Y_{7\text{-CR}}$ | −2.87 | | | | | | | |
| $Y_{1\text{-VC}}$ | 1.94 | | | | | | | |
| $Y_{2\text{-VC}}$ | −3.41 | | | | | | | |
| $Y_{3\text{-VC}}$ | | 3.49 | | 3.58 | | | | 2.34 |
| $Y_{4\text{-VC}}$ | −5.18 | −3.35 | | | | | | |
| $Y_{5\text{-VC}}$ | −2.69 | | −2.64 | | | | −2.22 | |
| $Y_{6\text{-VC}}$ | | | | | | | | |
| $Y_{7\text{-VC}}$ | | | | | | | | |

[a] $Y_1$, total dietary fibre TDF (%); $Y_2$, phytic acid (mg/g); $Y_3$, tannin (catechin units/g); $Y_4$, pH; $Y_5$, dialyzed copper; $Y_6$, dialyzed iron; $Y_7$, dialyzed zinc (%). From $Y_1$ to $Y_6$, results are expressed on a dry weight basis, while from $Y_9$ to $Y_{14}$ results are expressed on a wet weight ready-to-eat basis.

In Table 2, the trial column shows the order in which the experiments were carried out (a randomized order), while the run column shows the formal or systematic order developed to obtain the experimental design. Randomization by this means is essential to ensure that the average influence of noise factors, such as environmental factors, is lessened (Robison, 2000).

### 3.2. Fibre

Carrot was the main fibre source in both beikosts (Table 3); some authors have recently suggested that the carrot content in homogenized infant foods should be reduced (Olivares, Martínez, López, & Ros, 2001).

### 3.3. Phytic acid

Carrot and rice had a positive linear effect on phytic acid content in the CR beikost (Table 3); so increasing the proportion of these ingredients would thus give rise to higher phytic acid levels. In the VC beikost, however, carrot displayed a negative linear effect, signifying that an increased percentage of carrot would lead to a lower phytic acid content. This is simply because a higher proportion of carrot means a lower proportion of other ingredients rich in phytic acid, such as rice and potato, in the product formula (Table 1). In ready-to-eat food, however, both beikost types displayed very similar phytic acid contents (7.49 mg/g ± 5.76 for CR and 6.91 mg/g ± 5.69 for VC).

Although phytate is one of the factors involved in poor trace-element absorption, not all forms of phytate have the same mineral-binding capacity. A number of studies have shown that the maximum amount of metal ion bound by the various inositol phosphates is approximately the same for hexa-myoinositol (IP6) and penta-myoinositol (IP5) but decreases upon further dephosphorylation (Persson, Türk, & Nyman, 1998); for example, inositol phosphates below IP5 do not inhibit non-heme iron absorption (Allen & Ahluwalia, 1997). IP6 and IP5 make up over 90% of the total phytate in raw grains (Agte, Tarwadi, & Chiplonkar, 1999) but are degraded during processing and cooking, because at 120 °C the autohydrolysis of phytic acid gives rise to the formation of most phosphate esters of myo-inositol in varying amounts, depending upon the heating time (Hull, Gray, & Montgomery, 1999); when phytic acid (hexa-myoinositol, IP6) is dephosphorylated, other isomers of lower inositol phosphates, such as IP5, IP4, IP3 or IP2, are obtained (Torre, Rodriguez, & Saura-Calixto, 1991).

The sum of the linear effects of carrot and rice on phytic acid content in the CR beikost (3.35 and 3.05, respectively, Table 3) was greater than the resulting negative effect on Zn dialysability in the CR beikost (−2.87, Table 3). This difference may be accounted for in terms of two hypotheses, which are not mutually exclusive.

The first hypothesis is that heat treatment during industrial processing prompts the degradation of IP6 and IP5 to other more dephosphorylated and less inhibitory forms, such as IP1, IP2 and IP3 (Persson et al., 1998). The results obtained here agree with those of other reports, which suggest that the negative effect of phytic acid on the availability of trace elements such as Zn is governed by food type and phytate content (Bossecher et al., 2001). This should be borne in mind in the formulation of weaning foods. A high level of phytase activity as been shown, for example, in scallion leaves (Phillippy & Wyatt, 2001); the fact that phytases are able to dephosphorylate phytate in a stepwise manner to a series of lower inositol phosphate esters and ultimately, in theory at least, to inositol and inorganic P (Selle, Ravindran, Caldwell, & Bryden, 2000), would seem to justify the replacement of onion in the formula by this new ingredient as a way of improving Zn dialysability. Selle et al. (2000) found that most phytate was degraded by phytase within 30 min; phytase is therefore active during mixing and blending of ingredients, as well as during deairing (40 min) prior to inactivation by heat treatment.

The second hypothesis is that phytic acid may not have the same affinity for $Fe^{3+}$, $Fe^{2+}$, $Zn^{2+}$ and $Cu^{2+}$: some studies have shown that the maximum amount of metal ion bound by the various inositol phosphates is approximately the same for IP6 and IP5, but decreases upon further dephosphorylation (Persson et al., 1998); for example, inositol phosphates lower that IP5 do not inhibit non-heme iron absorption (Allen & Ahluwalia, 1997).

### 3.4. Tannins

In the CR beikost, the effect of ingredients on tannin content was wholly attributable to carrot (Table 3). In the VC beikost, observed effects were rather more complex: carrot, potato and liver displayed positive quadratic effects, so that when the level of these three ingredients rose from −1 to 0 (phase 1), tannin content increased; however, when the ingredient level rose from 0 to +1 (phase 2), tannin content fell, as shown in the perturbation plot (Fig. 1a). The effects of carrot and rice in phase 1 are attributable to the tannin levels in both ingredients (Table 3). With regard to the effects of veal liver, the phenol detoxification pathway in the liver implies the existence, in this ingredient, of phenolic substances arising from degradation of tannins; metabolites will therefore be quantified analytically, because, after absorption, flavonoids are usually conjugated in the liver and to a lesser extent in the kidney (Peterson & Dwyer, 1998), and gallic acid (3,4,5-dihydroxybenzoic acid) is the end-product of hydrolysis of tannins (Kazmi, Qureshi, & Maqsood, 1987). These residues would thus account for the positive effect of veal liver in increasing the tannin content of the VC beikost (Table 3). Moreover, results obtained by Yoshino et al. (2002) imply that the cuprous ion is reduced by gallate derivates, which may account for the negative effect (−2.2, Table 3) of liver on Cu dialysability.

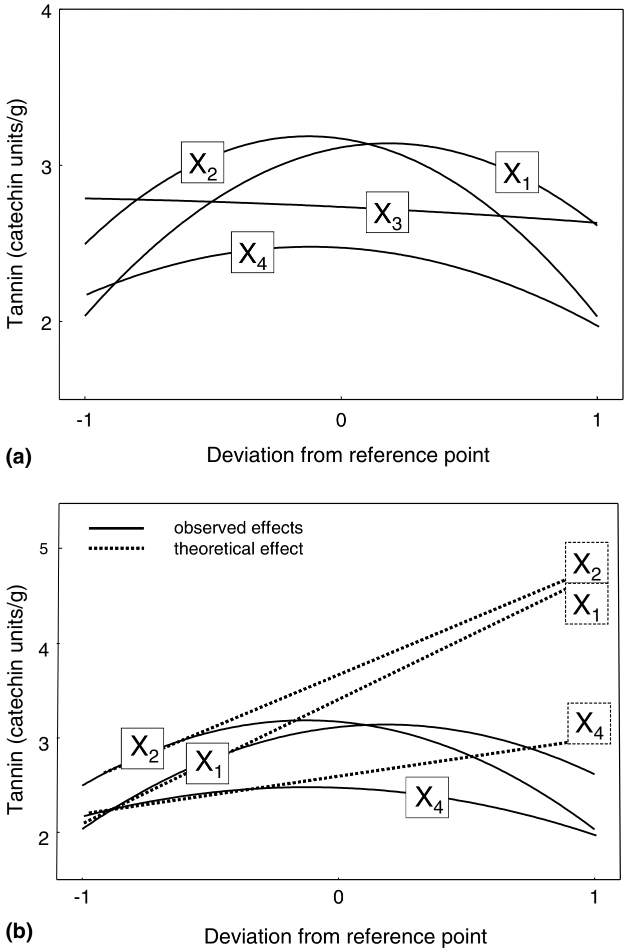

Fig. 1. Perturbation plot for tannin, and for VC beikost. Graph A plots perturbation for the results obtained. Graph B shows estimated perturbation on excluding the misleading effect of sunflower oil on tannin content, detected in the VC beikost.

To account for the fact that increased percentages of carrot, rice and liver in phase 2 lead to reduced tannin content in the final product, it is important to bear in mind the underlying tannin level contributed by the ingredients designated constant in the experimental design; an increase in these ingredients signifies an increase in product dry matter, so that, in relative terms, 250 g of product will contain a greater amount of variable ingredients (carrot, rice or liver); these will contribute less tannins than the sum of the constant ingredients (onion, celery, tomato and sunflower oil), since tannin content is expressed on a dry basis. However, since no quadratic effects were observed in the CR beikost, it would appear reasonable to assume that the constant ingredients are not formulated in the same proportions in the two beikosts. In fact, onion, celery, tomato and sunflower oil are formulated for both CR and VC beikosts as 1% vs. 0.5%, 0.67% vs. 0.67%, 0.57% vs. 0.50% and finally 0.60% vs. 2.30 %, respectively. Evidently, the main quantitative difference is for sunflower oil; we may therefore hypothesize that this is the ingredient responsible for the high underlying tannin level in the VC beikost. To account for the lowering of tannin levels in phase 2, it should first be made clear that the method used for tannin determination is based on the vanillin–HCl reaction, which is in turn based on condensation of phenolic aldehyde (vanillin) with the phloroglucinol structure of flavan-3-ols and proanthocyanidins under acidic conditions in methanol or ethanol (Price, van Scoyoc, & Butler, 1978; Reed, 1995); although sunflower seeds are known to be rich in phenols, most of which are lost during industrial processing to obtain oil, trace amounts are present in sunflower oil (De Leonardis, Macciola, & Di Rocco, 2003). Interference of these compounds in the analytical method could lead to an overestimation of tannin content in the VC beikost due to the high sunflower oil content; if this were so, the quadratic effects observed for carrot, rice and liver would not in fact exist. Thus, while Fig. 1a shows perturbation observed in the results, Fig. 1b shows estimated perturbation on excluding the misleading effect of sunflower oil; the resulting effects of carrot, rice and liver on tannin content in the VC beikost become linear, so that a increase in these ingredients would prompt a linear increase in beikost tannin content. Nevertheless, this hypothesis needs to be confirmed independently in future studies.

With the pea and potato proportions used here (1.40–2.6% and 2.20–4.6%, respectively, Table 1), no significant effect was observed on tannin content (Table 3).

### 3.5. pH

Both carrot ($-3.62$) and pea ($-3.20$) prompted a marked acidification of the CR beikost (Table 3), whereas, in the VC beikost, only carrot ($-5.18$) had a significant effect, although the effect was more intense than in the CR beikost, since the VC beikost contained a larger proportion of carrot (Table 1). Carrot is the main contributor of phytic acid (Table 3), a moderately strong acid with five to six $H^+$ dissociating with $pK$ about 1.5, two to three $H^+$ dissociating with $pK$ between 4 and 6 and four $H^+$ dissociating with $pK$ greater than 8 (Martin & Evans, 1986). In all probability, therefore, phytic acid was partly responsible for the increased acidification observed with increased carrot content and declining pH ($r = -0.45$, $p < 0.05$). The effect of ingredients on the pH of food is of major importance, since high gastric pH, such as occurs in infants, facilitates the formation of phytate–calcium–zinc chelates (Champagne & Phillippy, 1989).

### 3.6. Dialyzed Cu

In the CR beikost, Cu dialysability was negatively affected by rice and pea, whilst, in the VC beikost, it was negatively affected by carrot, rice and liver (Table 3).

The negative effect ($-2.2$, Table 3) of liver on Cu dialysability may be due to the fact that the cuprous ion is reduced by gallate derivates, as reported by Yoshino et al. (2002). Moreover, this negative effect was broadly similar, in quantitative terms, to the positive effect of liver on tannin content in the VC beikost (2.34, Table 3); this overlapping of negative effects again points to a possible relationship between the two. This hypothesis is borne out by the observation that, in the VC beikost, both the effect on tannin content and the effect on Cu dialysability were prompted by the same ingredients: carrot, rice and liver (Table 3, Fig. 2), suggesting that reduced Cu dialysability is due, first to the increased proportions of carrot and rice in the product formula, and, second to the presence in veal liver of degradation compounds from tannin metabolism.

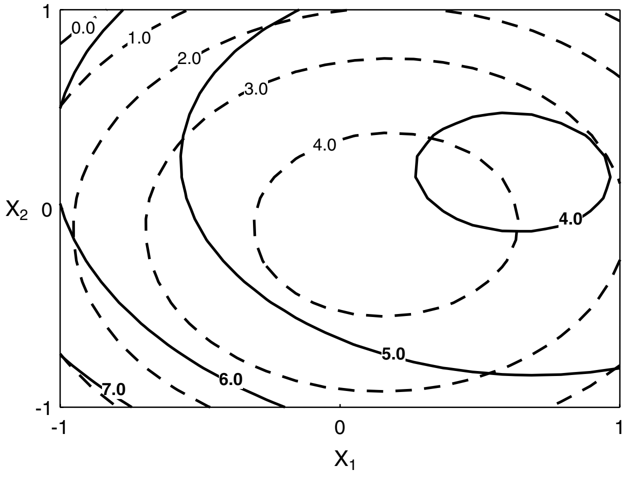

Fig. 2. Close overlapping of effects of carrot and rice on tannin content, expressed as catechin units/g on a dry weight basis (positive effect, broken line) and dialysability of copper, expressed as a percentage (negative effect, unbroken line) in the VC beikost.

On the basis of the experimental results (Table 1), phytic acid does not inhibit copper absorption, in accordance with other results reported (Lopez et al., 2000 and Lönnerdal, 2002), and indeed it has been shown to have no significant depressive effect on copper status in rats (Lopez et al., 2000); this would suggest that the negative effect of carrot on Cu dialysability can be wholly ascribed to tannin content. However, acidification (i.e. a fall in pH) of the VC beikost due to carrot may also be at least partly responsible, since a number of authors report that falling pH has a negative effect on Cu dialysability (Fairweather-Tait, 1992); here, a significant positive relationship was observed between the two variables, with acidification resulting in lower dialysability ($r = 0.56$, $p < 0.05$).

### 3.7. Dialyzed Fe

Significant negative effects on Fe dialysability were observed only in the CR beikost, for rice, pea and liver; in all cases, a negative quadratic effect was noted. This would appear to contrast with the findings of Mulvihill and Morrissey (1998), who report that lamb liver improves the dialysability of iron from FeCl. However, experimental conditions in the present study were quite different: no fortification was involved, and chicken liver rather than lamb liver was used. Moreover, the present study assessed changes in Fe dialysability on increasing liver content in the beikost (Table 1), but did not compare beikosts with and without liver; the results cannot therefore be held to contradict those reported by Mulvihill and Morrissey. Chicken liver had a negative quadratic effect on Fe dialysability: as liver content rose from level −1 to level 0 (phase 1), Fe dialysability diminished, but when content rose from level 0 to level +1 (phase 2), Fe dialysability increased.

The phase 1 findings may be accounted for as follows: most of the IP6 found in the organs of mammals such as rats is of dietary origin (Grases et al., 2002) and a level of $1.77 \times 10^{-3}$ mg/g of dry matter has been found in liver (Grases, Simonet, Prieto, & March, 2001); at the same time, gallic acid, the end-product of hydrolysis of tannin, forms a 3:1 complex with iron in the pH range 4–6 (Kazmi et al., 1987). The maximum amount of metal ion bound by the various inositol phosphates is approximately the same for IP6 and IP5 but decreases upon further dephosphorylation (Persson et al., 1998); inositol phosphates lower that IP5 do not inhibit non-heme iron absorption. However, IP3 and IP4 in processed foods contribute to the negative effect on iron absorption, presumably by binding iron between different inositol phosphates (Sandberg et al., 1999). Interaction of the phytate anion with a counter-cation may result in precipitation of the cation–phytate complex, although this process is dependent on the relative amount of cation, its valency and the pH (Nolan, Duffin, & McWeeny, 1987). Because most chelates with divalent mineral cations are soluble at pH $< 3.5$, but with a maximum insolubility occurring between pH 4 and 7 (Nolan et al., 1987), differences in the first complexes formed before ingestion may be attributed to differences in the pH of the product. However, since CR and VC beikost displayed similar mean pH values (6.03 ± 0.04 and 5.94 ± 0.06, respectively), the pH factor seems not to have affected trace-element dialysability, even though it is reported to cause variations in the dialysability of trace elements in weaning foods (Martínez, Rincón, & Ibánez, 2004).

In interpreting the phase 2 findings, it should be borne in mind that chicken liver contributes 7.40 mg Fe/100 g (Souci, Fachmann, & Kraut, 1994), and is a source of heme Fe in the beikost (Martínez et al., 2004); an increased supply of Fe in phase 2 would thus overlap with the IP6 in the liver itself. At the same time, the presence of thiol groups has been linked to improved Fe dialysability (Kapsokefalou & Miller, 1991; Mulvihill & Morrissey, 1998).

### 3.8. Dialyzed Zn

Only carrot displayed a negative effect on Zn dialysability in the CR beikost (−2.87, Table 3). Since carrot had a positive effect on phytic acid and TDF content (Table 3, Fig. 3), it may be assumed that these two components were directly responsible for reduced Zn dialysability ($r = -0.59$, $p < 0.05$ for the % TDF/% dialyzable Zn relationship and $r = -0.45$, $p < 0.05$ for the % phytic acid/% dialyzable Zn relationship). Other authors report that phytic acid significantly lowers Zn dialysability (Fairweather-Tait, 1992), exerting a strong inhibitory effect on IP6 and IP5, but a much weaker effect on IP4 and IP3 (Lönnerdal, 2002); this would account for the poor dialysability (only 19.5%) of Zn in carrot (Lucarini, Di Lullo, Cappelloni, & Lombardi-Boccia, 2000) and for the results obtained here for the CR beikost. In addition, since the number of free carboxyl groups on sugar residues and the uronic acid content of polysaccharides appear to be related to the cation exchange properties of fibres (Schneeman, 1986), it seems that some fibre types can lower dialysability; for example, a binding capacity of 220 μg Zn/50 g of fibre has been reported for some fibre types (Casterline & Ku, 1993).

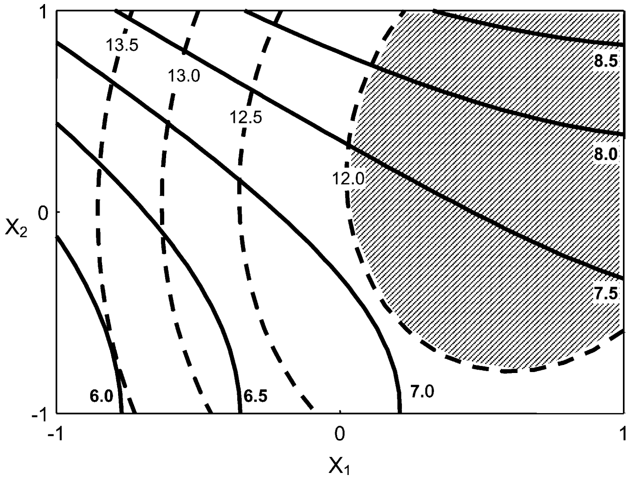

Fig. 3. Significant effects of carrot and rice on phytic acid content (unbroken line) and simultaneous lowest dialyzable zinc percentage (shaded area and broken line) determined only by effect of carrot for CR beikost.

To summarize, RSM revealed that carrot had a stronger effect on TDF and – together with rice – on phytic acid, while both carrot and pea prompted a significant decrease in the pH of the ready-to-use product. Inclusion of liver in the product formula generally lowered trace-element dialysability, a finding linked to the presence of metabolites from phytic acid and/or tannin. Liver should therefore not be included in the commercial form of these products.

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## References

Abellán, P., Rincón, F., Ros, G., & López, G. (1994). Mineral composition of meat-based infant beikosts. A preliminary study. *International Journal of Food Science, 45*, 209–215.

Agte, V. V., Tarwadi, K. V., & Chiplonkar, S. A. (1999). Phytate degradation during traditional cooking: significance of the phytic acid profile in cereal-based vegetarian meals. *Journal of Food Composition and Analysis, 12*, 161–167.

Allen, L. H. & Ahluwalia, N. (1997). Bioavailability of nonheme iron. In L. H. Allen & N. Ahluwalia (Eds.), *Improving iron status through diet. The application of knowledge concerning dietary iron bioavailability in human populations* (pp. 13–34). US Agency for International Development: Arlington, VA. Final Report of the Contract no. HRN-5122-C-00-3025-00. John Snow, Inc./OMNI Project.

AOAC. (1990). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists. The Association.

Bossecher, D., Lu, Z., Janssens, G., van Caillie-Bertrand, M., Robberecht, H., De Rycke, H., et al. (2001). In vitro availability of zinc from infant foods with increasing phytic acid contents. *British Journal of Nutrition, 86*, 241–247.

Box, G. E. P., & Behnken, D. W. (1960). Some new three level designs for the study of quantitative factors. *Technometrics, 2*, 455–475.

Casterline, J. L., & Ku, Y. (1993). Binding of zinc to apple fibre, wheat bran, and fibre components. *Journal of Food Science, 58*, 365–368.

Carnovale, E., & Lintas, C. (1995). Dietary fibre: effect of processing and nutrient interactions. *European Journal of Clinical Nutrition, 49*(Suppl. 3), S307–S311.

Champagne, E. T., & Phillippy, B. Q. (1989). Effects of pH on calcium, zinc, and phytate solubilities, and complexes following in vitro digestions of soy protein isolates. *Journal of Food Science, 54*, 587–592.

Cook, J. D., Reddy, M. B., & Hurrell, R. F. (1995). The effect of red and white wines on nonheme iron absorption in humans. *American Journal of Clinical Nutrition, 61*, 800–804.

De Leonardis, A., Macciola, V., & Di Rocco, A. (2003). Oxidative stabilization of cold-pressed sunflower oil using phenolic compounds of the same seeds. *Journal of the Science of Food and Agriculture, 83*, 523–528.

Fairweather-Tait, S. (1992). Bioavailability of trace elements. *Food Chemistry, 43*, 213–217.

Fomon, S. J. (1974). *Infant nutrition* (2nd ed.). Philadelphia, Pennsylvania, USA: W.B. Saunders.

Grases, F., Simonet, B. M., Prieto, R. M., & March, J. G. (2001). Phytate levels in diverse rat tissues: influence of dietary phytate. *British Journal of Nutrition, 86*, 225–231.

Grases, F., Simonet, B. M., Vucenik, I., Perello, J., Prieto, R. M., & Shamsuddin, A. M. (2002). Effects of exogenous inositol hexakisphophate (InsP(6)) on the levels of InsP(6) and of inositol trisphosphate (InsP(3)) in malignant cells, tissues and biological fluids. *Life Science, 71*, 1535–1546.

Hull, S. R., Gray, J. S. S., & Montgomery, R. (1999). Autohydrolysis of phytic acid. *Analytical Biochemistry, 273*, 252–260.

Hurrell, R. F., Davidsson, L., Reddy, M., Kastenmayer, P., & Cook, J. D. (1998). A comparison of iron absorption in adults and infants consuming identical infant formulas. *British Journal of Nutrition, 79*, 31–36.

Kapsokefalou, M., & Miller, D. D. (1991). Effects of meat and selected food components on the valence of non-haem iron during in vitro digestion. *Journal of Food Science, 56*, 352–358.

Kazmi, S. A., Qureshi, M. S., & Maqsood, Z. (1987). Reactivity of an iron (III) complex of gallic acid. *Inorganica Chimica Acta, 137*, 151–154.

Kersting, M., Kaiser, B., & Schoch, G. (1995). Nutrition of infants between 5th and 12th month of life. *Ernahrungs-Umschau, 42*, 18–21.

Lönnerdal, B. (2002). Phytic acid–trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology, 37*, 749–758.

Lopez, H. W., Coudray, Ch., Levrat-Verny, M. A., Feillet-Coudray, C., Demigné, C., & Rémésy, C. (2000). Fructooligosacharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. *Journal of Nutrition Biochemistry, 11*, 500–508.

Lucarini, M., Di Lullo, G., Cappelloni, M., & Lombardi-Boccia, G. (2000). In vitro estimation of iron and zinc dialysability from vegetables and composite dishes commonly consumed in Italy: effect of red wine. *Food Chemistry, 70*, 39–44.

Martin, C. J., & Evans, W. J. (1986). Phytic acid–metal ion interactions. II. The effect of pH on Ca (II) binding. *Journal of Inorganic Biochemistry, 27*, 17–30.

Martínez, B., Rincón, F., & Ibánez, M. V. (2000). Optimization of tannin extraction from infant foods. *Journal of Agriculture and Food Chemistry, 48*, 2097–2100.

Martínez, B., Rincón, F., & Ibánez, M. V. (2004). Effect of ascorbic acid and ferrous sulfate on trace element dialyzation in weaning foods. *Food Chemistry, 86*, 369–376.

Martínez, B., Rincón, F., Ibáñez, M. V., & Abellán, P. (2004). Improving the nutritive value of homogenized infant foods using response surface methodology. *Journal of Food Science, 69*, 38–43.

Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). An in vitro method for estimation of bioavailability from meals. *American Journal of Clinical Nutrition, 34*, 2248–2256.

Mulvihill, B., & Morrissey, P. A. (1998). Influence of the sulphydryl content of animal proteins on in vitro bioavailability of non-haem iron. *Food Chemistry, 61*, 1–7.

Myers, R. H., & Montgomery, D. C. (1995). Introduction to response surface methodology. In G. E. P. Box & D. W. Montgomery (Eds.), *Response surface methodology. Process and product optimization using designed experiments* (pp. 1–16). New York: Wiley.

Nolan, K. B., Duffin, P. A., & McWeeny, D. J. (1987). Effects of phytate on mineral bioavailability: in vitro studies of $Mg^{2+}$, $Ca^{2+}$, $Fe^{3+}$, $Cu^{2+}$ and $Zn^{2+}$ (also $Cd^{2+}$) solubilities in the presence of phytate. *Journal of the Science of Food and Agriculture, 40*, 79–85.

Olivares, A. B., Martínez, C., López, G., & Ros, G. (2001). Influence of the design of a production invitro mineral availability of homogenized weaning foods. *Innovative Food Science and Emerging Technologies, 2*, 181–187.

Persson, H., Türk, M., & Nyman, M. (1998). Binding of $Cu^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ to inositol tri-, tetra-, penta- and hexaphosphates. *Journal of Agricultural and Food Chemistry, 46*, 3194–3200.

Peterson, J., & Dwyer, J. (1998). Flavonoids: dietary occurrence and biochemical activity. *Nutrition Research, 18*, 1995–2018.

Phillippy, B. Q., & Wyatt, C. J. (2001). Degradation of phytate in foods by phytases in fruit and vegetables extracts. *Journal of Food Science, 66*, 535–539.

Plaami, S., & Kumpulainen, J. (1991). Determination of phytic acid in cereals using ICP-AES to determine phosphorus. *Journal of the Association of Official Analytical Chemistry, 74*, 32–36.

Price, M. L., van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*, 1214–1218.

Prosky, P., Aspm, N. G., Schweizer, T. F., Devries, J. W., & Furda, I. (1988). Determination of insoluble and soluble dietary fibre in food and food products: collaborative study. *Journal of the Association of the Official Analytical Chemistry, 68*, 677–679.

Reddy, M. B., Hurrell, R. F., & Cook, J. D. (2000). Estimation of nonheme bioavailability from meal composition. *American Journal of Clinical Nutrition, 71*, 937–943.

Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science, 73*, 1516–1528.

Rincón, F., Ros, G., Periago, M. J., & Martínez, C. (1996). Design of product as source of variance in composition of meat-based infant beikosts. *Meat Science, 43*, 99–109.

Robison, G. K. (2000). Plan a single experiment. In *Practical strategies for experimenting* (pp. 113–140). West Sussex, England: Wiley.

Ros, G., Abellán, P., Rincón, F., & Periago, M. J. (1994). Electrolyte composition of meat-based infant beikosts. *Journal of Food Composition and Analysis, 7*, 282–290.

Sandberg, A. S., Brune, M., Carlsson, N. G., Hallberg, L., Skoglund, E., & Rossander-Hulthén, L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in human. *American Journal of Clinical Nutrition, 70*, 240–246.

Schneeman, B. O. (1986). Dietary fibre: physical and chemical properties, methods of analysis, and physiological effects. *Food Technology, 40*, 104–110.

Selle, P. H., Ravindran, V., Caldwell, R. A., & Bryden, W. L. (2000). Phytate and phytase: consequences for protein utilization. *Nutrition Research Reviews, 13*, 255–278.

Souci, S. W., Fachmann, W., & Kraut, H. (1994). Meat and organs of slaughtered animals. In S. W. Souci, W. Fachmann, & H. Kraut (Eds.), *Food composition and nutrition tables* (pp. 193). Stuttgart, Germany: Medpharm GmbH Scientific Publishers.

Torre, M., Rodriguez, A., & Saura-Calixto, F. (1991). Effects of dietary fibre and phytic acid on mineral bioavailability. *Critical Review in Food Science and Nutrition, 1*, 1–22.

Vaquero, M. P., Van Dokkum, W., Bos, K. D., Wolters, M. G. E., Schaafsma, G., & Luten, J. B. (1992). In vitro availability of calcium, magnesium, iron, copper and zinc from white or brown breads separately or in combination with other foods. *Food Science and Technology International, 32*, 47–58.

Wolters, M. G., Schreuder, H. A., van den Heuvel, G., van Lonkhuijsen, H. J., & Voragen, A. G. (1993). A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. *British Journal of Nutrition, 69*, 849–861.

Yoshino, M., Haneda, M., Naruse, M., Htay, H. H., Iwata, S., Tsubouchi, R., et al. (2002). Prooxidant action of gallic acid compounds: copper-dependent strand breaks and the formation of 8-hydroxy-2′-deoxyguanosine in DNA. *Toxicology In vitro, 16*, 705–709.