

## Predicted Chemical Speciation of Essential Metals in Digested Food

PAUL ROBB, DAVID R. WILLIAMS

Department of Applied Chemistry, University of Wales, Institute of Science and Technology, PO Box 13, Cardiff CF1 3XF, U.K.

and DAVID J. McWEENY

Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Haldin House, Queen Street, Norwich NR2 4SX, U.K.

(Received April 1, 1986)

### Abstract

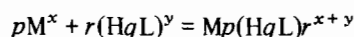
The bioavailability of essential elements liberated from food during digestion depends upon their chemical form *in vivo*. The concentrations of these species are often so low that analytical determinations cannot be carried out using existing methods, and so computerised simulation techniques have been used to investigate chemical speciation in digested food. The food is considered to be completely broken down into its constituent amino acids and metal ions which then take part in a series of complex equilibrium reactions. The computer simulation of these equilibria uses a thermodynamic approach to determine the concentrations of up to 1000 different species which may be formed *in vivo*.

### Introduction

Small amounts of metals are present in all of the foods we eat although they may exist in a number of different chemical forms. Some of these metals, for example calcium and zinc, are essential for the maintenance of good health but there are other metals, however, which have a deleterious effect if consumed in sufficient quantity, for example, lead and cadmium. The chemical reactions which occur during the digestion of food are very complex and are not well understood. Reactions can occur between metal ions and the products of digestion to produce several different chemical forms (*ie.* species) of each metal. The ability of the body to absorb these different species and to transport them through the blood (the bioavailability), is dependent upon the nature of the species formed *in vivo*. Since the components of the digested food may undergo equilibrium reactions with each other, a high level of one metal in a given diet may well affect the bioavailability of another [1–3]. Consequently, it is not sufficient simply to measure the total amount of metal in a given diet when assessing the problem of

metal bioavailability from digested foods. This has important implications if realistic maximum permitted levels for toxic metals in foods or recommended daily intakes of essential metals in diets are to be set [4].

The different species formed in digested food may be present at very low concentrations and direct measurements may not be possible even with modern analytical techniques, particularly when the species are in equilibrium. However, computerised simulation techniques have been developed for use in investigating chemical speciation in complex aqueous environments [5, 6]. Each species present in the simulation is assumed to be formed as a result of an equilibrium reaction between  $p$  metal ions  $M$ , of charge  $x+$  and  $r$  ligands  $L$  (each containing  $q$  protons) with a net charge  $y-$  according to the general equation:



A thermodynamic equilibrium constant,  $\beta$ , can be calculated for this reaction from the results of appropriate experimental techniques and the equation:

$$\beta = \frac{[Mp(HqL)_r^{x+y}]}{[M^x]^p \cdot [HqL]^r}$$

Once  $\beta$  has been measured, then the amount of complex which may be formed in aqueous solution at any given concentrations of metal ions and ligands can be calculated. Where more than one complex is formed, then a series of equations can be obtained which, when solved, produce the speciation profile for the metal under consideration [5].

In this work, the metal ions are assumed to react with chloride and hydroxide ions as well as amino acids, the latter being protonated to a greater or to a lesser extent according to the pH of the solution. The literature contains much information concerning the composition of foods and computerised techniques can be used to determine the amino acid composition of the protein fraction and the essential metal levels in these foods [7, 8]. This information can then be used to predict the nature of the essential metal species which may be formed *in vivo*.

## Experimental

### Computer Programs

All the computer programs used in this work have been written in FORTRAN 77 and run on DEC VAX11/780 computers in the University of Wales Institute of Science and Technology, Cardiff, U.K.

#### Food composition data

The nutritional composition of over 900 foods is given in the McCance and Widdowson food tables [7]. This database can be interrogated by the computer program, MINCE, which is described schematically in Fig. 1 (P. Robb, unpublished). In this work, the food composition data were stored in three discrete datafiles. The first, and largest, file was stored on magnetic tape and provided details of the metal ion content, the total amount of nitrogen present, and an amino acid reference number for each of the foods. The smaller second and third files were stored on disc and contained details of the amino acid content of the foods according to the amino acid reference numbers.

The program MINCE is interactive and requires the user to first specify the number of items in the diet under consideration, up to a maximum of 25 foods. The details of the diet can then be typed into the computer as a series of food reference numbers and food weights in grams. The program considers each food in turn but first a NAG routine (MO1AEF) is used to sort the food number and food weight data into ascending order [9]. This reduces the time required to interrogate the main data base. The program then finds the appropriate food number and reads in the amino acid reference number, total nitrogen and metal levels. In most cases, the amino acid data were retrieved directly from the second data file on the basis of the amino acid reference number. However, in the case of foods which contain several protein sources, a special amino acid reference number directs the program to a sub-routine which uses the third data file to obtain the nitrogen levels and amino acid reference numbers of the various proteins found in the food. The sub-routine then retrieves the required information from the second data file and calculates the total amino acid levels in the food before returning to the main program. The information in the second data file is stored as a numerical value which is a function of the total nitrogen content of the food and the program, therefore, has to calculate the amount of amino acid present in grams. A running total of component levels is calculated and the program then proceeds to search for the next food.

Once details of all of the food items have been found, the user can direct the program either to produce a data file listing the components of the meal (a .LOG file), or to produce a file for use with the

speciation program MINEQL (a .DAT file) as well as the .LOG file. The .LOG file contains details of the raw data read in from the database and can be used to verify the accuracy of the information used in the simulation. If a .DAT file is required, then the user is prompted to provide the volume of the digest in  $\text{cm}^3$  and the pH of the simulation. The program calculates the concentrations of the components of the digested food, includes  $150 \text{ mmol dm}^{-3}$  NaCl and the pH in the simulation, and sets the initial ionic strength of the solution to  $150 \text{ mmol dm}^{-3}$ . This information is recorded in the .DAT file. The inclusion of saline in the model allows a more accurate simulation of physiological conditions to be achieved. Table I lists the food components considered by the program in the preparation of the .DAT file.

The program MINCE will fail if either the food or amino acid reference numbers are not found and the user is advised of the reason for the error.

#### Chemical speciation program

A number of computer programs exist which can be used to investigate chemical speciation in aqueous solution [5, 6, 10]. In this work, the program MINEQL was used after slight modification to simulate chemical equilibria in physiological solutions at  $37^\circ\text{C}$  [6]. Up to 1000 different species and their appropriate thermodynamic constants can be considered in the calculations [10]. In this work, a simplified approach was used which considered that the protein in the food was completely dissociated into amino acids which then reacted with the metal ions found in the digested food. Iron may be present as either Fe(II) or Fe(III) and simulations could be carried out considering both oxidation states. The theory of the computerised simulation approach is described in detail elsewhere [5, 10]. The modified program MINEQL can calculate the ionic strength of the final aqueous solution and repeated simulations were necessary to achieve a situation where the ionic strength used in the computations was equal to that calculated on the basis of the predicted speciation profiles.

#### Example Simulation

The use of this approach is illustrated here by considering the speciation which may occur after the digestion of 100 g of raw tomato in a digest volume of  $1 \text{ dm}^3$  at pH 2 and at pH 7. The composition of the food was calculated using the program MINCE and speciation profiles were predicted using the program MINEQL.

## Results and Discussion

### Computer Predictions

The total metal ion and amino acid levels in tomato found using the computer program MINCE

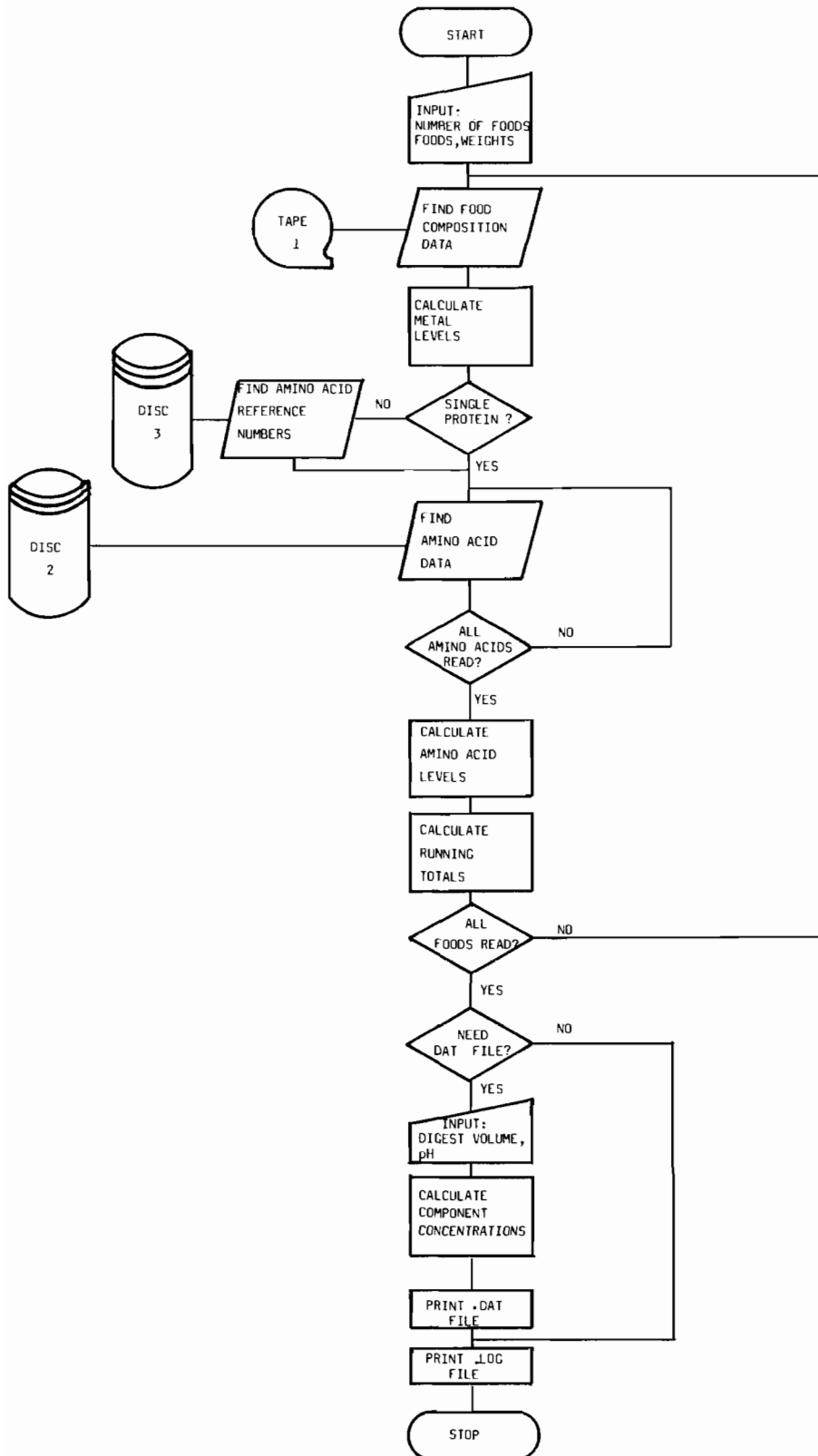


Fig. 1. Schematic Diagram of the Computer Program MINCE.

TABLE I. Components of Speciation Models

|           |               |
|-----------|---------------|
| Calcium   | Lysine        |
| Magnesium | Histidine     |
| Iron      | Aspartate     |
| Copper    | Serine        |
| Zinc      | Alanine       |
| Sodium    | Tryptophan    |
| Chloride  | Methionine    |
| Tyrosine  | Valine        |
| Glycine   | Threonine     |
| Glutamate | Phenylalanine |
| Cystine   | Iso-leucine   |
| Arginine  | Leucine       |
| Proline   |               |

TABLE II. Total Concentrations of Components in Model Obtained Using the Program MINCE

| Component     | Weight in 100 g Food  |
|---------------|-----------------------|
| Calcium       | $1.30 \times 10^{-2}$ |
| Magnesium     | $1.10 \times 10^{-2}$ |
| Iron          | $4.00 \times 10^{-4}$ |
| Copper        | $1.00 \times 10^{-4}$ |
| Zinc          | $2.00 \times 10^{-4}$ |
| Tyrosine      | $1.12 \times 10^{-2}$ |
| Glycine       | $1.54 \times 10^{-2}$ |
| Glutamate     | 0.00                  |
| Cystine       | $5.60 \times 10^{-3}$ |
| Arginine      | $1.82 \times 10^{-2}$ |
| Lysine        | $2.52 \times 10^{-2}$ |
| Histidine     | $1.26 \times 10^{-2}$ |
| Aspartate     | 0.101                 |
| Serine        | $2.24 \times 10^{-2}$ |
| Alanine       | $2.10 \times 10^{-2}$ |
| Tryptophan    | $7.00 \times 10^{-3}$ |
| Methionine    | $5.60 \times 10^{-3}$ |
| Valine        | $1.82 \times 10^{-2}$ |
| Threonine     | $1.96 \times 10^{-2}$ |
| Phenylalanine | $1.54 \times 10^{-2}$ |
| Iso-leucine   | $1.68 \times 10^{-2}$ |
| Leucine       | $2.38 \times 10^{-2}$ |
| Proline       | $1.40 \times 10^{-2}$ |

are shown in Table II. Each of the simulations carried out using the program MINEQL produced a list of over 400 different species which may be formed *in vivo*. Table III shows the major species which may exist at pH 2 and at pH 7 after digestion. Only those species which may exist in excess of 1% of the total metal concentration are given in Table III.

#### General observations

The speciation profiles differ markedly in most cases when the pH of the simulated digest is changed from 2 to 7. When the oxidation state of the iron is changed from Fe(II) to Fe(III) in these simulations, insignificant changes occur in the speciation profiles

of the other metals and these changes are not shown here. It is convenient to consider the speciation profiles of the individual metals in turn.

#### Calcium

Calcium appears to be exceptional in that only relatively minor changes are predicted when the pH is varied.

#### Magnesium

A slight reduction in the amount of  $Mg^{2+}$  and  $[MgCl]^+$  present is predicted when the pH is raised along with a small increase in the amount of amino acid complexes present at the higher pH.

#### Copper

Although very small amounts of amino acid complexes are likely to be formed at a pH of 2, an increase in the amount of these complexes present is predicted when the pH is raised. It is important to note that different complexes would be formed at the two pH values.

#### Zinc

Some amino acid complexes are liable to be formed at pH 7 although most of the zinc would be present as the dissolved hydroxide at pH 7. The free zinc ion is expected to predominate under acidic conditions.

#### Iron

Several species of iron(II) may be formed at pH 7 including small amounts of amino acid complexes. However, there are more iron(III) species predicted at pH 2 than at pH 7. This occurs since ferric hydroxide will readily precipitate in aqueous solution if present at a concentration in excess of  $10^{-17}$  mol dm<sup>-3</sup> at the higher pH [11]. Consequently, for iron(III) to be bioavailable, the iron must be complexed with a suitable ligand in the intestine since precipitated Fe(OH)<sub>3</sub> cannot be utilised by the body. In practice, iron(II) and iron(III) will both be present in the digested food. This problem can be solved in the future by measuring the redox potential of digested food and allowing the program MINEQL to determine the amount of each form of iron present in solution. This redox potential cannot be simply predicted since it depends upon the nature of the components in the digested food [11].

#### Future Method Development and Applications

Although this approach is in the early stages of development, the results discussed here show the feasibility of using computerised techniques to study chemical speciation in digested foods. However, even though a considerable amount of data can be obtained concerning the nature of the chemical species which may be formed *in vivo*, a substantial amount of

TABLE III. Computer Speciation Profiles of Essential Metals in 100 g of Digested Tomato

| At pH 2.0                             |  | At pH 7.0                                     |  |
|---------------------------------------|--|---|--|
| Species                               | Concentration<br>( $\mu\text{mol dm}^{-3}$ ) | Species                                       | Concentration<br>( $\mu\text{mol dm}^{-3}$ ) |
| Total Calcium                         | 320.0  |   |  |
| Ca <sup>2+</sup>                      | 269.0  | Ca <sup>+</sup>                               | 264.0  |
| [CaCl] <sup>+</sup>                   | 49.3   | [CaCl] <sup>+</sup>                           | 49.2   |
| Total Magnesium                       | 450.0  |   |  |
| Mg <sup>2+</sup>                      | 304.0  | Mg <sup>2+</sup>                              | 294.0  |
| [MgCl] <sup>+</sup>                   | 140.0  | [MgCl] <sup>+</sup>                           | 138.0  |
|                                       |  | [MgH <sub>2</sub> (lysinate)] <sup>3+</sup>   | 5.89   |
|                                       |  | [MgH(aspartate)] <sup>2+</sup>                | 4.70   |
| Total Copper                          | 1.60   |   |  |
| Cu <sup>2+</sup>                      | 1.20   | [Cu(histidinate) <sub>2</sub> ]               | 1.36   |
| [CuCl] <sup>+</sup>                   | 0.32   | [Cu(aspartate) <sub>2</sub> ]                 | 0.10   |
| [CuH(aspartate)] <sup>2+</sup>        | 0.02   | [CuH(histidinate) <sub>2</sub> ] <sup>+</sup> | 0.05   |
| [CuH(threoninate)] <sup>2+</sup>      | 0.02   | [Cu(histidinate)] <sup>+</sup>                | 0.03   |
| Total Zinc                            | 3.10   |   |  |
| Zn <sup>2+</sup>                      | 2.09   | Zn(OH) <sub>2</sub>                           | 2.96   |
| [ZnCl] <sup>+</sup>                   | 0.82   | [Zn(aspartate)] <sup>+</sup>                  | 0.06   |
| ZnCl <sub>2</sub>                     | 0.14   | [Zn(histidinate)] <sup>+</sup>                | 0.03   |
| Total Iron                            | 6.80   |   |  |
| Fe <sup>2+</sup>                      | 3.15   | Fe <sup>2+</sup>                              | 2.74   |
| [Fe(II)Cl] <sup>+</sup>               | 3.65   | [Fe(II)Cl] <sup>+</sup>                       | 3.23   |
|                                       |  | [Fe(II)(histidinate)] <sup>+</sup>            | 0.51   |
|                                       |  | [Fe(II)(aspartate)] <sup>+</sup>              | 0.18   |
| or                                    |  |   |  |
| Fe <sup>3+</sup>                      | 0.77   | Fe(OH) <sub>3</sub> ppt.                      | 6.80   |
| [Fe(II)Cl] <sup>2+</sup>              | 3.12   |   |  |
| [Fe(II)Cl <sub>2</sub> ] <sup>+</sup> | 2.13   |   |  |
| [Fe(II)OH] <sup>2+</sup>              | 0.59   |   |  |
| [Fe(II)(aspartate)] <sup>2+</sup>     | 0.09   |   |  |

further development is clearly desirable. The food composition database is limited and can be expanded, not only to cover more foods, but also to include more components such as the amount of iron bound in haem compounds, toxic metal levels and other ligands such as inorganic phosphate. The assumption that all of the protein is degraded into simple amino acids will be modified as the chemistry of digestion becomes better understood. Models can then be developed to include polypeptides in the metal–ligand equilibria once the necessary thermodynamic constants have been measured. In addition, secretions *in vivo* can also contribute to the metal and amino acid concentrations in the digest and these factors should be considered in future studies. A number of other food components, for example, ascorbic acid, may be included in future models to enhance the accuracy of the simulations.

In the example shown here, only two pH values are considered in the simulations but the program MINEQL is capable of calculating the speciation profiles of the metals in the digested food at any user

specified pH. At present, the models cannot account for the change in component concentrations which occur as nutrients are absorbed in the intestine. This problem arises because detailed information regarding nutrient absorption in the intestine is not readily available. Consequently, the approach can only be used at present to determine the species which are present immediately before absorption occurs.

The results of the simulations reported here indicate that many metal/amino acid complexes may be formed *in vivo* upon the digestion of tomato. It is relatively difficult to study the chemical reactions which occur during digestion *in vivo*, either in man or in animals, in such a way as to obtain reproducible results [12]. Alternative *in vitro* techniques can be used to investigate the reactions which occur during digestion [13, 14]. These chemical techniques have considerable potential for providing information relating to the release of metals from digested food, the efficiencies of protein degradation and a limited amount of data relating to speciation which will aid the design of improved models. The computerised

simulation technique can indicate whether certain species are liable to be present in detectable quantities and can help in the design of analytical techniques to find these species. This information will be of great value to researchers who are seeking to obtain a better understanding of the factors which influence the bioavailabilities of nutrients from foods.

## Conclusions

When foods are digested in the stomach and intestine, a complex mixture is produced which includes metal ions and amino acids. Computer programs can be used to investigate the nature of the species which may be formed *in vivo*, whereas existing chemical techniques can often only be used to determine the total amount of essential metal present in a food. The computerised simulation method is rapid and can indicate the directions in which research can progress to obtain a better understanding of the bioavailability of essential metals in digested foods. Further software development, the experimental validation of proposed models and the use of an expanded food composition database will all enhance the value of this technique in research into the speciation of trace metals in digested foods.

## Acknowledgements

One of us (P.R.) would like to acknowledge the financial support of the Ministry of Agriculture, Fisheries and Food. We would like to thank Dr.

H. Crews and Dr. R. Massey for their assistance in the preparation of this manuscript.

## References

- 1 J. W. Erdman Jr., *Cereal Chem.*, **58**, 21 (1981).
- 2 W. O. Caster and A. V. A. Resurreccion, *ACS Symp. Ser.*, **203**, 97 (1982).
- 3 S. J. Ritchey, *Cereal Chem.*, **58**, 18 (1981).
- 4 T. M. Florence, *Talanta.*, **29**, 345 (1982).
- 5 M. C. White, F. D. Baker, R. L. Chaney and A. M. Decker, *Plant Physiol.*, **67**, 301 (1981).
- 6 J. C. Westall, J. L. Zachary and F. M. M. Morel, 'MINEQL', a computer program for the calculation of chemical equilibrium composition of aqueous systems, Technical note no. 18, Department of Civil Engineering, Massachusetts Institute of Technology, 1976.
- 7 R. A. McCance and E. M. Widdowson, in A. A. Paul and D. A. T. Southgate (eds.), 'The Composition of Foods', HMSO, London 1978.
- 8 K. C. Day, 'Recipe', *J. Hum. Nutr.*, **34**, 181 (1980).
- 9 Numerical Algorithms Group, NAG FORTRAN Library Mark 11, Mayfield House, Oxford, 1983.
- 10 P. M. May, *Ph.D. Thesis*, University of St. Andrews, 1980.
- 11 P. M. May and D. R. Williams, in A. Jacobs and M. Worwood (eds.), 'The Inorganic Chemistry of Iron Metabolism. Iron in Biochemistry and Medicine II', Academic Press, London/New York, 1980, pp. 1-27.
- 12 K. T. Smith and J. T. Rotruck, in P. Saltman and J. Hegenauer (eds.), 'The Biochemistry and Physiology of Iron', Elsevier, Amsterdam, 1982, pp. 777-787.
- 13 H. M. Crews, J. A. Burrell and D. J. McWeeny, *J. Sci. Food Agric.*, **34**, 997 (1983).
- 14 H. M. Crews, J. A. Burrell and D. J. McWeeny, *Z. Lebensm. Unters. Forsch.*, **180**, 221 (1985).