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TECHNICAL NOTE

A chemical clean-up procedure to reduce trace metal contamination from laboratory blenders

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A simple and cost-effective method is described for minimising adventitious contamination from laboratory blenders prior to their use for the homogenisation of food samples that are to be analysed for mineral content. The blenders were treated with a hot aqueous solution of a mixture of 2% EDTA and 2% citric acid. The solution was allowed to mix in the blender at medium speed for a total period of 5 min before the bowls of the blenders were rinsed and the treatment repeated for a second time. A range of metals was determined from food samples homogenised in the treated blenders and the concentrations of metals were compared with those found in identical food samples homogenised in untreated blenders. Significant differences in metal level concentrations were observed for iron, zinc, copper, lead, cadmium and chromium — with the untreated blenders giving rise to a considerably higher degree of contamination. The results obtained indicate that laboratory blenders intended for homogenising food samples prior to analysis for metal content may need a chemical clean-up before use. The clean-up procedure described may provide a viable way of minimising contamination, particularly in the absence of specially modified equipment.

INTRODUCTION

When analysing food or other biological materials for mineral levels, whether in nutritional or toxicological studies, a preliminary preparative step to acquire a completely homogeneous sample is necessary. This may be carried out by mechanical homogenisation using various laboratory blenders and mixers, depending on the type of sample.

A problem associated with this sample preparation step is the possible metal contamination emanating from the metal parts of the homogenising equipment in contact with the sample. Certain precautionary measures to minimise contamination have been described in the literature, and these are reported to reduce contamination to acceptable levels. These include the use of homogenising equipment with the metal surfaces that are likely to be in contact with the sample coated with plastic (Keslay *et al.*, 1979) or with a nickel-gold plating (Engel *et al.*, 1967). More recently, homogenising equipment with titanium surfaces have also been reported to be effective in minimising metal contamination (Bunker *et al.*, 1982). The use of such equipment has been reported in some recent dietary analysis studies (Miller-Ihli & Wolf, 1986; Thomas *et al.*, 1986).

Homogenising equipment such as blenders, which are available in many laboratories, are commonly made from stainless steel rather than any other material; and as specially modified equipment may not be readily available commercially or accessible in view of cost, it may be necessary to take precautions to clean the blenders before use. This paper describes a simple chemical clean-up procedure that may be considered as a possible alternative method for minimising metal contamination.

MATERIALS AND METHODS

All glassware used was soaked overnight in 50% nitric acid, then rinsed with liberal amounts of distilled water.

Homogenising equipment

Four MSE Atomix Rotor blenders, equipped with stainless steel homogenising bowls and blades, were used. No modification to the original components was made.

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Chemicals

Analytical grade EDTA and citric acid (sodium salts) and hydrogen peroxide were obtained from Fison, Loughborough, UK. Sodium dodecyl sulphate (specially pure), nitric acid, calcium, magnesium, iron, copper, zinc, lead, cadmium, chromium standard solutions and lanthanum chloride (all spectrosol grade) were obtained from BDH, Poole, UK.

Food samples

Two types of a breakfast meal, consisting of the food items listed in Table 1, were used to obtain homogeneous samples of composite meals. All food items were purchased from local food stores.

Table 1. Composition of the two breakfast meals used to assess mineral contamination levels

Breakfast meal A		Breakfast meal B	
Component	Weight (g)	Component	Weight (g)
Cornflakes	40	Bread, wholemeal	70
Milk, UHT	150	Margarine	10
Sugar, white	15	Marmalade, orange	20
Coffee, instant	190	Orange juice, canned	200
		Coffee, instant	190
		Sugar, white	15

Procedure

The bowls of all blenders used were subjected to a standard clean-up procedure with a hot non-ionic detergent solution (1% sodium dodecyl sulphate), and rinsed with liberal amounts of distilled water.

Prior to the breakfast meal samples being homogenised, two of the homogenising bowls were further treated with 500 ml of a hot (80 ± 5°C) solution of EDTA (2%, w/v) and citric acid (2%, w/v). Mixing of the solution in each bowl was carried out at high speed for a total of 5 min, with intermittent stoppages. The solution was then allowed to stand for 5 min in the bowl before it was manually swirled and finally poured out. Each emptied bowl was then rinsed with distilled water, and the treatment repeated for a second time.

The two other bowls remained untreated and were used as controls for comparison of metal contamination levels.

To determine mineral contamination levels from the four homogenising bowls, a total of five duplicates of each of the breakfast meal samples (Table 1) were used. During each run, one duplicate of meal A was homogenised in the treated bowl HBA1, and one in the untreated bowl HBA2. Similarly, duplicate samples of breakfast meal B were homogenised; one in bowl HBB1 (treated) and one in bowl HBB2 (untreated). Each meal sample was allowed to mix at medium speed with occasional intermittent pauses, until a consistent homogeneous slurry was formed. Each homogenised sample was then poured onto a flat plastic drying tray, and the residual slurry in the bowl rinsed out with distilled water. All homogenised samples were freeze-dried until constant weight, and then stored at −20°C in polythene bags.

To ensure homogeneity of the freeze-dried sample, the content of each polythene bag was pulverised into a fine powder by a combination of pounding with a plastic hammer and hand-pressing, followed by agitation. An accurately weighed freeze-dried sample (1–2 g) was digested on a hot-plate with nitric acid and hydrogen peroxide in a 50 ml conical flask fitted with an air condenser. Digestion was considered complete upon the digestate becoming water-clear and reduced to about 1 ml in volume.

Each digestate was diluted with distilled water to 25 ml and used for analysis of calcium, magnesium, copper, zinc and iron by flame atomic absorption spectrophotometry (Perkin-Elmer model 372). Higher dilutions were needed for calcium and magnesium determinations after the addition of lanthanum chloride (active lanthanum concentration, 1% w/v). Chromium, cadmium and lead were determined by electrothermal atomisation atomic absorption spectrophotometry (Pye-Unicam model SP9). Operating conditions for both spectrophotometers are listed in Tables 2a and 2b. The reproducibility and precision of the analytical procedure used was evaluated by running a triplicate concurrent analysis of a standard reference material (National Bureau of Standards SRM 1577a). The results of the analysis are summarised in Table 3.

Table 2a. Instrumental parameters used for the determination of calcium, magnesium, iron, copper and zinc by flame atomic absorption spectrophotometry (Perkin-Elmer 372)

Parameter	Calcium	Magnesium	Iron	Copper	Zinc
Wavelength (nm)	422·7	285·2	248·3	324·8	213·9
Spectral bandwidth (nm)	0·7	0·7	0·7	0·7	0·7
Lamp current (mA)	7·0	7·0	10·0	5·0	5·0
Flame composition	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2
Air/fuel ratio	(45/55)	(60/40)	(60/40)	(60/40)	(60/40)
Optimum calibration range (μg litre^{-1})	2–12	0·2–1·0	1·5–10·0	0·3–10·0	0·4–2·0

Table 2b. Instrumental parameters used for the determination of lead, cadmium and chromium by electrothermal atomisation atomic absorption spectrophotometry (Pye-Unicam SP9)

Parameter	Element		
	Lead	Cadmium	Chromium
Wavelength (nm)	282·3	228·8	358·8
Spectral bandwidth (nm)	0·5	0·5	0·5
Lamp current (mA)	5·0	5·0	5·0
Flow gas used	N_2	N_2	N_2
Furnace programme			
Drying time (s)	25	25	25
temp. (°C)	150	100	100
Ashing time (s)	20	30	30
temp. (°C)	600	350	1200
Atomisation time (s)	10	5	3
temp. (°C)	2600	1800	2700
Optimum working range (μg litre^{-1})	20–100	0·5–200	20–200

RESULTS AND DISCUSSION

The data obtained from the mineral analysis of the meal samples homogenised in the treated and untreated bowls are shown in Table 4. With the exception of calcium and magnesium, the levels of zinc, copper, iron, lead, cadmium and chromium were consistently and significantly higher in concentration in the samples homogenised in the untreated blenders. The results appear, therefore, to suggest that treatment of the blenders according to the procedure described provides an effective method of reducing adventitious metal contamination during the homogenisation of the samples used in this study.

EDTA and citric acid are well-known chelating agents, forming highly stable complexes with transition metals. The deleterious effects of metal-catalysed oxidation, to which some food products are susceptible, is often counteracted by using such chelating agents as additives in such food products. This same characteristic formed the basis of the procedure described in this study. Such a procedure would probably be of benefit in other dietary analysis work where readily oxidisable nutrients, such as vitamin C, fat-soluble vitamins and unsaturated fatty acids, are being assayed, and which can be susceptible to metal-catalysed oxidation during sample homogenisation.

Table 3. Mineral levels obtained from the analysis of a standard reference material, SRM 1577a (bovine liver) (Results given are means of triplicate analysis ± SD.)

Element	Concentration (μg g^{-1})	
	Certified value	Actually found
Calcium	120 ± 7	124 ± 2·4
Magnesium	600 ± 15	609 ± 6·5
Zinc	123 ± 8	117 ± 2·5
Copper	158 ± 7	162 ± 0·9
Iron	194 ± 20	199 ± 9·2
Lead	0·135 ± 0·015	0·127 ± 0·018
Cadmium	0·44 ± 0·06	0·40 ± 0·09
Chromium	Not given	Not determined

The levels of contamination found in the blenders may have been partly the result of a history of previous use during which mineral species from other food samples homogenised accumulated over a period of time. This, nevertheless, would suggest that there is a need to examine laboratory blenders and probably other homogenising equipment before their use in mineral analysis work.

Furthermore, as many foods have a low pH, they are likely to cause the solubilisation of mineral species that may have been released during homogenisation and become bound onto the blender's surface. The use of citric acid was partly intended to act synergistically with EDTA as a chelating agent, but also to provide a low pH of the cleaning solution, in order to increase the solubility of any bound metallic deposits that may have resulted from previous use.

In conclusion, although the results obtained in this study suggest that the clean-up procedure used is effective in reducing trace metal contamination, the study was nevertheless confined to examining the procedure's effectiveness on a limited number of one type of commercial blender that was available in this laboratory. The wider applicability of the procedure needs a more comprehensive evaluation using a larger number of different commercial types of laboratory blenders in order to fully ascertain its effectiveness.

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Table 4. Mineral levels found in the breakfast meal samples homogenised in the four blenders used. Results are mean values obtained from the analysis of five duplicates of each of the breakfast meals A and B, homogenised in two treated (HBA1 and HBB1) and two untreated blenders (HBA2 and HBB2) (Values in parentheses represent range of values)

Element	Concentration (mg 100 g^{-1} freeze-dried weight)			
	HBA1	HBA2	HBB1	HBB2
Calcium	257 ± 3 (253–259)	260 ± 6 (253–269)	237 ± 7 (225–243)	234 ± 5 (229–236)
Magnesium	41 ± 2 (39–43)	41 ± 4 (38–45)	113 ± 2 (109–114)	117 ± 4 (102–120)
Iron[a]	3·9 ± 0·03 (3·85–3·92)	6·6 ± 0·42 (5·80–7·0)	3·0 ± 0·10 (2·90–3·20)	5·8 ± 0·10 (5·66–5·86)
Zinc[a]	0·9 ± 0·03 (0·92–1·0)	7·2 ± 0·3 (6·9–7·7)	2·59 ± 0·02 (2·58–2·63)	4·51 ± 0·42 (3·94–4·85)
Copper[b]	0·08 ± 0·01 (0·07–0·08)	0·16 ± 0·01 (0·14–0·19)	0·33 ± 0·01 (0·31–0·35)	0·51 ± 0·06 (0·42–0·61)
Lead[a]	0·68 ± 0·03 (0·64–0·73)	11·4 ± 1·0 (10·7–13·4)	0·61 ± 0·03 (0·57–0·65)	8·8 ± 1·2 (7·3–10·5)
Cadmium[a]	1·41 ± 0·09 (1·24–1·49)	3·3 ± 0·04 (3·2–3·4)	1·20 ± 0·05 (1·1–1·25)	2·6 ± 0·07 (2·52–2·7)
Chromium[a]	2·57 ± 0·03 (1·88–1·93)	23·8 ± 6·5 (16·9–18·2)	2·24 ± 0·03 (2·18–2·25)	32·2 ± 3·8 (29·2–39·4)

[a] HBA1 versus HBA2 and HBB1 versus HBB2 : significant difference $p < 0·001$.
[b] HBA1 versus HBA2 and HBB1 versus HBB2 : significant difference $p < 0·05$.

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